UNIVERSIDAD COMPLUTENSE DE MADRID
FACULTAD DE CIENCIAS QUÍMICAS

TESIS DOCTORAL

Estudio de la utilización del glucomanano en la reestructuración del músculo de pescado

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

Beatriz Solo de Zaldívar Gálvez

Directoras

Beatriz Herranz Hernández
Clara Asunción Tovar Rodríguez

Madrid

UNIVERSIDAD
COMPLUTENSE
MADRID

FACULTAD DE CIENCIAS QUÍMICAS

Estudio de la utilización del glucomanano en la reestructuración del músculo de pescado

Memoria para optar al Grado de Doctor presentada por:

Beatriz Solo de Zaldívar Gálvez

Dirigida por:

Dra. María Beatriz Herranz Hernández

Dra. Clara Asunción Tovar Rodríguez

Madrid, julio 2019

UNIVERSIDAD COMPLUTENSE MADRID

DECLARACIÓN DE AUTORÍA Y ORIGINALIDAD DE LA TESIS PRESENTADA PARA OBTENER EL TÍTULO DE DOCTOR

D./Dña. Beatriz Solo de Zaldívar Gálvez,
estudiante en el Programa de Doctorado D9BL - DOCTORADO EN QUÍMICA AVANZADA,
de la Facultad de Ciencias Químicas de la Universidad Complutense de Madrid, como autor/a de la tesis presentada para la obtención del título de Doctor y titulada:

Estudio de la utilización del glucomanano en la reestructuración del músculo de pescado

y dirigida por: Dra. Beatriz Herránz Hernández y Dra. Clara A. Tovar Rodríguez

DECLARO QUE:

La tesis es una obra original que no infringe los derechos de propiedad intelectual ni los derechos de propiedad industrial u otros, de acuerdo con el ordenamiento jurídico vigente, en particular, la Ley de Propiedad Intelectual (R.D. legislativo 1/1996, de 12 de abril, por el que se aprueba el texto refundido de la Ley de Propiedad Intelectual, modificado por la Ley 2/2019, de 1 de marzo, regularizando, aclarando y armonizando las disposiciones legales vigentes sobre la materia), en particular, las disposiciones referidas al derecho de cita.

Del mismo modo, asumo frente a la Universidad cualquier responsabilidad que pudiera derivarse de la autoría o falta de originalidad del contenido de la tesis presentada de conformidad con el ordenamiento jurídico vigente.

En Madrid, a 29 de julio de 2019

Fdo.:

Esta DECLARACIÓN DE AUTORÍA Y ORIGINALIDAD debe ser insertada en la primera página de la tesis presentada para la obtención del título de Doctor.

Dña. María Beatriz Herranz Hernández, Dra. en Ciencia y Tecnología de los Alimentos y Nutrición y Técnico Superior Especializado de Laboratorio, del Instituto De Ciencia Y Tecnología De Los Alimentos Y Nutrición del Consejo Superior de Investigaciones Científicas (ICTAN-CSIC) y **Dña. Clara Asunción Tovar Rodríguez**, Dra. en Física y Profesora Titular de la Universidad de Vigo, Departamento de Física Aplicada, HACEN CONSTAR QUE: el trabajo de investigación titulado **Estudio de la utilización del glucomanano en la reestructuración del músculo de pescado** que presenta **Dña. Beatriz Solo de Zaldívar Gálvez** por la Universidad Complutense de Madrid, ha sido realizado bajo nuestra dirección en el Departamento de Productos, en el grupo de Valorización de pescados y salud, del Instituto de Ciencia y Tecnología de los Alimentos y Nutrición (ICTAN), y que reúne las condiciones para optar al grado de Doctor.

Madrid, junio de 2019

Fdo.: Dra. M. Beatriz Herranz Hernández

Fdo.: Dra. Clara A. Tovar Rodriguez

La presente tesis doctoral ha sido financiada por el proyecto titulado "*Aplicación de konjac glucomanano en procesos de reestructuración de músculo de pescado*" del Ministerio de Ciencia, Innovación y Universidades (AGL2008-04892-C03-C3), a través de una Beca FPI concedida a Beatriz Solo de Zaldívar Gálvez (BES-2009-018321).

Agradezco en primer lugar, a mis directoras de tesis, las Dras. Beatriz Herranz y Clara Tovar, su confianza en mí. Muchas gracias por vuestra paciencia, tesón y apoyo. Al Prof. de Investigación Javier Borderías por la oportunidad que me ha brindado para llevar a cabo este trabajo bajo su dirección, así como sus enseñanzas.

Agradezco especialmente la colaboración del Prof. Pedro Carmona Hernández, Investigador Científico, y la Dra. Isabel Sánchez Alonso ambos del Departamento de Espectroscopía Vibracional y Procesos Multifotónicos del Instituto de Estructura de la Materia (CSIC). También a la Dr. Mª Teresa Solas Alados, profesora titular en el Departamento de Biología Celular de la facultad de Biología (UCM).

Muchas gracias a Silvia Díaz, por estar para lo que necesitara, por ser mi amiga. Gracias a mis compañeros, a todos los que se han cruzado en este largo camino y que han aportado mucho más de lo que seguramente imaginen.

Gracias Alicia, por todo lo que hemos disfrutado juntas, y lo que nos queda.

Alberto, siempre luchando y llegaron los momentos en los que nuestra lucha nos permitió lograr las mejores metas. Aún nos quedan muchas, llegaremos.

Martín y Diego, sois el motivo.

Hermanas, sería imposible sin vosotras. Papá, gracias por confiar en mí, quiero que estés orgulloso.

Juan Aguas, gracias por creer en mí, por contar conmigo. Familia, gracias por ser ejemplo, por ser muy grandes; los que nos acompañan sin estar, siempre serán un motivo de unión.

A mi madre

A mis hijos

ÍNDICE

RESUMEN

Esta tesis doctoral se centra en el diseño y la obtención de prototipos de reestructurados con aspecto de pescado fresco, con características funcionales y mínimamente procesado, elaborados a base de subproductos de pescado picado ("serrín"). Para ello se gelifica glucomanano, mediante desacetilación alcalina, en frío y de forma termoestable.

El pescado es un alimento saludable y nutritivo y su consumo diario está recomendado. Sin embargo, como recurso natural está sobreexplotado. Además, los procesos pesqueros llevan consigo la generación de subproductos, que podrían ser utilizados en la industria alimentaria en la elaboración de nuevos alimentos. La forma más habitual de elaborar productos reestructurados es la gelificación de la proteína miofibrilar aplicando calor, pero existe otra muy interesante, que consiste en la gelificación en frío mediante la incorporación al músculo de distintos tipos de ingredientes. Para llevar a cabo la gelificación en frío, se puede recurrir a tres ingredientes: alginato, transglutaminasa microbiana (MTGasa) y glucomanano. El incremento en la demanda de productos mínimamente procesados, ha hecho de esta tecnología una opción ventajosa, estos productos son muy versátiles y pueden comercializarse de diversas formas. Nos centramos en el glucomanano como gelificante.

El glucomanano (GM) es un polisacárido neutro y soluble en agua, obtenido principalmente de la raíz de *Amorphophallus Konjac*. Posee extraordinaria capacidad de formar geles termoestables mediante la adición de un álcali que retire sus grupos acetilo. Se estudió la posibilidad de utilizar GM como agente gelificante en la reestructuración del músculo de pescado, de tal manera que el GM formase una red acoplada con la proteína. Esto permitiría utilizar músculo cuya proteína tiene baja capacidad de gelificación.

En primer lugar, se lleva a cabo una caracterización físico-química, mecánica, y reológica, de los geles de GM, que permitirá elegir las mejores condiciones tecnológicas de gelificación para la posterior incorporación del pescado. Además, se analizan las interacciones que se establecen entre las cadenas de polisacárido. Una vez definidas, se adiciona el pescado para elaborar los reestructurados, y se estudian los parámetros tecnológicos anteriormente establecidos, en este nuevo sistema (GM -agua–pescado picado). También se analiza el efecto de la adición de sal y aceite como ingrediente, y se

llevan a cabo estudios de conservación: congelación, refrigeración, pasteurización, para establecer las condiciones idóneas que proporcionen más estabilidad a los productos.

Con el fin de lograr una dispersión homogénea del GM en agua que permita obtener geles de composición y estructura uniformes, se realiza un estudio para optimizar la temperatura, el tiempo y la estrategia de homogenización del sistema agua-GM. Las dispersiones acuosas de GM (AGD) más homogéneas se obtuvieron a 60 °C, con agitación continua en un homogeneizador mecánico, durante 30 min.

A continuación, se determina el gel con mejores características para la reestructuración de pescado en función de la concentración de GM y el tipo de álcali. Para ello se estudia la gelificación de las AGD a tres concentraciones, utilizando soluciones alcalinas de KOH, NaOH y Na_2CO_3 a diferentes concentraciones, adicionándolas a la AGD hasta un pH de 11,8–12,0, y son posteriormente neutralizados por inmersión en tampón. Los geles elaborados con Na_2CO_3 no son adecuados para el propósito que se persigue, por ser blandos y tener un color demasiado oscuro (gris). Los geles elaborados a partir de AGD con 3% de GM, desacetilados con NaOH y KOH 0,6N y 1N, se eligen como los más adecuados en base a las propiedades mecánicas y el color.

A continuación, se diseña un estudio de los geles seleccionados, tanto recién elaborados como después de 10 días en refrigeración (5 °C). La humedad de los geles se sitúa entre un 96–97% y no se ve significativamente afectada ni por el tipo y concentración de álcali utilizado, ni por el tiempo en refrigeración (10 días). Todos lo geles presentan una extraordinaria capacidad de retención de agua (CRA), encontrándose valores ligeramente superiores para los geles elaborados con NaOH, y manteniéndose después de 10 días en refrigeración. Las pérdidas de agua por cocción (PAC) también son menores en los geles elaborados con NaOH.

Para geles con una concentración de GM mayor (5% p/v), el contenido en humedad es similar a los del 3%, y los geles elaborados con NaOH siguen teniendo mayor CRA, aunque en este caso las diferencias no son significativas.

Respecto al color (luminosidad), cuando se lleva a cabo la gelificación de las dispersiones de GM ya sean del 3% o del 5% con KOH 0,6N, o con NaOH 1N, se obtienen

geles blancos y luminosos, y que no se ven afectados durante los 10 días de almacenamiento en refrigeración.

En todos los casos, los valores de amplitud de tensión (σ_{max}) y amplitud de deformación (γ_{max}) son más elevados con NaOH que con KOH a 0,6N y 1 N. El gel elaborado con KOH 0,6N muestra valores más altos de fuerza de rotura (FR), y deformación de rotura (DR). Además, los datos del ensayo de carga y recuperación, muestran que este gel es más estable, más elástico, y con mayor grado de conectividad (más bajo exponente *n*). Durante los 10 días de almacenamiento el gel elaborado con KOH 0,6N muestra una mejor estabilidad en el tiempo, ya que, aunque ambos geles pierden fuerza de gel (S), y sufren un aumento notable de *n*, el deterioro reológico es mayor en el gel elaborado con NaOH 1N.

Para seleccionar el pH que garantice una desacetilación del polisacárido adecuada, se realiza un análisis mediante espectroscopia de absorción infrarroja (IR) por transformada de Fourier (FTIR). Se preparan AGD al 3% y 5% (p/v). La gelificación se induce agregando KOH 0,6N (0,5-5%) de alcanzándose pH de 8,9 a 11,9 (para AGD al 3%), y de 7 a 11,4 (para AGD al 5%). Se obtiene los espectros normalizados a cada pH y los espectros muestran que la banda a 1730 cm^{-1} (vibración del grupo C=O), es visible en AGD a pH 5,6 (antes de la adición de álcali), y prácticamente desaparece cuando la AGD alcanza un pH 11,8. Se encuentra dependencia lineal entre el área relativa de las bandas de acetilo y el pH, mostrando discontinuad (*gap*) entre los pHs 9,3 y 9,8 para un AGD del 3%, y entre 9,2 y 10,7 para AGD del 5%. Con el fin de relacionar las características de la red de GM que se va formando con sus propiedades fisicoquímicas, mecánicas, viscoelásticas, y estructurales se realiza un estudio de las muestras. Antes del *gap* los geles obtenidos fueron débiles y blandos, y después fueron fuertes y consistentes, aptos para la reestructuración del músculo de pescado. A un pH bajo (~ 9,2) (antes del *gap*), a mayor concentración de GM (5%), las redes del gel son menos cohesivas (mayor $tan\delta$), más flexibles (γ_{max} alta), más dependientes de la frecuencia (convergencia entre G' y G'' a bajas frecuencias), y con menor conectividad (alto valor *n*), que las muestras en este mismo punto con la menor concentración de GM (3%). Por el contrario, a un pH alto (~ 10,7) (después del *gap*), tanto las muestras con 3% de GM como las del 5% son geles verdaderos, con poca dependencia de la frecuencia, y con flexibilidad conformacional parecida (γ_{max} similar), aunque las muestras del 5% fueron por naturaleza, más rígidas (valores mayores de BF, S y G^*). Por tanto, las condiciones de

gelificación mejores son a partir de pH ~10,7 para AGD de 3% y 5%, pH correspondiente a elevada (95%), y moderada (58%) desacetilación del GM, respectivamente. Estos geles resultantes poseen redes elásticas, cohesivas y estables en el tiempo. Las micrografías obtenidas por microscopía electrónica de barrido (SEM) muestran las interacciones entre las cadenas de polisacárido, que corroboran las características fisicoquímicas y reológicas de estos geles.

Para estudiar la termoestabilidad de los geles se elaboran geles de 3% y 5% (p/v) de GM con KOH 0,6 N y NaOH 1N. Se calientan a 50 °C, 70 °C y 90 °C y se comparan con los geles a 25 °C. Todos los geles presentan un comportamiento termoirreversible independientemente de la concentración de álcali y GM, consecuencia de las uniones permanentes que se establecen en la red del polisacárido. Se encuentran algunas diferencias estructurales según la concentración de álcali y GM. El aumento en la temperatura da lugar a redes más fuertes en los geles elaborados con un 3% de GM, que están más ordenadas en el gel elaborado con KOH. A altas temperaturas, estos geles tienen una red más elástica, con mayor capacidad de retener agua que los elaborados con NaOH. Al 5% de GM, las redes del gel están más compactas y el efecto de refuerzo con la temperatura es menor. El análisis de la microestructura mediante SEM corrobora estos resultados.

Los datos mecánicos, reológicos y estructurales, coinciden en que la gelificación del GM con KOH 0,6N proporciona mejores geles, con redes físicas menos rígidas, más elásticas, más estables con la temperatura con el tiempo. Los resultados muestran una matriz de gel ordenada y homogénea, y con alto nivel de conectividad capaz de sustentar en su estructura partículas finamente obtenidas de músculo de pescado de escasa funcionalidad.

Para la obtención de reestructurados, se añade a una AGD de 5% GM (p/v) el músculo de pescado finamente picado (25:75), y se homogeniza (T ≤ 5 °C). Se agrega KOH 0,6N hasta pH 11,8–12. Esta pasta, cuya concentración final de GM es de un 1,25%, se deja asentar (1h a 30 °C y 4h a 5 °C), y se neutraliza sumergiéndolo en tampón. El resultado es un gel termoestable con aspecto de músculo de pescado crudo, y propiedades sensoriales similares a las del pescado cuando se cocina. Los valores mecánicos de los reestructurados para deformación y fuerza de rotura, están en el mismo rango que los filetes de pescado. La CRA es similar a la del músculo fresco, y no disminuye después de un almacenamiento en

refrigeración. Los reestructurados incluyen más agua que el músculo fresco, y podrían catalogarse como alimentos bajos en calorías y con efecto saciante. Además, permiten la inclusión de ingredientes funcionales como el aceite de pescado rico en omega-3 y / o aceites vegetales ricos en ácido linoleico conjugado (CLA).

Se estudian sus propiedades y su estabilidad al ser congelados. Se elaboran prototipos de geles de pescado sin ingredientes adicionales, con aceite de pescado rico en ω-3 (5%) y con sal (0,8% NaCl). Se caracterizan después de 24h en refrigeración (5 °C), y después de 30, 90 y 150 días en congelación (-20 °C). El pescado-GM se sigue comportando como un verdadero gel después de la congelación, y durante todo el periodo de almacenamiento, no obstante, la congelación produce el endurecimiento debido a la deshidratación parcial de la matriz, causada por la formación de cristales de hielo., Es en el lote con sal, donde los geles son más rígidos, con menos capacidad de retención de agua (CRA), y presentan más pérdidas de agua por cocción (PAC), sobre todo después de 30 días de almacenamiento. Por el contrario, en las muestras sin ingredientes adicionales se establece cierta sinergia entre GM y las proteínas del pescado, que dan lugar a redes estables en el tiempo con mayor capacidad de retener agua después de la congelación. Las muestras con aceite, poseen menos PAC, y una tendencia más regular en los parámetros mecánicos y viscoelásticos, entre 90 y 150 días en congelación, lo que manifiesta el efecto estabilizador del complejo "aceite-proteína-agua".

Se estudia también el efecto de la pasteurización (80 °C, 20 min), y la estabilidad durante la posterior refrigeración (5 °C; 7, 21 y 35 días) de los tres lotes de reestructurados. Se examinan las propiedades fisicoquímicas, reológicas, microbiológicas y sensoriales. El calentamiento (pasteurización), produce respuestas estructurales diferentes en los tres lotes, pero reduce significativamente la CRA en todos, siendo menos evidente en los lotes con aceite y sal. La adición de sal favorece las asociaciones proteína-polisacárido, mejorando la conectividad en la red formando redes más firmes, más cohesivas y homogéneas. En el prototipo que contiene aceite, el relleno en la red se desorganiza parcialmente, liberando aceite, desarrollándose una textura más untuosa, suave y menos aceptable sensorialmente.

Durante la refrigeración tras la pasterización, el lote sin ingredientes mantiene la textura alcanzada después de la pasteurización. En el lote con sal la red se fortaleció debido

al refuerzo de los entrecruzamientos polares. En el lote con aceite hay reordenamientos estructurales del relleno de modo que las redes resultantes fueron menos homogéneas, pero con mayor capacidad de retener aceite y agua. La mejor puntuación en los análisis sensoriales se otorgó al lote pasteurizado con sal, tanto al día como a los 21 días de almacenamiento refrigerado.

El pescado picado así reestructurado se puede ofrecer en forma de producto congelado o fresco, precocinados o no, manteniendo una calidad sensorial similar a los alimentos pesqueros tradicionales, y que pueden resultar análogos a ellos.

SUMMARY

This doctoral thesis focuses on the design and production of restructured prototypes made from by-products of minced fish ("sawdust"). They are intended to have the same appearance as raw fish muscle, with sensory properties like fish muscle when they are cooked. For this, addition of glucomannan (a neutral hydrocolloid from Amorphophallus Konjac) helps to form thermostable hydrogels in the presence of alkali, entrapping the mince within the hydrocolloid matrix."

Fish is a healthy and nutritious food and daily consumption is recommended. However, as a natural resource it is over-exploited with not all sources are directly destined for human consumption. In addition, processing of fish involves the production of by-products, which have a use in the food industry as a raw material in the preparation of new food products. The most common way to produce restructured products is by gelling the myofibril protein because of heat. There is another less common, but interesting method consisting of cold gelation by incorporating different ingredients into the muscle. To conduct cold gelling, three ingredients can be used; alginate, microbial transglutaminase (MTGase), and glucomannan. In recent decades, the increased demand for minimally processed products has made this technology an advantageous option, since the products manufactured are versatile and have many marketable aspects.

Glucomannan (GM) is a neutral polysaccharide, obtained from the root of *Amorphophallus Konjac,* among other sources. Its importance lies in its extraordinary ability to form thermostable gels. For this, it is necessary to add an alkaline agent which removes acetyl groups from its molecule, which are responsible for its solubility in water. Based on the ability of glucomannan to form thermostable gels, the possibility of using glucomannan as a gelling agent in the restructuring of finely chopped fish muscle was studied. In this way, glucomannan and fish protein formed a coupled three-dimensional network. This would allow the incorporation of a greater variety of fish, traditionally not destined for human consumption or fish muscle from by-products of fishing processes, where heat had intervened and whose protein, being denatured, had little gelling capacity.

To elaborate the restructured products, physical-chemical, mechanical, and rheological characterisation of the GM gels was carried out. This allowed the determination of the best gelling conditions, for the subsequent incorporation of the minced fish muscle. In addition, the interactions established between the polysaccharide chains were analysed. Once the optimal technological conditions for gelling of the system were defined, the minced

fish was added to produce the restructured prototypes, and the previously established technological parameters were studied for the new systems (GM-water-minced fish). The effect of the addition of salt and oil as an ingredient was also analysed, while conservation studies carried out included: freezing, refrigeration, and pasteurisation. This was with the aim to establish the ideal conditions that provide more stability to the products.

To achieve a homogeneous dispersion of GM flour in water, allowing for gels of uniform composition and structure; a study was made, to optimise the temperature, time, and strategy of homogenisation of the water-GM system. Aqueous dispersions of different concentrations of GM (1 - 6% w / v) were prepared at various temperatures (50 - 100 °C), with different mechanical stirring times (30 - 90 min). The most homogeneous GM dispersions were obtained at 60 °C, with continuous agitation in a mechanical homogeniser, for 30 min.

Next, the gel with better characteristics for fish restructuring is determined according to the concentration of GM and the type of alkali. For this, the gelation of the aqueous glucomannan dispersions (AGD) at three concentrations (1, 3 and 6% (w / v) was studied. Using three alkaline solutions (KOH, NaOH, and Na_2CO_3) at different concentrations (0.2 M, 0.6 M, and 1 M, respectively), adding them to the AGD until reaching a pH of 11.8 - 12.0, which are subsequently neutralised by immersion in a buffer. The gels elaborated with Na_2CO_3 were not suitable for the purpose of this investigation, as a soft texture and a dark (grey) colour was produced. Gels made from AGD with 3% GM, deacetylated with 0.6 N NaOH and 1 N KOH were chosen as the most suitable based on observed mechanical properties and colour.

A complete study of these gels was designed, for both newly developed and those stored for 10 days in refrigeration (5 °C). It was observed that the humidity of the gels was between 96 - 97%. This water content was not significantly affected by either the type or concentration of alkali used for gelling, neither by the storage time in refrigeration. All gels have an extraordinary water binding capacity (WBC), with slightly higher values found in gels made with NaOH, after remaining 10 days in refrigerated storage at 5 °C. Water losses by cooking (WLC) are also lower in gels made with NaOH.

The experimental design was repeated for gels with a higher GM concentration (5% w / v). It was observed that the moisture content was not affected by the concentration of polysaccharide. The gels made with NaOH showed the same trend but still had higher WRC, although in this case the differences were not significant.

Regarding the colour (luminosity) of the gels, when the gelation of the GM dispersions was conducted with either 3% 0.6 N KOH, or with 5% 1 N NaOH, white and luminous gels were obtained. Over the 10 days of storage in refrigeration, this luminosity was not affected.

In all cases, the values of stress amplitude (σ_{max}) and strain amplitude of deformation (γ_{max}) were greater with NaOH compared with KOH at concentration 0.6 N and 1 N, respectively. The gel made with 0.6 N KOH shows greater breaking force (BF) and breaking deformation (BD). In addition, the creep and recovery data show that this gel is more stable, more elastic, and with a greater degree of connectivity (lower *n* exponent). Over the 10 days of storage, the gel made with 0.6 N KOH showed better stability over time, although both gels lose gel strength (*S*), and suffer a remarkable increase in n, the rheological deterioration is greater in the gel made with 1N NaOH.

To select the pH value that guarantees a complete deacetylation of the polysaccharide, that allows from the AGDs , thermostable gels; an analysis was performed by Fourier-transform infrared spectroscopy (FTIR). Several batches of AGD at 3% and 5% (w / v) were prepared. The gelation was induced by adding different amounts (0.5 - 5%) of 0.6N KOH, reaching pH from 8.9 to 11.9 (for AGD at 3%), and from 7 to 11.4 (for AGD at 5%). The normalised spectra were obtained at each pH and it was observed that the band at 1730 cm^{-1} (vibration of group C = O), was visible in glucomannan dispersions at pH 5.6 (before the addition of alkali), and it practically disappears when the dispersion reaches a pH = 11.8. A linear dependence was found between the relative area of the acetyl bands and the pH, showing linear discontinuity (gap) between the pH 9.3 and 9.8 for an AGD of 3%, and between 9.2 and 10.7 for AGD of 5%. To relate the characteristics of the GM network formed as the pH increases (deacetylation), with its physicochemical, mechanical, viscoelastic, and structural properties, a complete study of the samples was carried out. It was observed that, before the gap, the gels obtained were weak and soft; after they were strong and consistent, suitable for the restructuring of the fish muscle. At a low pH (≈ 9.2) (before the gap), at a higher GM concentration (5%), gel networks are less cohesive (larger tan δ), more flexible (high γ_{max}), more dependent on the frequency (convergence between G' and G'' at low

frequencies), and had less connectivity (high *n* value). This was when compared to the samples at this same point with the lowest concentration of GM (3%). On the contrary, at a high pH (≈ 10.7) (after the gap), both samples with 3% GM and 5% are true gels, with little dependence on frequency, and with similar conformational flexibility (similar γ_{max}). Samples of 5% GM were, by nature, more rigid (higher values of BF, *S*, and *G**). Therefore, the best gelation conditions were pH 10.7 for AGD of 3% and 5%, pH corresponding to high (95%), and moderate (58%) deacetylation of GM, respectively. These resulting gels have elastic, cohesive, and stable networks over time. The microphotographs obtained by scanning electron microscopy (SEM) show the interactions established between the polysaccharide chains, which corroborate the physicochemical and rheological characteristics of these gels.

A study of the thermostability of 3% and 5% (w / v) GM gels made with 0.6 N KOH and 1N NaOH was conducted. They were heated to 50 °C, 70 °C, and 90 °C for 20 min, and compared with the gels at 25 °C. All the gels presented a thermo-irreversible behaviour, independent of alkali concentration and GM, a consequence of the cuasi-permanent unions established in the polysaccharide network. Structural differences were found according to the concentration of alkali and GM. The increase in temperature gave rise to stronger networks in gels made with 3% GM, which are more ordered in gels made with KOH. At high temperatures, these gels have more elastic networks, with greater capacity to retain water than those made with NaOH at the same concentration. At 5% GM, the gel networks are more compact and the effect of reinforcing with temperature, is lower. The analysis of the microstructure by SEM corroborates these results.

The mechanical, rheological, and structural data agrees that the gelation of GM with 0.6 N KOH provides better gels, with less rigid physical networks, were more elastic, more time- and temperature-stable and over greater storage times. The results show an ordered and homogenous gel matrix, with a high level of connectivity, able to hold particles in its structure, finely obtained, from minced fish muscle with little or scarce functionality to the produced restructured product.

To produce the restructured fish product, fish muscle was finely chopped and added to a dispersion of 5% GM in water (w / v) in the proportion (75:25) and the mixture was homogenised for 10 min at low temperature (T ≤ 5 °C). 0.6 N KOH was added until a pH of 11.8 - 12 was achieved. This paste was introduced into moulds, stored at 30 °C for 1 h and

then at 5 °C for 4 h. This fish-GM system was subsequently neutralised by immersing it in a buffer, resulting in a thermostable gel with the same appearance as raw fish muscle. The fish-GM system also had the sensory properties similar to those of fish when cooked, with the final concentration of 1.25% GM. The mechanical values of the restructured product for the breaking force and the deformation were in the same range as the fish fillets. The water retention capacity is similar to that of fish muscle and does not decrease after refrigeration storage. These restructured products include more water than fish muscle and could be categorised as low-calorie and satiating foods. They also allow the inclusion of functional ingredients such as fish oils rich in ω-3 and / or vegetable oils rich in conjugated linoleic acid (CLA).

The minced fish being restructured can be offered to consumers as a fresh or frozen product, maintaining a nutritional and sensory quality similar to traditional foods. They can be presented in different formats, from ready-to-heat products, skewers, carpaccio, sushi, prepared dishes, as well as marinated and / or smoked products. Therefore, its properties and stability were studied when frozen. Three batches of fish gel prototypes were prepared at a final GM concentration of 1.25%, without additional ingredients, with fish oil rich in ω-3 (5%) and with salt (0.8% NaCl). They were characterised after 24 hours of refrigerated storage (5 °C), and after 30, 90 and 150 days of storage in freezing conditions (-20 °C). The GM continued to behave like a true gel after freezing and throughout the storage period. However, freezing caused a hardening of the gels due to partial dehydration because of the formation of ice crystals, particularly in the batch with salt. Batches with salt showed gels were more rigid, with less water retention capacity (WRC), and presented more water losses by cooking (WLC), especially after 30 days of storage. In contrast, samples without additional ingredients show that some synergy was established between GM and fish proteins, which resulted in networks that were stable over time with a greater capacity to retain water after freezing. Samples with oil showed less water loss by cooking, and more regular behaviour in the mechanical and viscoelastic parameters between 90 and 150 days of frozen storage. This reveals the stabilising effect of the "oil-protein-water" complex in the GM-fish protein matrix. These samples were sensory evaluated, and those containing oil were scored the worst because they were unctuous and a little bland.

The effect of pasteurisation (80 °C, 20 min), and the stability during the subsequent refrigerated storage (5 °C for 7, 21, and 35 days) of the three restructured fish prototypes

were also studied. The physicochemical, rheological, microbiological, and sensory properties were examined. Pasteurisation significantly reduced the water retention capacity (WRC), but was less evident in batches with oil and salt. In the batch with oil an emulsion was formed in the gel network, which makes this sample less sensory acceptable. The addition of salt favoured protein-polysaccharide associations, thus reinforcing the gel network. Heating (pasteurisation) produced different structural responses in the three batches depending on their composition. In the sample without additional ingredients, pasteurisation produced more damage to the network, evidenced by a greater decrease in the capacity to retain water, giving a sensorial drier sensation. In the prototype that contains oil, the filling in the network is partially disorganised, releasing oil and, consequently, produces a lubricating effect, developing a smoother more unctuous texture. The addition of salt helped to form a uniform colloidal dispersion with the myofibrillar protein. This improved the connectivity in the gel network during gelation, producing firmer, more cohesive, and homogeneous networks showing the stabilising role of the salt. During refrigerated storage, the strengthening of the GM network continued in the batch with salt, due to the reinforcement of the polar interactions. This is because the networks tend to become more rigid, although a little less homogeneous, at low temperatures. The batch without ingredients maintained the texture achieved after pasteurisation. Finally, structural rearrangements of the filling were observed due to the low temperatures in the GM-protein network in the batch with oil, so that the resulting networks were less homogeneous, but with greater capacity to retain oil and water. All these structural changes were also reflected in the physicochemical parameters and sensory analysis. The best score in sensory analyses was given to the batch with salt, just after pasteurisation (day 1), and after 21 days of refrigerated storage.

The minced fish thus restructured can be offered in the form of a fresh or frozen product, precooked or not, maintaining a sensory quality similar to that of traditional fishery foods and which may be analogous to them.

I.- INTRODUCCIÓN GENERAL

BLOQUE 1.- EL GLUCOMANANO (GM)

1.1.-DEFINICIÓN Y ESTRUCTURA QUÍMICA DEL GLUCOMANANO (GM)

El glucomanano (GM) es un polisacárido neutro constituido por unidades de D-manosa y de D-glucosa unidos por enlaces glicosídico β-1,4 con una relación de 1,6:1 o 1,4:1 (D-manosa: D-glucosa) (Kato y Matsuda, 1969; Shimahara, Suzuki, Sugiyama, y Nisizawa, 1975a), dependiendo de los genotipos de la planta de la que procede. La estructura detallada del GM se ha determinado mediante hidrólisis ácida y enzimática (Kato y Matsuda, 1969; Shimahara y col., 1975a). La secuencia representada en la Figura 1 constituye la unidad básica y repetitiva de la cadena polimérica: GGMMGMMMMM, donde G sería D-glucosa y M representaría a la D-manosa.

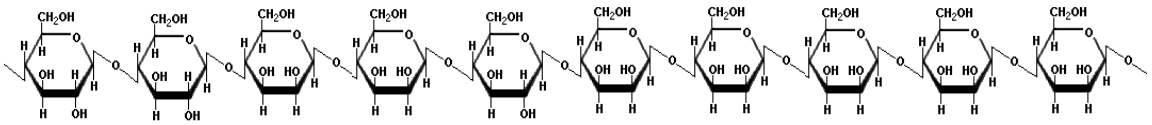

Figura 1- Unidad estructural del polisacárido de GM. Imagen tomada de "Glucomanano: propiedades y aplicaciones terapéuticas" (*Fuente* González Canga y col., 2004).

El esqueleto del polisacárido tiene un grado de ramificación (Figura 2) de aproximadamente un 8%; las ramificaciones están unidas a la cadena principal a través de un enlace glicosídico β-1,6 (Shimahara, Suzuki, Sugiyama, y Nisizawa, 1975b). Un grupo acetilo sustituye a un grupo hidroxilo en la posición C-6 de la cadena principal, por cada 9–19 residuos de monosacárido, el grupo acetilo es el que le confiere la solubilidad en agua (Maekaji, 1974; Williams y col., 2000). El peso molecular del GM varía de 500 kDa a 2000 kDa, dependiendo de la procedencia y de los métodos de obtención (Shimahara y col., 1975a), y tiende a tener un tamaño de cadena homogéneo, con una polidispersidad de 1,21 (Peso molecular promedio en masa (Mw)/peso molecular medio en número (Mn)) (Behera y Ray, 2016; C. Zhang y Yang, 2014; Zhu, 2018).

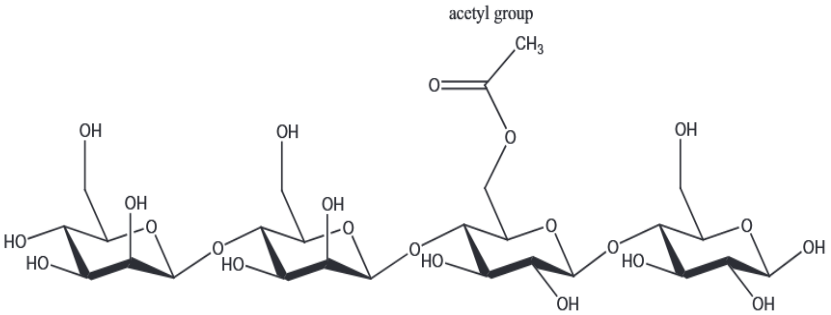

Figura 2.- Estructura del GM: D-glucosa y D-manosa unidas por enlaces β-(1-4)-glicosídico con la posición C-6 acetilada por cada 9–19 residuos de monosacárido.

1.2.- CAPACIDAD DE GELIFICACIÓN DEL GLUCOMANANO

El glucomanano tiene capacidad de formar un gel con interesantes características, es biodegradable, no tóxico, inocuo, biocompatible y funcional, lo que permite su aplicación en alimentos, productos químicos, fármacos y materiales de diversas aplicaciones, como resinas adsorbentes. Las cadenas de GM desacetiladas se agregan entre sí y forman una estructura de red tridimensional (Maekaji, 1978), dando lugar a un gel termoestable. Las redes tridimensionales formadas por GM son estructuras físicamente formadas por regiones ordenadas localmente (zonas de unión), estabilizadas por interacciones físicas como enlaces de hidrógeno e interacciones hidrofóbicas (Huang, Takahashi, Kobayashi, Kawase, y Nishinari, 2002; Nishinari y Zhang, 2004).

1.2.1. – Grado de acetilación del glucomanano

El número y la frecuencia de sustitución de grupos hidroxilo de las manosas por grupos acetato en la cadena polimérica del GM, varían en función de su origen y su método de obtención (Figura 3).

Numerosos estudios han puesto de manifiesto que la gelificación del GM se realiza desacetilando las cadenas del polisacárido (Figura 3), mediante diferentes mecanismos. La sustitución de grupos –CO-CH_3 por grupos –OH en las cadenas (mucho menos voluminosos), reducen el impedimento estérico lo que facilita la libre asociación de las cadenas para formar el gel (Chen, Li y Li, 2011; Du y col., 2012; Gao y Nishinari, 2004; Hu y col., 2019; Yang y col., 2017) .

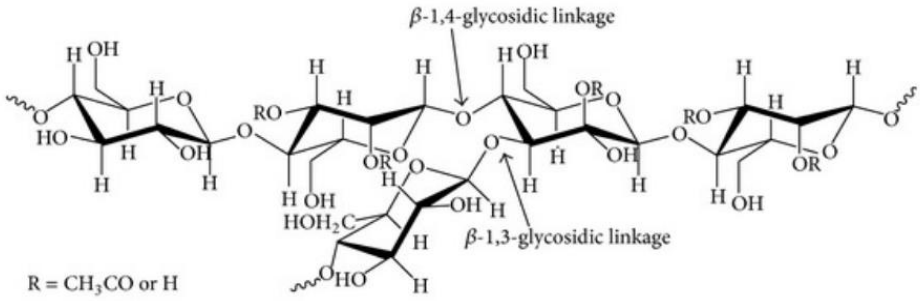

Figura 3.- GM, unidades de D- glucosa y D- manosa unidas por enlaces glicosídicos β-1,3 o β-1,4y donde R pueden ser H o grupos acetilos dando lugar a polisacáridos con distinto grado de acetilación.

Muchos geles físicos se forman principalmente por enlaces de hidrógeno cruzados entre sus cadenas, formando hélices o láminas, como es el caso del gel de gelatina y agarosa. Estos geles son termo-reversibles, ya que estos enlaces se desestabilizan y se destruyen al aumentar la temperatura, alcanzando el estado sol. Sin embargo, los geles de GM son termo-irreversibles, ya que una vez formados, si se incrementa la temperatura, conservan el estado gel. Dependiendo del grado de desacetilación también se pueden comportar como el caucho (*rubber-like*), ya que el módulo elástico aumenta durante el calentamiento (Herranz, Piñeiro, Borderías, y Tovar, 2017; Tovar, Piñeiro, y Herranz, 2017; Tye, 1991). El fenómeno de la termoestabilidad de los geles de GM sugiere que algún otro tipo de uniones se deben establecer entre cadenas del polisacárido gelificado. Las interacciones hidrofóbicas y el fuerte acoplamiento de red por el elevado número de interacciones físicas, que actúan cooperativamente entre las cadenas desacetiladas, desempeñan un papel importante (Du y col., 2012). Por lo tanto, el grado de acetilación del GM nativo influye en la capacidad de gelificación de las dispersiones acuosas, y en las características de los geles obtenidos.

1.2.2.- Métodos de gelificación del glucomanano

Se han descrito diversos métodos de gelificación del GM, entre ellos, el más habitual es la gelificación inducida por tratamiento alcalino (Du y col., 2012; Huang y col., 2002). Además se ha descrito: la formación de gel mediante la interacción de iones de boro con los grupos hidroxilo de las manosas que constituyen el polisacárido (Gao, Guo, y Nishinari, 2008; Gao, Guo, Wu, y Wang, 2008), mediante métodos de copolimerización con otros polímeros como la acrilamida (Fan y col., 2016; Li, Su, Haq, Xie, y Wang, 2016), el carragenato (Williams, Clegg, Day, Phillips, y Nishinari, 2010), la goma xantana (Annable, Williams, y Nishinari, 1994) o la goma gellan (Miyoshi, Takaya, y Nishinari, 1996). También métodos físicos, mediante la aplicación de un campo eléctrico de alto voltaje (Wang, Jiang,

Lin, Pang, y Liu, 2016; Wang, Zhuang, Li, Pang, y Liu, 2016) y otros métodos que dan lugar a microesferas de gel como la inmovilización sobre iones metálicos (Wang, Liao, Huang, y Cheng, 2012; Wu, Lin, Wu, Zhou, y Luo, 2016). Los geles de GM preparados mediante diferentes métodos, son significativamente diferentes en términos de microestructura (Moreno-Conde y col., 2016; Ji y col., 2017; Park, 1994; Zhang y col., 2016). La microestructura de los geles de GM determina sus propiedades físicas, y, por tanto, sus aplicaciones. Esta microestructura viene dada por el grado de acetilación del GM nativo, la concentración de las dispersiones acuosas y el mecanismo de gelificación empleado.

1.2.3.- Gelificación del glucomanano inducida por álcali

En esta memoria nos centraremos en la **gelificación del glucomanano inducida por álcali.** Es bien conocido que el GM forma un gel térmicamente irreversible en presencia de un álcali (Hu y col., 2019). Ya las primeras investigaciones acerca del mecanismo de gelificación del GM inducido por álcali en 1974 (Maekaji, 1974), pusieron de manifiesto que es la pérdida de grupos acetilo de las cadenas del polisacárido durante el tratamiento alcalino, la causa de la formación de agregados intermoleculares. Por tanto, en función de los valores de pH de las dispersiones acuosas de GM modificado con diferentes concentraciones de álcali, se disminuye el grado de acetilación de las cadenas nativas, dando lugar a geles con microestructuras diferentes, mantenidas en gran medida, por interacciones intermoleculares de corto alcance, como enlaces de hidrógeno e interacciones hidrofóbicas (Gao y Nishinari, 2004; Nishinari y Zhang, 2004; Yin, Zhang, Huang, y Nishinari, 2008).

Los geles de GM elaborados para esta memoria fueron estudiados y caracterizados fisicoquímica, microestructural y reológicamente. Se buscan las mejores características para obtener una textura adecuada, con el fin de ser utilizados en la reestructuración del músculo de pescado picado con casi nula funcionalidad. Para ello, se tuvieron en cuenta las siguientes variables, que se optimizan a lo largo de este trabajo:

1) Grado de acetilación del GM nativo;
2) Concentración de GM en las dispersiones acuosas;
3) Tipo y concentración de álcali que induce la desacetilación.

1.3.- LA PLANTA DE KONJAC Y SU HISTORIA

El **GM** es un polisacárido neutro, abundante en la naturaleza, específicamente en maderas blandas (hemicelulosa), raíces, tubérculos o bulbos de algunas plantas. A pesar de que se puede obtener de variadas fuentes, el tipo de GM comúnmente utilizado es el denominado **Konjac Glucomanano** (KGM), que se obtiene del tubérculo de la planta *Amorphophallus konjac*.

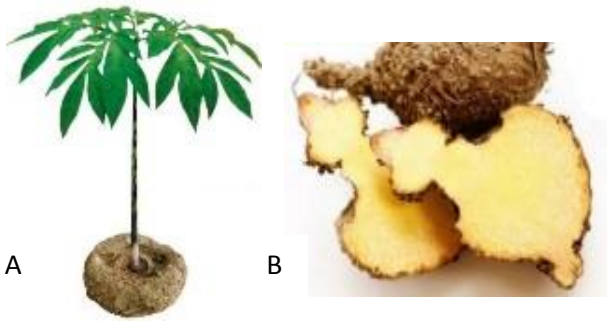

Figura 4.- Amorphophallus konjac. (A) planta de konjac (B) Tubérculo de Konjac*. Imágenes tomadas de* https://www.comycebaleares.com/que-es-el-konjac/.

La planta de *Amorphophallus Konjac* es de hoja perenne con un tallo subterráneo en forma de bulbo (tubérculo), y hojas altamente diseccionadas en forma de paraguas (Figura 4). En general, el tubérculo de *Amorphophallus konjac* contiene entre un 49 y un 60% (p/p) de GM, 10–13% (p/p) de almidón, 2,6–7% (p/p) de elementos inorgánicos (aluminio, calcio, cromo, cobalto, hierro, magnesio, manganeso, fósforo, potasio, selenio, silicio, sodio, estaño y zinc), 5–14% de proteína, 3–5% (p/p) de carbohidratos simples solubles, 3,4%–5,3% de cenizas y una pequeña cantidad del alcaloide trigonelina (N-metil-betaina del ácido nicotídico), y algunas saponinas en la base de su tallo (Liu y col., 2005; Usda, 2004). En el tejido fresco del tubérculo también se han encontrado compuestos orgánicos como β-caroteno, colina, niacina, rivoflavina y tiamina (Usda, 2004), así como serotonina y trans-N-(p-coumaroil) serotonina (Niwa, Etoh, Shimizu, y Shimizu, 2000). La composición del tubérculo varía con la especie, el origen y las condiciones de cultivo.

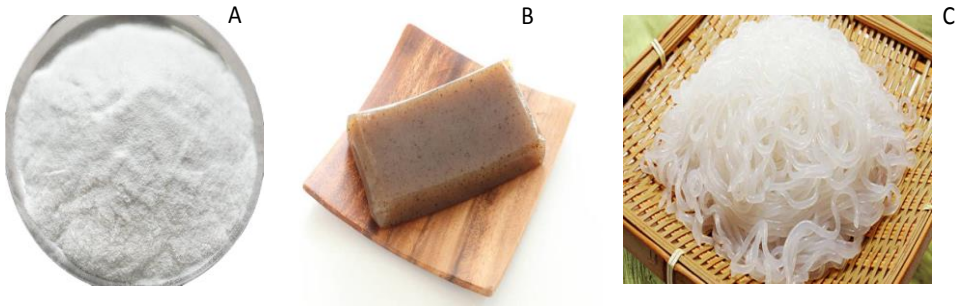

Figura 5. -(A) Harina de Konjac (B) Gel de harina de Konjac (C) Noodles Shirataki, elaborados con al menos un 40% de harina de Konjac.
Fuente: https://www.alimente.elconfidencial.com/nutricion/2018-07-30/productos-konjac-glucomanano_1598240/.

Los chinos fueron los primeros en estudiar y utilizar la planta y el tubérculo de Konjac, cuyo uso como hierba medicinal fue descrito por primera vez en el "Shen Nong Materia Medica "durante la dinastía occidental Han (206 AC-08 DC). Tradicionalmente, los tubérculos eran lavados, pelados, troceados, secados y molidos para producir la **harina de konjac**, la cual era consumida en forma de pastel (o gel) después de hervir la harina con otras plantas. En el siglo VII d.C. la harina de konjac fue introducida en Japón como uso medicinal pero finalmente se hizo popular en la cocina vegetariana (Pereira y col., 2004), siendo el ingrediente principal de alimentos como los noodles (*shirataki*) (Figura 5).

1.4.- OBTENCIÓN DEL GLUCOMANANO DE KONJAC (KGM)

Del tubérculo de la planta de *Amorphophallus Konjac* se extrae una **harina** soluble en agua, comúnmente conocida como konjac glucomanano (KGM). Tradicionalmente los cultivadores preparaban la harina de konjac secando con aire láminas del tubérculo previamente machacadas.

Las diferentes aplicaciones comerciales del KGM requieren diversos grados de pureza de la harina, siendo muy alto en el caso de aplicaciones alimentarias (Fang y Wu, 2004), por lo que actualmente, el proceso de obtención de la harina de konjac se ha implementado y es el siguiente:

Figura 6.-(A) Lavado de los tubérculos- (B) Secado de las láminas. Imágenes tomadas de Traditional uses and potencial health benefits of "Amorphophallus konjac" (Chua, Baldwin, Hocking, y Chan, 2010).

El tubérculo se lava para eliminar la suciedad y los sólidos. A continuación, los tubérculos limpios se laminan y seguidamente se secan con aire (Figura 6). Los trozos secos son pulverizados para producir la harina de konjac cruda, la cual, tiene olor a pescado, color blanquecino y sabor acre. La harina de konjac común para uso alimentario se produce eliminando de la harina cruda pulverizada, las impurezas, el almidón, la celulosa y los carbohidratos de bajo peso molecular, mediante tamizado o por precipitación con alcohol (Liu, 1998; Takigami, 2009). Este último implica la inmersión de la harina en una solución 50% (v/v) de agua- metanol durante 3h seguido de un secado a 50 °C bajo presión reducida. La harina cruda se trata 5 veces con benceno-alcohol absoluto (4:1 v/v) y triclorometano-n-butanol (Método Sevag) (Sevag, 1934). Esta harina así desengrasada y desproteinizada, se disuelve en agua a 40 °C, y posteriormente se enfría hasta temperatura ambiente. A continuación, se centrifuga a 16000 rpm durante 20 min. Se añade acetona al sobrenadante, se obtiene un precipitado blanco con aspecto de algodón que se filtra a través de un tamiz de tela, se escurre y se liofiliza (Li y Xie, 2006).

La harina de konjac así purificada está lista para uso alimentario, aunque posteriormente se realizan varias inspecciones antes de ser formulada como suplementos nutricionales (Chua y col., 2010). El proceso de extracción y purificación de la harina de konjac es crucial para la calidad final y afecta a sus propiedades fisicoquímicas y funcionales. Por ejemplo, se observó un importante incremento de la bioactividad anti-obesidad de la harina molida frente a la no molida (disminución del tamaño de partícula de 765 a 24 μm) (Li, Xia, Wang, y Xie, 2005). Un menor tamaño de partícula de la harina de konjac conlleva una mayor velocidad de hidratación, incrementándose así la actividad saciante.

1.5.- REGULACIÓN, CALIDAD Y DEFINICIONES DEL GLUCOMANANO DE *KONJAC* (KGM)

El GM procedente de *A. Konjac* (KGM) tiene uso como aditivo alimentario autorizado en Europa desde 1994, y le fue concedido el número E425 por la European Food Safety Authority (EFSA, 2007), que le asignó una dosis máxima específica de 10 g/kg en alimentos. Está clasificado como GRAS (Generally Recognized as Safe) por la U.S. Food and Drugs Administration (FDA) (Zhang, Xie, y Gan, 2005). En 1996 fue aceptado como ligante en carnes y productos avícolas por U.S Department of Agriculture (USDA).

Diferentes métodos de obtención de los compuestos bioactivos de las plantas pueden afectar a su concentración y a su funcionalidad, por lo que la Comisión Europea en la Comisión Directiva 2001/30/EC, en lo que respecta al GM procedente de A. Konjac, ha hecho una distinción entre la *"goma de konjac*" y el "*glucomanano de Konjac*". Las definiciones de ambos son:

E 425 (i) GOMA de KONJAC es un hidrocoloide soluble en agua obtenido de la harina de konjac mediante extracción en fase acuosa. El principal componente de la "goma de konjac" es el GM (más de un 75%), que está constituido por unidades de D-glucosa y D-manosa con una proporción molar de 1,6:1 - 1,4:1 en unidas por enlaces glicosídico β-(1-4).

E 425 (ii) GLUCOMANANO de KONJAC (KGM) es un hidrocoloide soluble en agua obtenido de la harina de konjac mediante extracción en fase alcohólica (etanol-agua). El principal componente KGM es el GM (más de un 95% sobre el peso en seco).

En la Tabla 1, se describen las composiciones de la goma de Konjac y el KGM dadas por el ministerio de agricultura chino, la Comisión Europea, y la Food Chemicals Codex (EEUU).

Los productos que incluyen GM en su composición se presentan actualmente al consumidor en general, como suplemento dietético, planta medicinal, nutracéutico o alimento funcional, dependiendo de la licencia establecida en el país concreto. Por tanto, es necesario que se armonicen los estándares para asegurar la calidad de los productos de GM (Chan, 2009) en todos los países.

Tabla 1.- Estándares (en % p/p) para la clasificación de la goma de konjac y KGM (*Fuente:* Ministerio de Agricultura Chino, 2002; Comisión europea, 2001; Food Chemicals Codex, USA, 1996;).

	Goma de konjac			Glucomanano de konjac	
	M.Agricultura Chino	Comisión Europea	U.S.A. FDA	M. Agricultura Chino	Comisión Europea
Glucomanano (%)	> 70	> 75	> 75	> 90	> 95
Dióxido de azufre (g/Kg)	< 1,6	–	–	< 0,3	< 0,004
Pérdidas por desecación (%)	< 11,0	< 12	< 15	< 10	< 8
Cenizas totales	< 4,5	< 5	< 5*	< 3	< 2
Arsénico (mg/Kg)	< 3	< 3	< 3	< 2	–
Plomo(mg/Kg)	< 1	< 2	< 2	< 1	< 1
Almidón (%)	–	< 3		–	< 1
Proteínas (%)	–	< 3	< 8	–	< 1,5
Materia soluble en éter	–	< 0,1	–	–	< 0,5%
Cloruro	–	–	–	–	< 0,02%
Salmonella spp.	–	Ausencia en 12,5 g	–	–	Ausencia en 12,5g
E. coli	–	Ausencia en 5g	–	–	Ausencia en 5 g

*800 ºC, 3-4h

1.6- FUNCIONALIDAD Y APLICACIONES DEL GLUCOMANANO

En los últimos años el GM está siendo objeto de investigaciones que ponen de manifiesto sus propiedades funcionales, y sus numerosas aplicaciones, tanto en estado nativo como modificado en su estructura por diversos tratamientos (Behera y Ray, 2017; Guerreiro, Pontes, da Costa, y Grenha, 2019; Ren, Zhang, Qin, y Li, 2016; Tester y Al-Ghazzewi, 2016; Yang y col., 2017; Zhang y Yang, 2014; Zhu, 2018; Zia, Zia, Zuber, Ahmad, y Muneer, 2016). La aplicación tradicional del GM en occidente se limitaba a ser suplemento (nutracéutico) y aditivo alimentario, pero en los últimos años se está utilizando también para usos biomédicos y medioambientales. Si se considera que los polímeros basados en compuestos fósiles o sintéticos están siendo cada vez más cuestionados, los polisacáridos naturales (biopolímeros), incluido el GM tienden a ser cada vez más utilizados por ser renovables, ecológicos (biodegradables) y biocompatibles (Zia y col., 2016).

Las propiedades nutricionales (funcionalidad) y los efectos en la salud de GM también se han revisado en los últimos años (Behera y Ray, 2016, 2017; Devaraj, Reddy, y Xu, 2018;

Ho y col., 2017; Jiang, Li, Shi, y Xu, 2018; Shah y col., 2015; Tester y Al-Ghazzewi, 2016). En lo referente a la funcionalidad, se han puesto de manifiesto, diversas investigaciones biomédicas realizadas sobre GM que abarcan actividades como la reducción del colesterol, (Lee y Dugoua, 2011), normalización de la concentración de triglicéridos (TG) en la sangre (Martino y col., 2013), mejora de los niveles de azúcar en la sangre (Chen, Sheu, Tai, Liaw, y Chen, 2003), mejora de la actividad intestinal (Chiu y Stewart, 2012) y de la función inmune (Onitake y col., 2015), e incluso se usa el GM como apósito para heridas (Chen y col., 2018). El GM se considera fibra dietética, su ingesta mostró un incremento significativo en la frecuencia de defecación (Chen y col., 2006), y es resistente a la hidrólisis por la acción de enzimas digestivas en el intestino humano (Anderson y col., 2009). De hecho, se ha catalogado como ingrediente alimentario funcional con potencial prebiótico (Al-Ghazzewi, Khanna, Tester, y Piggott, 2007; Chen, Fan, Chen, y Chan, 2005; Gómez, Míguez, Yáñez, y Alonso, 2017). Chen y col. (2005) presentaron un estudio de evolución en el tiempo y de dependencia con la dosis, para examinar los efectos de GM en la microbiota del colon de ratones. En este estudio se observó que el GM incrementa significativamente el recuento de bifidobacterias, comparado con la celulosa (control), y reduce la presencia de patógenos como *Clostridium perfringens*. Por su parte, Gómez et al. (2017), hicieron una revisión de las aplicaciones y propiedades del GM donde se puso de manifiesto la falta de investigación que aún existe acerca del potencial prebiótico del GM, y la necesidad de realizar estudios para demostrar y reproducir con ensayos en animales y humanos sus efectos sobre la microbiota intestinal.

A raíz de todas estas alegaciones de salud y aplicaciones, la Comisión Europea pidió en 2010 al Panel sobre Productos Dietéticos, Nutrición y Alergias (EFSA, 2010) que emitiera un dictamen científico en conformidad con el artículo 13 del Reglamento (CE) nº 1924/2006, sobre una lista de declaraciones de propiedades saludables del GM. Estas propiedades se relacionan con la reducción del peso corporal, la reducción de las respuestas glucémicas postprandiales, el mantenimiento de las concentraciones normales de glucosa en sangre, el mantenimiento de las concentraciones sanguíneas normales de triglicéridos y colesterol , la función intestinal normal, y la capacidad de disminuir la población de microorganismos gastrointestinales potencialmente patógenos.

Respecto a la reducción de peso, sobre la base de los datos presentados, el Panel de científicos de la EFSA, concluyó que existía una relación de causa y efecto entre el consumo

de GM y la reducción del peso corporal, en el contexto de una dieta con restricción energética en una población adulta con sobrepeso. El Panel considera que, para obtener el efecto declarado, se deben consumir al menos 3 g de GM diariamente en tres dosis de al menos 1 g cada una, junto con 1–2 vasos de agua antes de las comidas. Por el momento no se ha establecido una relación causa–efecto ni en caso de la reducción de la respuesta glucémica postprandial, ni en el mantenimiento de niveles saludables de glucosa en sangre, ni de los niveles fisiológicos de lípidos en la sangre, ni de la función intestinal, ni en la disminución de microorganismos patógenos (en este caso no se presentaron estudios en humanos). En 2017, la EFSA revisó parámetros como la genotoxocidad del GM, tanto de KGM como de la goma de Konjac y determinó que no hay evidencias de que su consumo produzca ningún tipo de toxicidad. Se estableció que la ingesta aceptable de GM en alimentos es de 10 g/kg, siempre que la ingesta total de todas las fuentes se mantenga por debajo de 3 g/día. Este informe también revisa aspectos como la hipersensibilidad, alergenicidad e intolerancia alimentaria. En general, considerando los datos tanto en humanos como en animales, se consideró que no hay ninguna indicación de preocupación por la inmunotoxicidad o alergenicidad con el KGM (EFSA, 2017).

En el campo de la biotecnología, el GM ha permitido desarrollar membranas termoestables que pueden encapsular enzimas, células, agentes biológicos, farmacéuticos, inmunológicos o mezcla de ellos (Nussinovitch, 2004, 2009).

En el área de la química fina, el GM se ha mostrado como un magnífico agente para la formación de películas acompañado de polímeros como la poliacrilamida (Xiao, Gao, Wang, y Zhang, 2000). La termoestabilidad y las propiedades mecánicas de la poliacrilamida se mejoran con la adición del GM. Existe una aplicación tecnológica en la que se utiliza un recubrimiento de GM para preservar productos frescos como melones y manzanas (Yang y col., 2004). Este recubrimiento protege al producto fresco de los gases, particularmente oxígeno, etileno, dióxido de carbono y vapor de agua, tanto de dentro a fuera, como de fuera a dentro, permitiéndose así el control de la maduración de los frutos.

El GM nativo tiene una funcionalidad tecnológica limitada, pero posee capacidad para ser modificado mediante técnicas químicas como la copolimeración de injerto y, físicas como las altas presiones o el electrohilado (“electrospinning”), que amplían la funcionalidad del GM para ser utilizado en las aplicaciones deseadas (Zhu, 2018) incluso tras largo periodo de congelación (Tovar et al., 2017). La copolimerización de injerto permite, incluir en la

estructura del GM radicales de otros polímeros que aportan características especiales al GM, por ejemplo, la biodegradabilidad del GM combinada por copolimerización con la dependencia de pH de polímeros, como ácido poliacrílico (PAA), para la administración específica de medicamentos en colon. El electrohilado (*electrospinning*) es una técnica que permite obtener fibras a partir de soluciones de polisacáridos puros o mezclas de ellos, para obtener las propiedades deseadas. El GM preparado en solución acuosa (con pH ligeramente básico), mediante electrohilado, da lugar a fibras altamente estables y biocompatibles con numerosas aplicaciones en biomedicina (Nie y col., 2011).

1.7.- CARACTERISTICAS TECNOLÓGICAS DEL GLUCOMANANO

El reciente desarrollo de las aplicaciones del GM en la industria y en la medicina está incrementando su demanda, proliferando estudios para caracterizar totalmente sus propiedades fisicoquímicas y tecnológicas, mejorar la agronomía del konjac, y aumentar su potencial de mercado. Las características tecnológicas más apreciadas del GM en la industria alimentaria son , su **solubilidad** en agua, su capacidad de **hinchamiento y retención de agua,** y su capacidad de **gelificar de forma termoestable** además de sus propiedades emulsionantes, estabilizantes y espesantes (Commission EU, 2011).

Respecto a la **solubilidad,** Chen, Li y Li (2011) publicaron que la solubilidad del GM procedente de *Konjac* (PM = 7.47×10^5 g mol^{-1}) es de un 83%. Como se describió anteriormente, la cadena principal del polisacárido de GM posee uno de cada 9–19 monómeros sustituidos en su carbono 6 por un grupo acetilo (en función de su origen y su método de obtención). La presencia de los grupos acetilo le confiere solubilidad al polisacárido en solución acuosa (el GM no acetilado, con enlaces β-1-4 es insoluble en agua) (Dea y Morrison, 1975). El grado de acetilación del GM es, por tanto, determinante para obtener dispersiones acuosas homogéneas.

La elevada **capacidad de absorber agua** del GM se analizó en numerosos estudios. Como polisacárido hidrófilo, 1 g de GM puede absorber entre 50 y 100 g de agua (Doi, 1995; Geng, Lin, Chen, Liu, y Wang, 2009; Koroskenyi y McCarthy, 2001), en función del grado de acetilación. La gran capacidad de absorber agua se debe a la formación de enlaces de hidrógeno entre los grupos hidroxilo de GM y las moléculas de agua (Koroskenyi y McCarthy, 2001).

Cuando el GM gelifica, es capaz de retener entre el 70 y el 80 % de agua, por lo que el gel tiene muy buenas características hidrofílicas. Los grupos hidroxilo que sustituyen a los grupos acetilo cuando se induce su gelificación con álcali, podrán ligar agua formando enlaces de hidrógeno y, además, la red formada puede atrapar agua en su estructura, de aquí la alta **capacidad de retención de agua (CRA)** que tienen los geles de GM. Ésta es una de las aplicaciones ya comentada, pero también constituye una propiedad muy interesante para la aplicación alimentaria junto con la baja **pérdida de agua por cocción (PAC),** son dos parámetros que se estudian y se valoran a la hora de elaborar productos reestructurados a base de GM. La CRA y la PAC se usan comúnmente como indicadores de la aceptación general de productos reestructurados, tanto de los consumidores (percibiendo mayor jugosidad) como de la industria.

La **capacidad de gelificar de manera termoestable** del GM es muy apreciada en la industria alimentaria, y depende del tratamiento térmico utilizado en su elaboración (Herranz, Borderías, Solas y Tovar, 2012). La termoestabilidad se manifiesta principalmente en el valor prácticamente invariante de la componente elástica (G') en la red del gel, incluso a elevadas temperaturas (Mezger, 2006). Como se verá a lo largo de este trabajo, el GM permite obtener productos pesqueros reestructurados procedentes de subproductos de la industria pesquera o de músculo de pescado picado, dando lugar a productos con aspecto de crudos, que pueden ser cocinados sin perder su estructura, basándose en la capacidad de gelificar de forma termoestable del GM y en las buenas características tecnológicas que se han descrito.

BLOQUE 2.- PRODUCTOS PESQUEROS

En los hábitos alimentarios de la humanidad siempre han jugado un papel importante los productos marinos, entre ellos el pescado. Actualmente, para unos 3000 millones de personas en el mundo cerca del 17% de su aporte de proteínas animales proviene del pescado, y de productos derivados de la pesca. En 2016 se registró un máximo histórico en el consumo de pescado per cápita (20,3 kg de pescado por habitante y año), en parte, gracias a que también se alcanzó un máximo histórico en las capturas (171 millones de toneladas). España se encuentra entre los primeros países europeos a nivel de consumo de pescado, con un índice anual de consumo que en 2017 alcanzó los 42 kg/ habitante y año, por detrás de Portugal, con aproximadamente unos 57 kg/ habitante y año (FAO, 2018).

En 1967 se publicó el Decreto 2484/1967 por el que se aprobaba el texto del Código Alimentario en el que se definen los grupos de alimentos. Respecto a los pescados y derivados (3ª parte "Alimentos y bebidas", capitulo XII. 3.12.01) se diferencian dos grandes secciones: los **pescados,** definidos como animales vertebrados comestibles, marinos o de agua dulce (peces, mamíferos, cetáceos y anfibios) frescos o conservados por distintos procedimientos autorizados y, los **derivados** definidos como productos obtenidos a partir de pescados de buena calidad y frescos, por procedimientos técnicos adecuados (Figura 7).

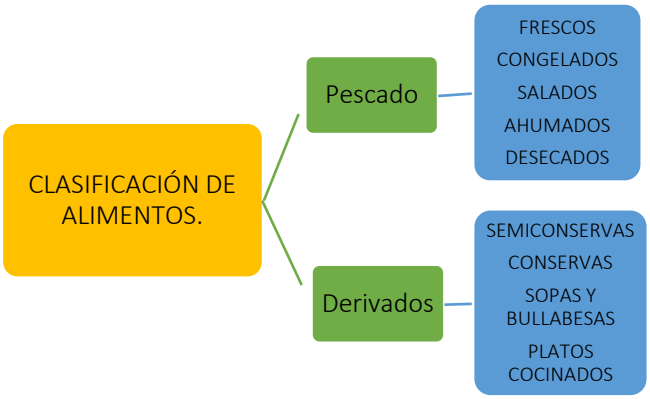

Figura 7.-Clasificación de alimentos. Pescados y Derivados (BOE, 1967).

2.1.- PRODUCTOS Y SUBPRODUCTOS DE LA PESCA

2.1.1.- Valor nutricional del pescado

La gran mayoría de la población percibe el pescado como un alimento saludable y nutritivo independientemente de la especie, y se considera que comer pescado es esencial para una dieta equilibrada y saludable. Las recomendaciones dadas por la *American Heart Association* a cerca del consumo de pescado es de, al menos, dos veces por semana en raciones de 125–150 g (peso neto). La ingesta de dos porciones de pescado por semana se ha asociado con una disminución en un 23–25% de muerte por enfermedad cardiovascular (ECV) (Smith y Sahyoun, 2005; Weichselbaum, Coe, Buttriss, y Stanner, 2013).

La **composición química** del pescado difiere de acuerdo a los siguientes aspectos: especie, estación del año, época de captura, edad, condiciones del medio y tipo de alimentación. Los principales componentes químicos del músculo del pescado son: agua, proteínas y lípidos. El contenido de carbohidratos en el músculo de pescado es muy bajo, generalmente inferior al 0,5%. El contenido en agua varía entre 60–80% y es inversamente proporcional al contenido graso siendo éste último fluctuante (Gil, 2010). Según el contenido graso los pescados se clasifican en: blancos, semi-grasos y grasos (Astiasarán y Martínez, 2000). El contenido en proteínas es constante (Badui, Guerrero, López, y Armenta, 2006). La cantidad de vitaminas y minerales es propia de la especie, puede variar con la estación del año, y representa aproximadamente el 2% en peso del músculo del pescado. Cabe destacar las vitaminas del grupo B y las vitaminas A y D en el caso de pescados grasos. También son buena fuente micronutrientes como hierro, zinc, calcio, fósforo, selenio, potasio y yodo y, tiene, en general, bajo contenido en sodio (Tabla 2).

Los compuestos de nitrógeno no proteico (NPR) también están presentes en el pescado, en diversos grados, según la especie. Los músculos oscuros de los peces generalmente contienen una mayor cantidad de compuestos NPR que los músculos claros. Los compuestos NPR en los tejidos musculares están formados por aminoácidos libres, aminas, nucleótidos, guanidina y sus productos de degradación, urea y sales de amonio (Velankar y Govindan, 1958). La contribución de los compuestos NPR al sabor de los pescados es importante (Gram y Huss, 1996).

Tabla 2.- Composición nutricional por 100 g del pescado. AGS (ácidos grasos saturados), AGM (ácidos grasos monoinsaturados), AGPI (ácidos grasos poliinsaturados). (Fuente: Chalamaiah y col., 2012, Love, 1970; Murray y Burt, 1977; Venugopal y Shahidi, 1996).

	Bacalao fresco	**Trucha**	**Salmón**	**Arenque**
ENERGÍA (KCAL)	79,8	135	191	233
PROTEÍNA (g)	17,7	18,75	20,62	18,8
GRASA (g)	1,01	6,7	12,1	17,8
AGS	0,13	1,7	2,1	3,3
AGM	0,1	2	3,7	2,87
AGP	0,39	2,2	3,29	3,08
AGUA (g)	81,3	74,6	67	64
NIACINA (mg)	1,7	5,1	9,25	4,1
TIAMINA (mg)	0,06	0,07	0,2	0,04
VITAMINA B12 (µg)	1,2	5,23	3,93	8,5
HIERRO (mg)	0,32	1,16	0,84	1,1
CINC (mg)	0,4	1,39	0,72	0,58
SELENIO (µg)	28	25	29	43
SODIO (mg)	72	56	59	117
POTASIO (mg)	352	480	336	360

Numerosas investigaciones han puesto de manifiesto que los productos pesqueros tienen características nutricionales saludables (Harris, 2004; Hosomi, Yoshida y Fukunaga, 2012; Mozaffarian y Rimm, 2006; Weichselbaum y col., 2013). Los beneficios nutricionales del consumo de pescado se deben principalmente a la presencia de proteínas de alto valor biológico, ácidos grasos poliinsaturados (AGPI), minerales (calcio, hierro, selenio, zinc, etc.) y vitaminas como la A, la B3 (nicotinamida), B6 (piridoxina), B12 (cobalamina), E (d-tocoferol) y del grupo D.

Las proteínas del pescado son de alto valor biológico ya que contienen aminoácidos esenciales en proporciones adecuadas (por ejemplo, con la ingesta de 200 g de pescado se cubren y superan las necesidades de treonina, valina, leucina, isoleucina, lisina y un 80 % de las de triptófano), además de otras sustancias nitrogenadas no proteicas (Astiasarán y Martínez, 2000; Gil, 2010; Ordoñez, y de la Hoz, 1999).

Sus beneficios nutricionales también derivan de la abundancia de los ácidos grasos poliinsaturados (AGPI) (PUFAs, siglas en inglés) de cadena larga, principalmente de la serie **ω-3** —ácido docosahexaenoico (DHA) o 22:6 ω-3 y ácido eicosapentaenoico (EPA) ó 20:5 ω-3. Una buena salud cardiovascular se ha asociado en numerosas investigaciones con una

dieta rica en AGPI (PUFAs) (Ruxton, Reed, Simpson, y Millington, 2004; Sidhu, 2003; Weichselbaum y col., 2013). Además, los ácidos grasos ω-3 también tienen potencial para proteger la piel de las lesiones producidas por radiación ultravioleta a través de una variedad de mecanismos (Pilkington, Watson, Nicolaou, y Rhodes, 2011).

Los beneficios para la salud del consumo de pescado también se han puesto de manifiesto en lo que respecta a enfermedades inflamatorias (Gopinath y col., 2011; Rosell, Wesley, Rydin, Klareskog, y Alfredsson, 2009), ciertos cánceres (Szymanski, Wheeler, y Mucci, 2010), demencia (Cederholm y Palmblad, 2010) y depresión (Appleton, Rogers, y Ness, 2010; Peet y Stokes, 2005). En la actualidad se presta cada vez más atención al pescado como fuente de nutrientes esenciales para el desarrollo, en las etapas iniciales, del cerebro y el sistema nervioso ya que, nutrientes esenciales para las funciones cerebrales como el yodo se encuentran casi exclusivamente en alimentos procedentes del medio marino.

Sin embargo, a pesar de los efectos beneficiosos del pescado, existe una preocupación creciente acerca de la relación del consumo frecuente de pescado con ciertos riesgos para la salud, derivados de la exposición a contaminantes químicos en ciertas especies. El metilmercurio y los bifenilos policlorados (PCB) son los contaminantes a los que se ha prestado más atención. En términos generales, la mayoría de las especies marinas no deben significar efectos adversos para la salud de los consumidores. Sin embargo, el tipo de pescado, la frecuencia de consumo y el tamaño de las raciones son cuestiones esenciales para el equilibrio de los beneficios y los riesgos para la salud (Cohen y col., 2005; Domingo, 2016; Hoekstra y col., 2013; Mozaffarian y Rimm, 2006; Sidhu, 2003; Tur y col., 2012).

2.1.2.- Consumo de pescado y derivados

A nivel mundial, Asia es el continente que más pescado consume, el 79% del pescado mundial. En la Unión Europea, España presenta el mayor consumo de pescado con un 42% del total, seguido de Italia y Francia. En nuestro país se consumen 24 kg de pescado por persona y año (Figura 8) (FAO, 2016, 2018).

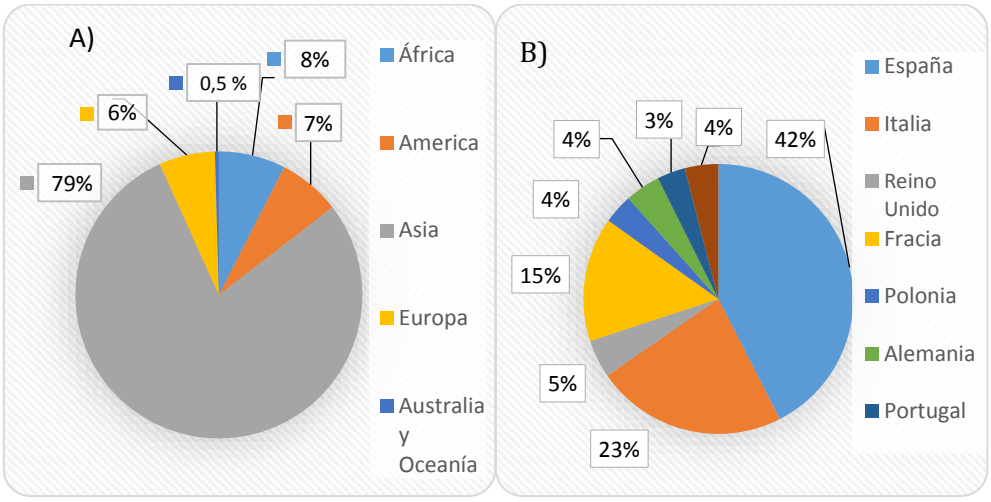

Figura 8.- A) Consumo de pescado fresco a nivel mundial, por continentes (FAOSTAT, 2016). B) Consumo de pescado fresco por países europeos (EUMOFA, 2018).

En 2007 se llevó a cabo una encuesta de consumo transversal en cinco países europeos (Bélgica, Dinamarca, Países Bajos, Polonia y España), con una muestra representativa por edad y región, acerca de la percepción que se tiene del pescado en la dieta. Los resultados muestran que en general, la población tiene una percepción nutritiva y saludable del pescado (Pieniak, Verbeke, Scholderer, Brunsø, y Olsen, 2007). Esto pone de manifiesto que el consumo de pescado se incrementará o al menos no descenderá en las próximas décadas. Los pescados frescos representan la mayor parte del pescado destinado al consumo humano directo (45%), seguido del pescado congelado y curado (seco, salado, en salmuera, fermentado, ahumado) (12%). La congelación constituye el principal método de conservación de pescado para consumo humano. En 2016 representó el 56% del total de pescado dirigido al consumo humano y el 27% de la producción total de pescado. Por otro lado, la proporción de las poblaciones de peces que se encuentran explotadas a niveles biológicamente insostenibles se incrementó del 10% en 1974 al 33,1% en 2015. Actualmente se ha conseguido una mejora significativa de la estabilidad de la pesca de captura, mediante la mejora de las técnicas de refrigeración y congelación, y una reducción del descarte impuesto por la Política Pesquera Comunitaria (Comisión Europea, 2016), no obstante, este dato de sobreexplotación presenta una situación preocupante. Se calcula que todavía se pierde o desperdicia un 27% del pescado capturado. En parte, el crecimiento de la producción mundial de pescado de acuicultura es un factor importante para paliar la sobre-explotación marina. En 2016 se alcanzaron los 80 millones de toneladas de peces de acuicultura (FAO, 2016).

El sector del congelado vive un buen momento desde el punto de vista industrial, que pasa por fortalecer su posicionamiento en origen, y el desarrollo de nuevos productos de valor añadido para atraer a los consumidores de pescado, con iniciativas para mejorar el sabor o ampliar la oferta de productos pesqueros, valorándose especialmente aquellos que se comercializan mínimamente procesados.

2.1.3.- Revalorización de subproductos

Los precios de los productos pesqueros procedentes de especies nobles son elevados, y suelen ser un factor limitante para su consumo, por lo que aunque el pescado goza de una alta demanda, no están al alcance de toda la población (James y Bligh, 1992). España es el primer productor industrial de la UE en productos de pesca con el 20% de la producción. La flota española capturó 898.333 Tm de pescados y mariscos en 2016, y ocupa el primer lugar de la UE tanto en volumen como en valor. Pero no todas las capturas van destinadas directamente al consumo humano, cientos de toneladas de productos pesqueros sin valor comercial, son destruidas o enviadas a empresas harineras, ya que no son consideradas comercialmente aptas para el consumo directo. Por ejemplo, especies que poseen muchas espinas, de músculo muy blando, de pequeño tamaño, de muy alto contenido en grasa, subproductos del fileteado, etc. Por lo tanto, es necesario buscar alternativas a la situación actual de "descartes" en la que se encuentra el sector pesquero. Los procesos de congelación y fileteado de grandes piezas llevan consigo la producción de "desperdicios", que podrían ser utilizados en la industria alimentaria como materia prima para la elaboración de nuevos productos pesqueros. Estos productos tendrán determinadas características, según los diferentes mercados a los que se enfocan, pudiéndose, además, aportar ingredientes funcionales tras un proceso tecnológico adecuado, ya que existen menos restricciones técnicas para ser incluidos en el reestructurado que por ejemplo, en el músculo de pescado mediante la modulación de su dieta (Moreno, Herranz, Pérez-Mateos, Sánchez-Alonso, y Borderías, 2016).

Estos procesos tecnológicos constituyen una forma de aumentar la oferta de productos pesqueros para el consumidor. La disminución de los desperdicios del procesado del pescado, ayudaría también a minimizar los residuos generados por la industria pesquera. El músculo extraído de estos restos o del procesado habitual del pescado a través de diferentes técnicas, necesita ser reestructurado para su consumo de forma que se

constituyan texturas, aspectos y sabores deseados por el consumidor. A estos nuevos productos se les agrupa bajo la denominación de "*productos reestructurados*".

2.2.-PRODUCTOS PESQUEROS REESTRUCTURADOS

La introducción de los productos pesqueros reestructurados en el mercado tuvo un auténtico auge en los años 50 del pasado siglo, en los países del norte de Europa y, a partir de los 70 en España. La principal razón para la reestructuración del músculo del pescado es cubrir la demanda de productos pesqueros, utilizando mejor los recursos existentes.

Se consideran **reestructurados** aquellos productos elaborados a partir de materias primas cárnicas o pesqueras, que tras un cierto proceso de desintegración estructural (troceado, picado, etc.), son sometidas a diversos tratamientos de reestructuración con el objetivo de impartirles las características propias de los productos que pretenden imitar (Borderías y Pérez Mateos, 1996; Sánchez Alonso, Pérez Mateos, y Borderías, 2004). Suelen ser comercializados como productos crudos (refrigerados o congelados) y precocinados o cocinados. La elaboración de los reestructurados se fundamenta en procesos tecnológicos que permiten obtener productos con diferente composición química, tamaño de partícula, e ingredientes, dando lugar a productos análogos o totalmente diferentes de la materia prima de la que proceden (Ordóñez y col., 1998), y han surgido por demanda específica del consumidor. Los reestructurados de pescado pertenecen al grupo de alimentos formulados y se definen en el Código Alimentario (Codex Alimentarius, 2003) como "aquellos que se obtienen por mezcla de ingredientes". Incluyen cuatro grandes grupos: alimentos reestructurados, alimentos enriquecidos, alimentos funcionales y alimentos de bajo contenido en algún componente o propiedad. El desarrollo de este tipo de productos tiene como objetivo elaborar alimentos con texturas, olores, sabores, colores y apariencias atractivas para los consumidores de diferentes mercados.

En la década de 1970 se comenzó a desarrollar la tecnología de reestructuración como un nuevo concepto para mejorar la utilización de carne y, posteriormente, esta tecnología se trasladó al pescado, permitiendo la elaboración de productos con valor añadido, partiendo de cortes y pedazos de baja calidad. Se estudiaron los factores que afectan a: la calidad de los productos reestructurados, la trituración y el método de conformación, el tamaño de las partículas, el tiempo de mezcla, el tipo de músculo y el contenido en grasa. También se describieron las funciones de otros ingredientes, como sal, fosfato, proteína de

soja y transglutaminasa (TG) (Sun, 2009). Los aglutinantes como k-carragenano, alginato y TG se usan ampliamente y con éxito en la producción de reestructurados tanto de carnes como de pescados (Borderías y Pérez Mateos, 1996; Carballo, Ayo, y Colmenero, 2006; Jiménez Colmenero, Serrano, y Cofrades, 2004; Moreno y col., 2016; Moreno, Carballo, y Borderías, 2008; Serrano, Cofrades, y Jiménez Colmenero, 2004).

Actualmente pueden distinguirse varias familias de productos pesqueros reestructurados, pudiendo diferenciarse en función de la materia prima (filetes o músculo de pescado picado), y en función de la tecnología de reestructuración (sin gelificación o con gelificación). A continuación, se describen detalladamente.

2.2.1.- Productos formados por reestructuración de trozos de filetes de pescado

Los productos típicos destinados a la reestructuración son las porciones de músculo de pescado (casi siempre especies blancas), que no han perdido su estructura en miotomos y mioseptos. Estos reestructurados están compuestos de trozos de filetes que se congelan en bloques, se sierran en porciones y, posteriormente, se rebozan o empanan, después se prefríen para fijar el rebozado y se congelan. Estos productos adquieren diversas denominaciones comerciales como son: delicias, varitas, palitos, imitaciones de rodajas de pescado fresco rebozado, etc, …La mayoría de las veces no se les añade ningún aditivo, y la cohesividad se consigue solubilizando con sal y polifosfatos la proteína de la superficie de los filetes de pescado, por lo que es necesario que la proteína esté nativa, es decir, que sea funcional.

2.2.2.- Productos formados por reestructuración de músculo de pescado picado

Las ventajas de utilizar músculo de pescado picado son varias, entre ellas que se pueden usar especies pequeñas, poco apreciadas o con muchas espinas, o aprovechar restos de fileteado. Además, de esta forma, el músculo se pone en contacto directo con aditivos como los estabilizantes, por lo que la eficacia de éstos es máxima y también pueden ser integrados en el músculo picado otros ingredientes interesantes. El proceso está sujeto a variaciones según el producto final al que vaya destinado, pero básicamente consta de las siguientes etapas:

1. Separación mecánica. - La mayoría de las técnicas de separación están basadas en la extrusión del músculo, a partir de los pescados descabezados y eviscerados, a través de placas perforadas, separándolo de piel y espinas.
2. Lavado. - No siempre se lava el pescado picado; pero sí en el caso de músculos con mucha grasa, aclarando el color o disminuyendo el sabor.
3. Estabilización del pescado picado. - Dada la baja estabilidad del músculo de pescado, la mayoría de los tecnólogos recomiendan para prácticas comerciales, temperaturas por debajo de -18 °C. Los problemas de conservación del músculo picado, son principalmente los derivados de los altos niveles de lípidos poliinsaturados, y el efecto de la oxidación de estos lípidos en el sabor y la textura. Asimismo, los cambios estructurales asociados a la desnaturalización y agregación de las proteínas miofibrilares, que alteran su funcionalidad y la textura, principalmente con la congelación. Para evitar este problema, se emplean crioprotectores como aditivos, mayoritariamente carbohidratos (glucosa, fructosa, sacarosa, lactosa) y polialcoholes (sorbitol).

Una vez estabilizado el pescado picado, la industria ha desarrollado diferentes tecnologías para llevar a cabo la reestructuración, diferenciando si el proceso se realiza sin gelificación, o gelificando, tanto en frío como en caliente.

2.2.2.1.- Reestructuración del pescado picado sin gelificación

En muchos casos al músculo de pescado picado, con o sin ingredientes adicionales, se le aplica una presión dándole la forma deseada y se congela o se refrigera. Este es el caso de hamburguesas, albóndigas, etc.

Se han desarrollado tecnologías de reestructuración más sofisticadas como la formación de cristales de hielo que se pueden aprovechar tecnológicamente, siendo beneficiosa para texturizar músculo homogeneizado al darle un aspecto fibroso parecido al del músculo de pescado (Mackie, 1993). El proceso consiste en aplicar ciclos de congelación-descongelación a una dispersión acuosa del músculo picado, de tal forma, que los cristales de hielo se alineen predominantemente en una dirección. Dependiendo de la velocidad de congelación, del número de ciclos congelación-descongelación, el efecto será más o menos intenso. Por supuesto, el producto final es más duro y menos jugoso que el original, lo que limita su aplicación a determinados casos o bien requiere su combinación con otros

métodos por los que se pueda ligar agua "a posteriori".

Por otra parte, se está experimentando sobre la forma de elaboración de filetes a base de pescado picado con la tecnología más apropiada para lograr las características texturales del músculo entero. Para ello, existen métodos que estructuran el pescado picado en capas a manera de miotomos y mioseptos o en fibras. En el primer caso se forman láminas de pescado picado texturizadas y se juntan con sustancias ligantes, después se cortan verticalmente de forma que toma la apariencia de un filete con sus miotomos y mioseptos (Figura 9) (Borderías, Carballo y Moreno-Conde, 2008). También se utiliza el músculo picado emulsionado en la fabricación de salchichas, y picado directamente en diversos platos preparados, como croquetas, empanadillas, "crêpes", etc.

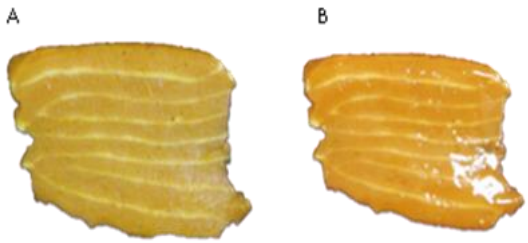

Figura 9.- Láminas de pescado picado reestructurado tipo "salmón ahumado" . (A) Sin aceite (B) Con aceite.(Fuente: Borderías y col., 2008).

2.2.2.2.-. Reestructuración de pescado picado mediante gelificación

Muchos de los productos reestructurados fabricados a base de pescado picado se consiguen mediante la tecnología de **gelificación,** ya sea a partir del músculo de **pescado picado** tal cual, ligeramente lavado, o bien, a partir de **surimi**. Este producto es un concentrado de proteínas miofibrilares que se obtiene a través de una serie de lavados del músculo picado de pescado. Debido al poco poder gelificante del pescado picado, especialmente en el caso de pescado no recién capturado, se suelen utilizar una serie de ingredientes, como hidrocoloides y proteínas no musculares que refuerzan la capacidad de formar gel. Se han desarrollado métodos de gelificación inducidos por calor y métodos gelificación en frio:

A) Gelificación inducida por calor. *Surimi*

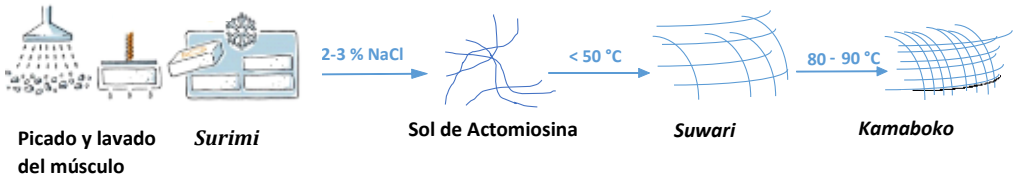

Figura 10.- Formación del gel (Kamaboco) a partir de surimi.

La interrelación térmica de las proteínas miofibrilares, es la responsable de la gelificación de los productos elaborados a base de pescado desmenuzado como el surimi. La principal característica de las proteínas miofibrilares es su capacidad de formar geles, formándose estructuras fuertes y deformables, lo que permite utilizar el surimi como un ingrediente intermedio en la elaboración de diversos productos (palitos de cangrejo, sucedáneos de angulas, gambas o colas de langosta). Además, debido a que el surimi no tiene sabor, color, ni olor, permite dar al producto final los atributos deseados con relativa facilidad. El surimi se definió en 1960 como una pasta de pescado picado, obtenido mecánicamente, lavado y al que se le añaden crioprotectores para mejorar su conservación en congelación (Nishiya, Takeda, Tamoto, Tanaka, y Kubo, 1960). Para que se formen los geles a partir de proteína miofibrilar es necesario que la miosina se solubilice con la adición de sal (2 a 3%), para que las miofibrillas del músculo se separen, la actomiosina pierda su estructura original, interaccionen entre sí y formen un gel en cuya red se integran agua e ingredientes adicionados. Si se somete esta pasta a una T< 50 °C (temperatura de asentamiento), se forma un compuesto semi-sólido traslúcido, blando y con alta capacidad de retención de agua, el gel «Suwari» (Suzuki, 1987). A medida que se aumenta la temperatura y se prolonga el tiempo de calentamiento, se obtiene un gel más opaco y rígido, el «Kamaboko» (Hamann, Amato, Wu, y Foegeding, 1990; Lanier y Lee, 1992; Park, 1994) (Figura 10).

Las condiciones ideales para la gelificación varían según la especie (conformación nativa proteica, número y localización de puntos de unión), y de los cambios de la proteína durante su manipulación (captura, procesado y conservación), por lo que frecuentemente se requiere la adición de coadyuvantes de la gelificación, como son hidrocoloides y/o proteínas no musculares. Como se ha especificado, la gelificación se induce térmicamente,

originando un producto reestructurado que no tiene aspecto de crudo, y además, muchas especies de peces muestran degradación de proteínas durante el calentamiento (Ramírez, Uresti, Velazquez, y Vazquez, 2011; Sanchez-Gonzalez y col., 2008; Seki, Nozawa, y Ni, 1998). De este modo, el calor puede producir una desnaturalización parcial de la proteína miofibrilar, disminuyendo la calidad del gel obtenido (An, Peters, y Seymour, 1996; Ramírez, García-Carreño, Morales, y Sánchez, 2002). Por tanto, se buscan alternativas para la gelificación aplicando, por ejemplo, altas presiones hidrostáticas (APH) (Moreno y col., 2015). Los tratamientos de APH a temperaturas no desnaturalizantes (por debajo de 10 °C), han demostrado ser eficientes para mejorar las propiedades mecánicas de los geles de pescado (Borderías, Pérez-Mateos, Solas, y Montero, 1997; Cando, Borderías, y Moreno, 2016; Colmenero, 2002; Moreno y col., 2015; Pérez-Mateos, Lourenço, Montero, y Borderías, 2002; Uresti, Velazquez, Ramírez, Vázquez, y Torres, 2004). Hay que tener en cuenta que la aplicación de alta presión modifica el color del músculo de pescado, se vuelve más blanquecino y opaco, aunque estos efectos dependen de la especie utilizada. Este efecto puede restringir considerablemente su aplicación en los productos pesqueros, sobre todo en aquellos en los que se quiere preservar el aspecto de crudo (Hurtado, Montero, y Borderias, 2000). Una opción que conserva el aspecto de crudo del producto pesquero, consiste en adicionar polisacáridos que forman geles termoestables como el GM. Se observa que, partiendo de surimi de calamar gigante, cuya proteína es poco funcional, la adición de GM mejora la propiedad del gel de surimi (Iglesias-Otero, Borderías, y Tovar, 2010). Esta tecnología se puede utilizar para obtener geles de surimi y productos pesqueros reestructurados.

B) Gelificación en frío

Una forma menos habitual, pero muy interesante, es reestructurar el músculo de pescado picado mediante la gelificación en frío, incorporando distintos ingredientes como el alginato, o la transglutaminasa de origen microbiano (MTGasa). Con este tipo de tecnología se pueden elaborar tanto, productos con aspecto de crudo, como ahumados o marinados.

El alginato se ha utilizado ampliamente en productos cárnicos, pero no demasiado en productos de pescado. El alginato es un polisacárido constituido por dos tipos de monosacáridos, el ácido gulurónico y el ácido manurónico. Se extrae de las “algas marrones” y se usa en los sistemas alimentarios como estabilizador y para modificar la reología de los

alimentos (Moreno y col., 2008, 2016), pero una de sus propiedades más interesantes es la gelificación. El alginato es diferente de otros hidrocoloides gelificantes, ya que forma geles termoestables sin tratamiento térmico. Los geles de alginato se forman por asociación intermolecular de cationes polivalentes (principalmente calcio), a las regiones de ácido gulurónico de la molécula de polisacárido (Means y Schmidt, 1986). La proporción del alginato de sodio, la fuente de iones polivalentes (calcio), el tiempo de fraguado, y el tiempo de reacción, son variables que se utilizan para lograr diferentes tipos de textura (Tolstoguzov, 1998).

La MTGasa, que es comercial, constituye una opción muy adecuada para elaborar productos reestructurados (Moreno y col., 2016). Su actividad se basa en catalizar la formación de enlaces covalentes ε-(γ-glutaminil), lisina entre proteínas que contengan glutamina y lisina (Kuraishi y col., 1997), y entre ellas, se encuentra la miosina. Por otra parte, esta actividad puede darse a temperaturas lo suficientemente bajas (30 °C) (Ajinomoto, 1994), como para evitar cambios en las propiedades sensoriales que debe tener un producto crudo. Su inactivación es irreversible a temperaturas de más de 80 °C (Menéndez, Rawel, Schwarzenbolz, y Henle, 2006), lo cual, debe ser tenido en cuenta ante un posible tratamiento térmico que quiera aplicarse al producto. La calidad funcional (funcionalidad desde un punto de vista tecnológico) de la materia prima, juega un papel muy importante en el empleo de la MTGasa. Así, si la proteína del músculo de pescado ha perdido cierta funcionalidad, será necesario un tamaño de partícula de picado mucho más pequeña para facilitar la actividad de la enzima (Moreno, Carballo, y Borderias, 2009).

En la elaboración de productos en los que se requiera un pH< 6, la enzima pierde actividad, lo que se puede paliar con la adición cantidades de cloruro sódico (NaCl) relativamente elevadas (4%), para obtener las propiedades físico-químicas esperadas, pero estas concentraciones de sal solo se pueden utilizar en determinados productos, como por ejemplo las anchoas (Moreno, Carballo, y Borderías, 2010; Moreno y col., 2016).

Sin embargo, se ha observado que el contenido graso del músculo de pescado, condiciona la acción de la enzima, principalmente ralentizando su actividad. En este sentido, falta por esclarecer la relación entre la incorporación de MTGasa y la evolución de los procesos oxidativos en el producto elaborado (Moreno, Carballo, y col., 2009). Por otra parte, se ha comprobado que los geles a partir de surimi de calamar gigante (*Dosidicus gigas*), mejoran notablemente sus propiedades con la inclusión de MTGasa (Moreno,

Cardoso, Solas, y Borderías, 2009). Su empleo permite también mejorar el proceso de reestructuración de músculo de pescado, de diferentes especies reduciendo el contenido en NaCl (Ramirez, Uresti, Téllez, y Vázquez, 2002), e incluso de fosfatos (Min y Green, 2008), obteniéndose así productos más saludables.

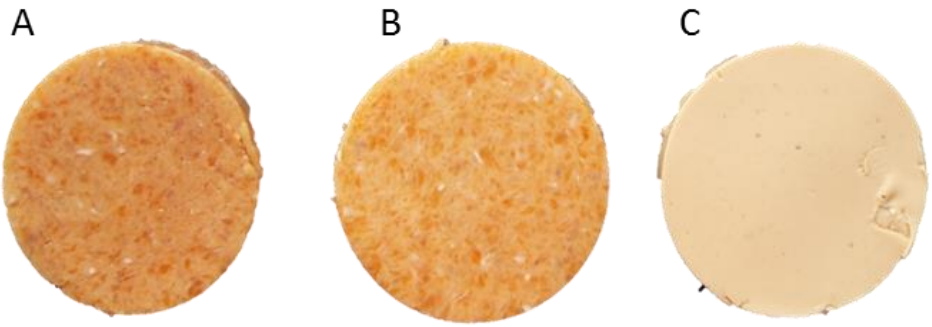

Figura 11.- Reestructurados de pescado picado con MTGasa. A. Pescado picado B. Pescado picado y lavado C. Pescado homogeneizado en masa**.**

2.2.3.-. Reestructurados de pescado con características funcionales

Los productos pesqueros reestructurados, además de lo anteriormente expuesto, tienen ventajas añadidas ya que pueden ser modificados en su composición, incorporando nuevos ingredientes que les confieran una capacidad funcional (Borderías, Sánchez-Alonso, y Pérez-Mateos, 2005; Sánchez-Alonso y Borderías, 2008; Sánchez-Alonso, Haji-Maleki, y Borderias, 2007; Sánchez Alonso y col., 2004; Shahidi y Ambigaipalan, 2015). En la actualidad, los consumidores buscan no solo que los alimentos cumplan con los requisitos nutricionales básicos, sino que además les permita mantener un estilo de vida sano, una mejora de salud y calidad de vida. Por ello, está adquiriendo una gran relevancia el desarrollo de productos más saludables que se caracterizan por poseer alguna de las siguientes características: prevenir o limitar la presencia de ciertos compuestos potencialmente perjudiciales (mediante cambios en su composición o procesado), y/o favorecer la presencia de determinadas sustancias (naturalmente presentes o adicionadas), que ejerzan un papel beneficioso para la salud (Jiménez-Colmenero, Carballo, y Cofrades, 2001; Siro, Kápolna, Kápolna, y Lugasi, 2008).

Con todo, los productos reestructurados a base de pescado presentan muchas ventajas y posibilidades, entre las que cabe destacar las que se describen a continuación

(Boles y Shand, 1999; Means y Schmidt, 1987; Ordóñez y col., 1998; Resurreccion, 2004; Tarrant, 1998):

1. *Revalorización de las materias primas pesqueras*:

Fue uno de los primeros objetivos al permitir el empleo de subproductos (coproductos), o partes de los pescados de bajo valor comercial para su transformación, proporcionando al consumidor productos de calidad y a mejores precios.

2. *Posibilidad de ampliar la gama de productos pesqueros ofertados*.

Esta tecnología permite obtener productos prácticamente de cualquier forma o tamaño, diversificando la oferta. La comercialización más frecuente de los reestructurados pesqueros es congelada o precocinada. La utilización de agentes de gelificación como el GM permite gelificar en frío, posibilitando la elaboración de reestructurados frescos refrigerados.

3. *Control exacto y reproducible del peso, atributos sensoriales y propiedades tecnológicas del producto*.

Esto hace posible elaborar reestructurados con características uniformes y convenientes en cuanto a tamaño y forma, (permitiendo además la automatización de su elaboración), comportamiento a la cocción, textura, etc. Esto significa acercar sus características a las exigencias actuales del mercado, que demanda porciones regulares e individuales, de calidad constante, preparación sencilla y rápida, para una mayor comodidad de consumo. Entre los productos derivados del pescado picado se encuentran las porciones libres de espinas y piel (palitos, hamburguesas, albóndigas, nuggets, etc.), muy apreciadas por el consumidor (Documentos COTEC, 1995; Gonçalves y Passos, 2010). Japón, por ejemplo, utiliza gran parte de sus capturas para la producción de alimentos no convencionales, del tipo de pastas de pescado, budines, croquetas, embutidos y jamones (Melgarejo y Maury, 2002).

4. *Formulación de productos de composición garantizada y ajustada. Alimentos funcionales*.

Durante la reformulación del producto pesquero original se pueden eliminar algunos constituyentes o incorporar otros nuevos ingredientes o aditivos, Lo que supone un valor añadido al consumo de pescado. Estos se pueden dividir en ingredientes: a) que favorecen la conservación, b) funcionales desde el punto de vista tecnológico y c) funcionales desde el punto de vista nutricional, dando lugar a nuevos **alimentos funcionales** (Sánchez Alonso y col., 2004). Los alimentos funcionales fueron regulados por la Unión Europea mediante el Reglamento Europeo 1924/2006 sobre las declaraciones nutricionales, y de propiedades saludables en los alimentos, que se complementó en 2012 con el RE 432/2012 de la Comisión, de 16 de mayo de 2012 (Comisión Europea, 2006, 2012), y se refiere a "todo aquel alimento semejante en apariencia física al alimento convencional, consumido como parte de la dieta diaria, pero capaz de producir efectos metabólicos o fisiológicos, útiles en el mantenimiento de una buena salud física y mental, en la reducción del riesgo de enfermedades crónico-degenerativas, además de sus funciones nutricionales básicas" (Schärer y Fenton, 1998). Por tanto, convertir los reestructurados en alimentos funcionales es una posibilidad de dar un valor añadido a los productos pesqueros, los cuales, aparte de su contenido nutritivo, podrían contener ingredientes que desempeñan una actividad específica en las funciones fisiológicas del organismo humano, favoreciendo la capacidad física y el estado mental.

2.2.4-El glucomanano procedente de Amorphophallus konjac (KGM) como alternativa en la reestructuración del músculo de pescado

Como resultado del serrado de los bloques de pescado congelado se genera un subproducto al que denominamos "serrín" de pescado. Esta materia prima, debido a la pérdida de funcionalidad de la proteína por el proceso de serrado, para ser empleada en la obtención de reestructurados, necesita una serie de ingredientes, como hidrocoloides y proteínas no musculares, que refuercen la capacidad de formar gel. En general, los hidrocoloides se utilizan como espesantes, gelificantes y estabilizantes. En el papel de gelificante se utilizan hidrocoloides como pectinas de bajo metoxilo, κ-carragenato y ι-carragenato que son solubles en agua caliente y gelifican al enfriarse a temperatura ambiente. Los geles así obtenidos son termorreversibles, solo el caso de los alginatos y las pectinas de bajo metoxilo en presencia de sales cálcicas, y las pectinas de alto metoxilo, en presencia de azúcares pueden dar lugar a geles termoestables (Pérez-Mateos, 1998). Los

reestructurados de pescado con geles termorreversibles no podrían ser calentados para el cocinado, ya que perderían su estructura (Chen, Liu, y Zhuo, 2005; Pereira y col., 2004).

En este sentido, el GM procedente de *Amorphophallus Konjac* (KGM) ofrece una nueva e interesante forma de obtener productos pesqueros reestructurados, procedentes de músculos de pescado cuyas proteínas han perdido su funcionalidad. Esta menor funcionalidad podría ser debida al cocinado, congelamiento prolongado, calentamiento por tratamientos mecánicos, originando productos con aspecto de crudos pudiendo ser comercializados como frescos. La gelificación termoestable del GM permite reutilizar y reestructurar subproductos de la industria pesquera como los restos de atún cocido para enlatar, el "serrín" generado en los cortes de grandes bloques de pescado congelado, las cabezas de los pulpos previamente cocidas, y en general, cualquier tipo de músculo sobrante en un proceso, en el que su proteína ha podido desnaturalizarse por calor, y poder elaborar estructuras que traten de imitar productos frescos o cocinados. De igual forma, como se ha mencionado anteriormente, cuando se utiliza músculo de pescado con un elevado contenido graso, la MTGasa no actúa correctamente, produciendo una débil gelificación (Moreno, Carballo, y col., 2009), por lo que la utilización de GM como agente gelificante podría ser una alternativa.

Una ventaja de estos productos reestructurados con GM, es que al incluir más agua que el músculo normal, puede actuar como elemento saciante bajo en calorías. Por otro lado, estos reestructurados ofrecen la posibilidad de incluir ingredientes funcionales, como el aceite de pescado, rico en ω-3 y/o aceites vegetales ricos en ácido linoleico conjugado (CLA).

2.3.- IMPORTANCIA DE LA TEXTURA Y REOLOGÍA EN LA CARACTERIZACIÓN, CALIDAD, Y DESARROLLO DE NUEVOS ALIMENTOS

La calidad de un alimento es el "conjunto de características de un producto alimenticio relativas a materias primas, ingredientes, naturaleza, composición, pureza, identificación, origen, y trazabilidad, así como a los procesos de elaboración, almacenamiento, envasado y comercialización. También a la presentación del producto final, incluyendo su contenido efectivo, y la información al consumidor especialmente en el etiquetado" (Ley 28/2015, del 30 Julio, BOE-A-2015-8563). Todas estas propiedades determinan el grado de aceptabilidad del consumidor.

Los atributos sensoriales (aspecto, sabor, aroma y textura) integran la calidad sensorial de un alimento. La evaluación sensorial se define como una disciplina científica, empleada para evocar, medir, analizar e interpretar reacciones características del alimento, percibidas a través de los sentidos de la vista, olfato, gusto, tacto y audición. La mayoría de las características sensoriales sólo pueden ser medidas significativamente por humanos. El término calidad sensorial implica el grado de excelencia de un producto y es una percepción humana que abarca muchas propiedades.

La importancia que se concede a cada atributo de la calidad sensorial depende del tipo de alimento y del consumidor. En general, el aspecto (color, forma y tamaño) perceptible con la vista, resulta determinante en el momento de elegir el alimento, pero son los restantes atributos (sabor, aroma, y textura) los que determinan finalmente el grado de satisfacción del consumidor. La **textura** es uno de los atributos de los alimentos que está relacionado directamente con su calidad, y que determina en gran medida la aceptabilidad final por parte de los consumidores.

2.3.1. Medida objetiva de la textura

La textura debe entenderse como una propiedad multifactorial compleja. Existen varias definiciones de textura, la Norma Española de Análisis Sensorial sobre vocabulario (UNE, 1995), correspondiente a la norma ISO 5492 (1992), la define como el "conjunto de propiedades reológicas y de estructura (geométricas y de superficie) de un producto, perceptibles por los mecano-receptores, los receptores táctiles y, en ciertos casos, por los visuales y auditivos." Por otra parte, Szczesniak, (2002) define la textura como la "manifestación sensorial y funcional de la propiedad estructural, mecánica y de superficie de los alimentos detectada a través de los sentidos de la vista, el oído, el tacto y la cinestesia. El proceso productivo o de transformación de un producto le confiere diferentes propiedades texturales, dependiendo de sus componentes, interacciones moleculares, colocación espacial y concentración. En cualquier caso, las propiedades texturales son factores determinantes de la calidad de un producto, siendo clave a la hora de ser aceptado o rechazado.

Para conseguir la caracterización textural se pueden hacer tres tipos de ensayos, de los cuales, el último es el más aplicado en la industria alimentaria:

- *Ensayos fundamentales*: son objetivos porque los resultados son independientes del método de medida. Proporcionan valores de las propiedades físicas básicas, como elasticidad, flexibilidad conformacional, solidez, y resistencia a la deformación (Mezger, 2006), utilizando unas condiciones de medida dentro de los límites del comportamiento viscoelástico lineal.

- *Ensayos empíricos*: sin poseer base científica real, miden, en condiciones definidas, variables relacionadas con alguno de los atributos característicos de la textura de los alimentos. Estos ensayos incluyen, técnicas de penetrometria, cizallamiento y extrusión (Park, Noh, y Park, 2005).

- *Ensayos imitativos*: este tipo de ensayos se dirigen a la experimentación simulada de procesos sufridos por los alimentos durante su consumo.

Entre los ensayos imitativos, el **análisis de perfil de textura instrumental** (TPA) y el **ensayo de penetración o penetrometria**. Son los más simples y comúnmente empleados en la determinación de la textura de los alimentos. Los estudios de texturas de alimentos mediante el TPA se utilizan para medir las propiedades mecánicas de los alimentos (Friedman, Whitney, y Szczesniak, 1963).

El TPA se realiza mediante una compresión uniaxial de dos ciclos (Pons y Fiszman, 1996) que comprime los alimentos con un grado de deformación controlado, que varía según el objetivo de la medición (Pons y Fiszman, 1996). El perfil de textura, es una curva fuerza-tiempo (Figura 12) de la que se obtienen datos de la deformabilidad (de compresión) del alimento que se puede utilizar para interpretar algunas de las principales características de la textura mecánica, como dureza, fragilidad, elasticidad, gomosidad y adhesividad (Chen y Stokes, 2012).

El TPA permite hacer una medida de textura sensorial (Chen y Opara, 2013). En la Tabla 3 quedan recogidos los principales parámetros que la interpretación de los perfiles de TPA permite obtener. La dureza sensorial percibida en un alimento, se ha relacionado con la fuerza de compresión máxima durante el primer ciclo de compresión y se interpreta como la fuerza requerida para deformar un alimento. Es uno de los atributos de textura más

importante en los alimentos; en el caso de los productos pesqueros, junto con el sabor y la apariencia, constituye una de las características en las que el consumidor basa su decisión de elección del alimento. El resto de parámetros de textura, por lo general determinan el estado reológico que predomina en el alimento (Torres, González-Morelo, y Acevedo, 2015).

Por otro lado, el ensayo de penetración mide la fuerza que se necesita para ocasionar la ruptura del alimento, simulando las altas deformaciones que sufre en el proceso de masticación. Obtenemos en este caso, la fuerza y deformación de rotura, el trabajo de penetración por volumen desplazado, y la fuerza media de penetración.

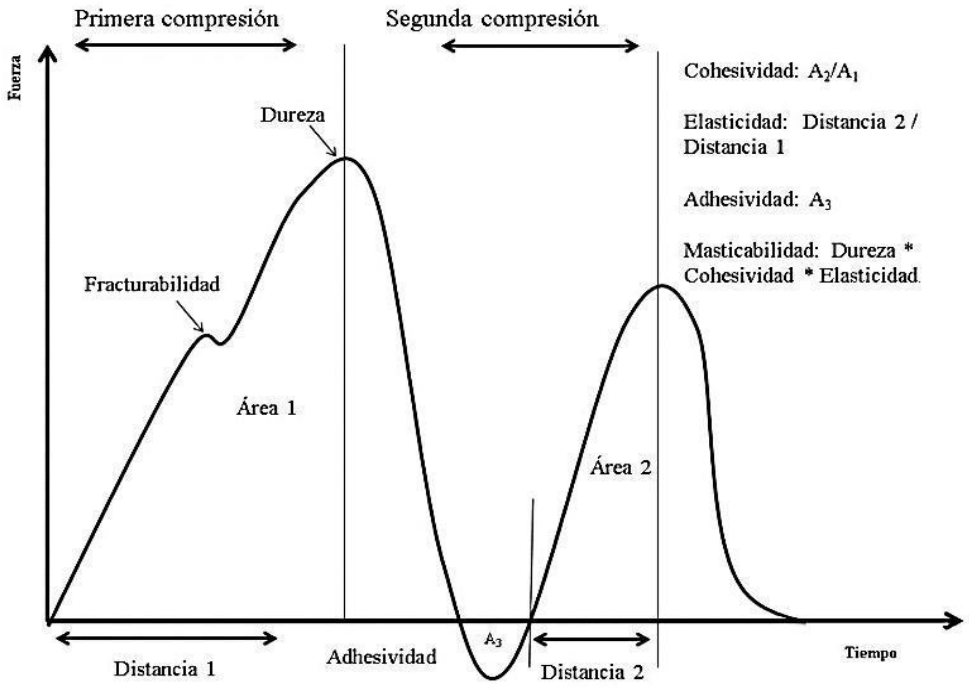

Figura 12.-Gráfica general del análisis de perfil de textura (Fuerza-Tiempo) (Fuente: Hleap y Velasco, 2010).

Tabla 3.-Parámetros básicos a determinar en el análisis del perfil de textura (Fuente: adaptado de Pons y Fiszman, 1996).

Propiedades	Definición	Significado
Dureza sensorial	Máxima fuerza de la primera compresión	Deformabilidad compresiva del alimento
Adhesividad	Área negativa después de la primera compresión	Capacidad de adhesión del alimento a la sonda al retirarse después de la primera compresión
Cohesividad	Área 2 / Área 1	Relación entre una primera y una segunda deformación en relación con su resistencia a la rotura.
Elasticidad	Distancia de altura durante la segunda compresión por la primera distancia de compresión	Capacidad de recuperación de un alimento después de que se deforme durante la primera compresión.
Go mosidad	Dureza x cohesividad	Cuánta fuerza y trabajo se requiere para comprimir el alimento
Masticabilidad	Gomosidad x elasticidad	Cuánta fuerza, trabajo y distancia se requiere para comprimir el alimento

2.3.2. La reología como herramienta para el estudio de la calidad de alimentos

La reología se define como la ciencia de la deformación y el flujo de la materia (Malkin y Isayev, 2017). La reología se aplica en el ámbito alimentario en cualquier etapa del proceso de elaboración de un producto, ya sea para el diseño de los procesos industriales, la formulación de los productos, o la evaluación de la textura final del alimento, permitiendo analizar los cambios estructurales que ocurren durante el proceso. El desarrollo de esta ciencia ha permitido determinar mediante métodos instrumentales, una serie de parámetros objetivos que se pueden relacionar con las características sensoriales y subjetivas que definen la textura de un alimento y su aceptación por parte del consumidor. De este modo, los estudios reológicos en alimentos son importantes ya que aportan información sobre la estructura del alimento, son rápidos, precisos y aportan datos reproducibles. Los parámetros obtenidos son objetivos a la hora de definir la textura de un alimento. Se han correlacionado estadísticamente, propiedades de textura sensorial y empírica con propiedades reológicas en el rango viscoelástico lineal, que han servido para establecer el fundamento experimental de la correlación entre textura sensorial e

instrumental en geles lácteos (Bargiela, 2014). En resumen, una clara comprensión de las propiedades reológicas y texturales de un alimento es fundamental en la investigación y desarrollo de nuevos productos, su control de calidad, el diseño de equipos, y la optimización o de los diversos procesados (Steffe, 1996).

2.3.2.1. Ensayos reológicos dinámicos

Los ensayos dinámicos u oscilatorios son ensayos reológicos en los que la materia se somete a un esfuerzo que varía armónicamente con el tiempo, a frecuencia fija y/o variable (Sahin y Sumnu, 2006). Constituyen una de las herramientas fundamentales y de uso creciente, para la caracterización de las propiedades **viscoelásticas** de los alimentos, en relación con su estructura. Son ensayos que mantienen la estructura de la muestra ya que se aplican pequeños esfuerzos de cizalla llamados **SAOS,** acrónimo de las siguientes palabras: *Small Amplitude Oscillatory Shear*. La muestra se sitúa entre dos placas paralelas, la inferior fija y atemperada y la superior, conectada al motor del reómetro, aplica un esfuerzo oscilante (Figura 13).

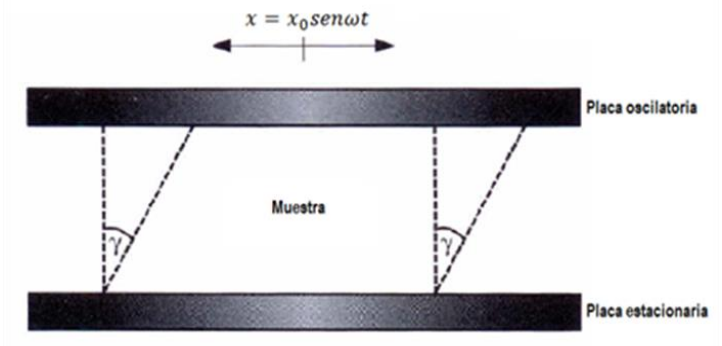

Figura 13.- Esquema simplificado de una prueba oscilatoria.
(Fuente: Norton, Spyropoulos, y Cox., 2010).

La **posición de la placa oscilatoria (*x*)** respecto al tiempo *t* está dada por la ecuación 1:

$$x = x_o \cdot sen\omega \cdot t \qquad (1)$$

Siendo *x* la posición instantánea de la placa y x_o la amplitud de oscilación.

El **esfuerzo de cizalla armónico (σ),** de amplitud σ_o, a frecuencia angular (ω) que se aplica al material, viene dado por la ecuación 2:

$$\sigma = \sigma_0 \cdot sen\omega t \qquad (2)$$

De este modo, el uso de bajas amplitudes de tensión, dentro del intervalo viscoelástico lineal (IVEL), produce una respuesta de **deformación** (γ) retrasada un cierto valor llamado **ángulo de fase** (δ) según la ecuación 3:

$$\gamma = \gamma_0 \cdot sen(\omega \cdot t - \delta) \quad \textbf{(3)}$$

Donde γ_0 es la amplitud de deformación de cizallamiento.

El ángulo δ, es 0° para los materiales puramente **elásticos** (sin retraso en la respuesta), y 90° para los **fluidos** (puramente viscosos). Un material **viscoelástico ideal** tendría δ=45 °, indicando la misma proporción de carácter elástico que viscoso(Steffe, 1996).

El cociente entre el **esfuerzo de cizalla armónico** (σ), y la deformación (γ) proporciona el **módulo de cizalla complejo** (G^*). Utilizando la notación exponencial, según la ecuación 4, se obtiene G^*, un parámetro que habitualmente se da como medida de fuerza de gel (Gabriele, de Cindio, y D'Antona, 2001).

$$G^* = \frac{\sigma_0 \cdot e^{j\omega t}}{\gamma_0 \cdot e^{j(\omega t - \delta)}} \quad \textbf{(4)}$$

$$G^* = \frac{\sigma_c}{\gamma_0}(\cos\delta + j sen\delta) \quad \textbf{(5)}$$

De la ecuación 5 se deducen las ecuaciones que proporcionan la parte real o **componente elástica** (G') (ecuación.6), y la parte imaginaria o **componente viscosa** (G'') (ecuación. 7).

$$G' = \frac{\sigma_0 cos\delta}{\gamma_0} = \frac{amplitud\ de\ estrés\ o\ esfuerzo\ en\ fase}{amplitud\ de\ deformación} \quad \textbf{(6)}$$

$$G'' = \frac{\sigma_0 sen\delta}{\gamma_0} = \frac{amplitud\ de\ estrés\ o\ esfuerzo\ fuera\ de\ fase}{amplitud\ de\ deformación} \quad \textbf{(7)}$$

Y la relación entre la componente viscosa y elástica proporciona el **factor de pérdida** (tanδ), según la ecuación 8:

$$tan\delta = \frac{G''}{G'} \qquad \textbf{(8)}$$

En la Tabla 4 se presenta un resumen del significado físico de los principales parámetros viscoelásticos.

Tabla 4.-Definiciones de los principales parámetros viscoelásticos. (Fuente: adaptado de Tunick, 2000).

Término	Símbolo	Definición	Información proporcionada
Módulo complejo	G^*	$[(G')^2 + (G'')^2]^{0.5}$	Resistencia promedio a la deformación (elástica y viscosa)
Módulo elástico o de almacenamiento	G'	$\frac{\sigma_0}{\gamma_0} \cdot \cos\delta$	Energía almacenada por la muestra durante el proceso de cizalla: comportamiento sólido o elástico
Módulo viscoso o de pérdida	G''	$\frac{\sigma_0}{\gamma_0} \cdot sen\delta$	Energía disipada durante el proceso de cizalla: comportamiento líquido o viscoso
Factor de pérdida	$tan\delta$	G''/G'	Grado de solidez
Viscosidad compleja	∩*	G*/ω	Flujo viscoelástico

En este trabajo se llevaron a cabo tres tipos de ensayos oscilatorios para caracterizar los geles: **ensayo de barrido de tensión** (Figura 14), donde *G'* y *G''* se obtienen en función de la tensión aplicada para determinar los valores límite que caracterizan el intervalo viscoelástico lineal (IVEL) (Campo-Deaño y Tovar, 2009).

La Figura 15 representa un ensayo en barrido de frecuencia en el que se obtiene el

espectro mecánico, es decir, *G'* y G" se determinan en función de la frecuencia a baja deformación dentro del IVEL, y temperatura fija. El espectro mecánico se considera la "*huella dactilar*" de la muestra ya que proporciona los valores de *G' y G''* a distintas escalas de tiempo (Steffe, 1996).

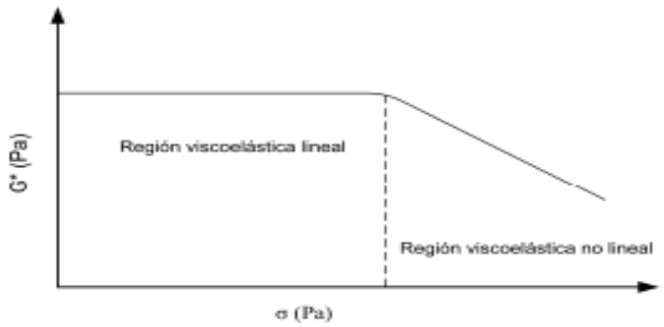

Figura 14.- Límite del intervalo viscoelástico lineal obtenido mediante ensayo en barrido de tensión a frecuencia constante. (Fuente: Tabilo-Munizaga y Barbosa-Cánovas, 2005).

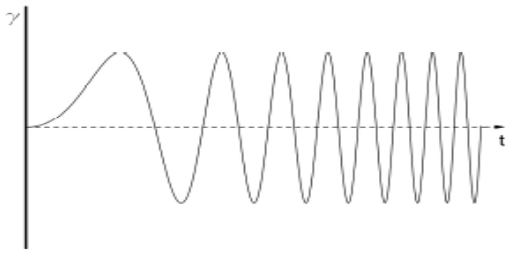

Figura 15.- Ensayo oscilatorio en barrido de frecuencia a amplitud de deformación constante. (Fuente: Tabilo-Munizaga y Barbosa-Cánovas, 2005).

La información proporcionada por los espectros mecánicos sirve para caracterizar o clasificar estructuralmente una dispersión en: disoluciones diluidas, concentradas, geles débiles y fuertes (Rao, 2010). Las disoluciones diluidas serían aquellas en las que *G''*>>*G'* en todo el rango de frecuencias, aunque a altas frecuencias los módulos se van aproximando, y además, ambos módulos son fuertemente dependientes de la frecuencia. Las disoluciones concentradas poseen módulos *G'* y *G''* que pueden cruzarse en algún valor característico de frecuencia, indicando una clara tendencia hacia un comportamiento más sólido a frecuencias más altas, y más fluido a frecuencias más bajas. La frecuencia (ω) a la cual *G'* se iguala a *G''*, se denomina **frecuencia de superposición** o cofluencia (Rao, 2010), y el tiempo $t=1/\omega$ es un valor característico del material.

Los geles se pueden definir como sistemas coloidales de dos fases, formados por una matriz sólida (fase continua) y un líquido, normalmente agua (fase dispersada) (Sahin y Sumnu, 2006) y cada fase puede estar interconectada. Se pueden clasificar en geles fuertes o débiles, los fuertes se caracterizan porque $G' >> G''$ (al menos un orden de magnitud mayor), en todo el rango de frecuencias. Siendo ambos módulos G' y G'' prácticamente independientes de la frecuencia (Rao, 2010). Un gel débil es aquel que siendo $G'>G''$ en todo el rango de frecuencias, ocurre que ambos módulos poseen mayor dependencia de la frecuencia (Rao, 2010), y podría considerarse como el sistema intermedio entre un gel fuerte y una disolución concentrada. Su comportamiento es similar a los geles fuertes a altas frecuencias, pudiendo observarse el cambio de estado de gel a sol al disminuir la frecuencia. Si hablamos de geles formados por polisacáridos, el comportamiento es peculiar debido al elevado número de enlaces de hidrógeno e interacciones polares, que pueden formar "zonas de unión" más o menos largas, y de diferente grosor entre las cadenas poliméricas, dando lugar a geles físicos, altamente estables con la frecuencia y la temperatura (Lapasin y Pricl, 2012).

La Figura 16 representa el ensayo en barrido de temperatura, que permite obtener los perfiles térmicos de las muestras, es decir, los valores de G' y G" en función de la temperatura a frecuencia y deformación fijas. Estos ensayos son relevantes por la gran información estructural que proporcionan, ya que permiten caracterizar el comportamiento termo-reológico de las muestras (Mezger, 2006).

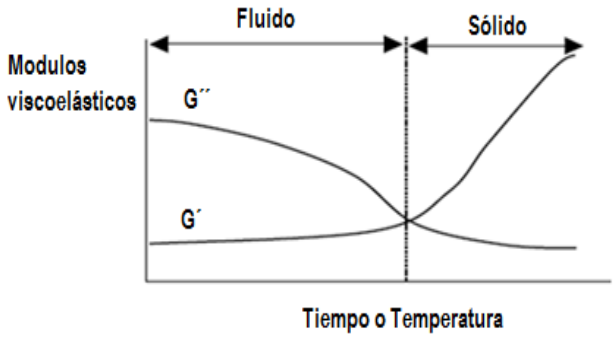

Figura 16.- Perfil térmico, mediante ensayo en barrido de temperatura, del cambio de fase sol-gel (Fuente: Tabilo-Munizaga y Barbosa-Cánovas, 2005).

2.3.2.2. Ensayo de carga y recuperación

El ensayo de carga y recuperación consiste en aplicar un esfuerzo constante a una muestra, y medir su deformación en función del tiempo. Normalmente los ensayos de carga y recuperación en los geles se realizan a tensiones dentro del IVEL, y las escalas de tiempo son superiores a las que se emplean en los ensayos oscilatorios. Por lo tanto, proporcionan información sobre la secuencia de ruptura de los enlaces cruzados en la red del gel: al principio del ensayo (tiempos cortos) se rompen las interacciones cruzadas más débiles (*short-range intractions*). A continuación, cuando la deformación sigue incrementando con el tiempo, la ruptura de nuevas interacciones se extiende a más "*zonas de unión*" (*long-range*), pues a tiempos más elevados unas interacciones se rompen y la estructura puede re-organizarse, para finalmente seguir rompiendo progresivamente interacciones más fuertes (Lapasin y Pricl, 2013).

Finalizado el tiempo de carga (Figura 17 superior), se retira la tensión, dejando evolucionar el sistema libremente, y se mide la deformación que se recupera en función del tiempo. Para un material elástico ideal sometido a una tensión constante, al retirar el esfuerzo, el material es capaz de retornar a su forma original. En el caso de un material viscoso ideal, el flujo continuo hace provoca respuesta lineal de la deformación instantánea con el tiempo, y el material es incapaz de recuperar su estructura cuando la tensión se ha eliminado. En los materiales viscoelásticos, la respuesta frente al esfuerzo no será lineal, y al retirar la carga, tendrá cierta capacidad de recuperación (Figura 17, inferior), porque libera parcialmente la energía absorbida en el proceso de carga (Steffe, 1996).

Los datos durante la fase de carga y en la recuperación, están escritos en función de la capacitancia de carga *(J)* (ecuación 9) como se indica en el apartado de materiales y métodos:

$$J(t) = \frac{\gamma(t)}{\sigma_0} \qquad \textbf{(9)}$$

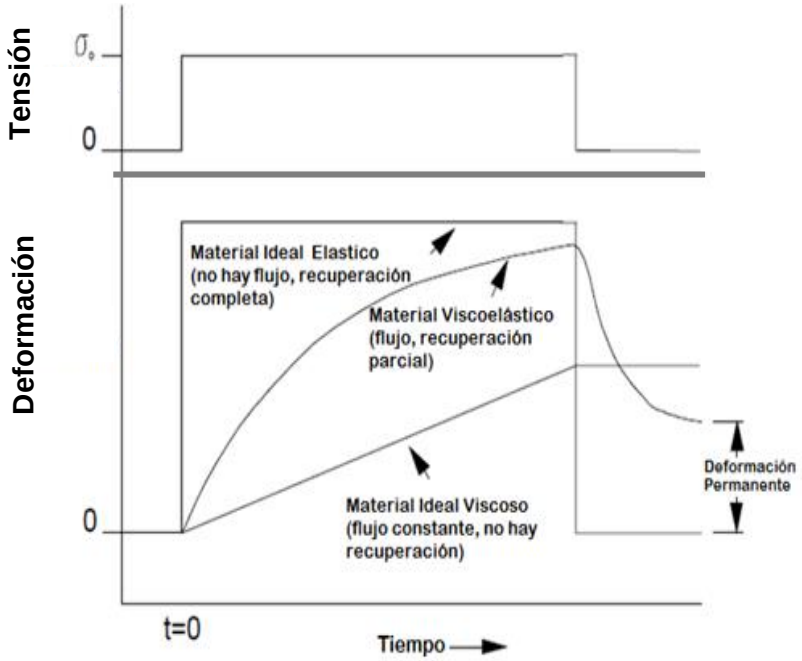

Figura 17.- Curvas de carga y recuperación de un material elástico ideal, viscoso ideal y viscoelástico. (Fuente: Steffe, 1996).

Las curvas de capacitancia (deformabilidad) a distintos niveles de tensión, se solapan cuando los valores del esfuerzo aplicado están dentro del IVEL. Este hecho permite examinar y comparar las propiedades estructurales de los geles de surimi a escalas de tiempo más largas y al mismo tiempo, corroborar los valores límite del rango viscoelástico lineal (Steffe, 1996).

2.3.3.- Reología de geles mixtos (inclusión del músculo de pescado picado en los geles de GM)

En los reestructurados de pescado, la gelificación es crítica para la obtención de la textura deseada, y en ello tienen un papel determinante tanto las características mecánicas como las reológicas. A nivel molecular, la gelificación consiste en la formación de una red tridimensional continua de moléculas poliméricas, que forman la matriz sólida responsable de la resistencia a la tensión (Sahin y Sumnu, 2006). La gelificación de hidrocoloides se produce por la asociación intermolecular entre las cadenas y conlleva un reordenamiento estructural, que origina las denominadas "zonas de unión", unidades estructurales básicas que forman la matriz de la red tridimensional característica del gel (Lapasin y Pricl, 2013). Varios parámetros como temperatura, presencia de iones, y la estructura del hidrocoloide influyen en la disposición física de las zonas de unión dentro de la red y en su grosor (Milani y Maleki, 2012). La mayoría de los biopolímeros, en particular los polisacáridos, forman geles físicos, estructurados por interacciones débiles (enlaces de hidrógeno, interacciones electrostáticas, dipolo-dipolo, e hidrofóbicas). Todas ellas aunque son individualmente

débiles, cuando actúan cooperativamente, originan estructuras permanentes predominantemente desordenadas (agregados físicos), pero con regiones cristalinas de orden local (Lapasin y Pricl, 2013). Estos agregados son los responsables de la rigidez y del elevado grado de solidez en las redes de gel y, por tanto, definen su comportamiento termo-reológico. En el caso concreto que se estudia en esta memoria, el gel de GM completamente desacetilado es un gel termoestable y por tanto termoirreversible. "Geles termoestables" son aquellos que mantienen prácticamente constante los valores de *G'* al incrementar la temperatura (Mezger, 2006), son lógicamente termoirreversibles porque no revertirán el estado sol tras un tratamiento térmico, que va desde un calentamiento suave hasta una fritura profunda.

Resumiendo, la caracterización reológica de los geles y los reestructurados de pescado es importante, ya que se correlaciona con los atributos texturales del producto, que, a su vez, determinan sus características sensoriales y su aceptabilidad por parte del consumidor.

2.3.4.- Características físico-químicas de los geles mixtos

Las principales características fisicoquímicas que definen al gel de GM, al reestructurado de pescado, y que determinan en gran parte su textura son: **humedad, capacidad de retención de agua** (CRA), **pérdida de agua por cocinado** (PAC) y **color** (CIELAB). En los casos en los que durante la elaboración de los reestructurados de pescado con GM se adicionó aceite de pescado (Omevital™ 1812 TG Gold), como fuente de ácidos grasos ω-3 de cadena larga se determinó la **capacidad de retención de aceite (CRAc)**. Todas ellas son de gran importancia, y sirven de fundamento, para entender el comportamiento reológico de los geles, tanto individuales (solo de GM) como mixtos (GM y pescado).

Humedad

El contenido de humedad en un alimento es un índice de estabilidad del producto, además influye en sus características sensoriales. Se ha descrito que el GM en agua constituye una de las dispersiones coloidales de mayor viscosidad (Kök, Abdelhameed, Ang, Morris, y Harding, 2009; Shinzato, Broussalis, y Ferraro, 1996), pudiendo absorber hasta 200 veces su peso de agua (Shinzato y col., 1996). Los geles de GM retienen en su estructura

un elevado contenido en agua (96-97%) sin detrimento de su textura, esta propiedad se aprovecha en los reestructurados de pescado, dándole al producto jugosidad que puede haberse perdido en el pescado picado, por el tratamiento desnaturalizante al que ha sido sometido ("serrado").

Capacidad de retención de agua (CRA)

La CRA puede determinar las propiedades sensoriales del producto, como jugosidad, textura y sabor (Wierbicki, Kunkle, y Deatherage, 1957). Es un parámetro físico-químico que se utiliza para describir la capacidad del músculo (ya sea de carne o de pescado), o de un producto procedente de él para unir el agua en unas condiciones específicas. La CRA está directamente relacionada con la microestructura del sistema, depende del tamaño de los poros donde se aloja el agua, y de la existencia de moléculas que aporten cargas y permitan establecer interacciones dipolo-dipolo con las moléculas de agua (Moore, 1978; Wismer-Pedersen, 1994). La CRA en el caso del pescado es un buen indicador, de la idoneidad de los métodos de conservación y de la funcionalidad de la proteína miofibrilar.

En general los geles de GM y proteína son estructuras altamente hidratadas, dependiendo de la concentración de biopolímero. Una cantidad de agua pudiera estar retenida dentro de la matriz sólida a modo de relleno (*filler*). Considerando las posibles interacciones electrostáticas según las diferentes densidades de carga en las cadenas de proteína o GM. De este modo el "*agua de relleno*" forma una fase cuasi-cristalina debido a la red de enlaces de hidrógeno intermoleculares (Damodaran, 2017). Esta red se extiende en mayor o menor medida dependiendo del tamaño del hueco producido por los diversos segmentos poliméricos, que a su vez depende de la distribución de carga entre ellos. Cuanto menor es el tamaño del hueco, más rígida será la estructura resultante ya que la "micro-red de agua" estará más compactada en su interior. En la red del gel existe otra fase acuosa (liquida), que está dispersada en diminutas gotas a lo largo de toda la red, y que puede ser agua de capilaridad, agua hidrodinámica y agua físicamente atrapada (Damodaran, 2017).

Pérdida de agua por cocinado (PAC)

Este parámetro mide el fluido liberado tras el calentamiento del músculo sin aplicar fuerzas externas y es importante sensorialmente ya que la jugosidad del alimento cocinado varía inversamente con PAC. Durante el calentamiento, las proteínas del músculo de carne

y pescado, se desnaturalizan, este efecto produce cambios estructurales como la destrucción de membranas celulares, el encogimiento longitudinal y transversal de las fibras, la agregación de las proteínas sarcoplásmicas, y el encogimiento del tejido conjuntivo. Todos los fenómenos citados, y especialmente el último, originan "pérdidas de agua por cocción".

Capacidad de retención de aceite (CRAc)

Con esta determinación se pretende conocer si es posible la adición de aceites con un perfil lipídico saludable que haga de los reestructurados de pescado alimentos funcionales ricos en ω-3. Se ha descrito que el GM tiene cierta capacidad para retener aceites y grasas, de modo que actuaría como un agente de carga. Los agentes de carga están constituidos por partículas de aceite, inmovilizadas en una red hidrogelificada, en este caso la estructura del gel del GM podría inmovilizar las partículas de aceite mejorando el perfil lipídico de los alimentos que lo contienen (Sandoval, 2015). Otro ejemplo representativo del empleo de esta tecnología es la elaboración de salchichas y hamburguesas con geles de GM, llevada a cabo por Jiménez-Colmenero y col. (2013) y Triki y col. (2013), dirigido al desarrollo de productos alimenticios con mejor contenido lipídico en los que además los geles de GM actúan como sustituto de grasa.

Se determinará la CRAc de los reestructurados de pescado- GM frescos y después de ser sometidos a métodos tradicionales de conservación (refrigeración, congelación, y pasteurización).

Color

La medida del color *L*a*b**, también referido como CIELAB, es actualmente uno de los métodos de caracterización más populares y uniformes usado para evaluar el color de un objeto o alimento. Es ampliamente usado porque correlaciona los valores numéricos de color consistentemente con la percepción visual humana. *L** indica la luminosidad y, *a** y *b** son las coordenadas cromáticas (McGuire, 1992).

*L**: luminosidad: *L**=0 en cuerpo negro (absorbente perfecto de radiación), y *L**=100 para cuerpo blanco (difusor perfecto), pues la luz incidente, penetra en el material yes reflejada de forma difusa (en muchos ángulos), por los diversos componentes del material

*a**: coordenadas rojo/verde (*+a* indica rojo, *-a* indica verde)

*b**: coordenadas amarillo/azul (*+b* indica amarillo, *-b* indica azul)

En el pescado blanco se aprecia el color como indicador de calidad, en concreto la "blancura" del pescado. Así, se considera al producto de mejor calidad cuanto más "blancura" presente, mientras que cuanto más amarillo es el color del músculo de pescado, se considera que el producto es más viejo y rancio. El color, la textura, y el sabor son parámetros determinantes en la calidad de procesados de pescados como el Surimi (Park y Morrissey, 1994) y conviene establecer la influencia de los distintos ingredientes y procesos tecnológicos sobre el color (Park, 2000). En general, el gel de GM tiene alta luminosidad y es translucido, por lo que puede ser utilizado sin interferir en el color del producto reestructurado (Rohm, 1990).

2.3.5.- Características sensoriales de los geles mixtos

En última instancia, son las características sensoriales lo que van a hacer del reestructurado de pescado un producto aceptado por los consumidores, de ahí la importancia del análisis sensorial, para evaluar la calidad de los alimentos desde el punto de vista de sus atributos.

Según (Fernández de Simón y col., 2008), el análisis sensorial es una medida multifactorial (color, olor, sabor y apariencia), que nos da una idea general de la aceptación o rechazo del producto por parte de los consumidores. Llega a ser tan importante que determina en muchas ocasiones la aceptación o fracaso de avances e innovaciones en alimentos (Costell y Durán, 1981), apareciendo empresas destinadas a ofrecer los servicios de un panel de catadores.

El análisis sensorial es un método de evaluación que emplea personas (panel de catadores), que pueden estar entrenados o no. Mediante sus sentidos expresan su opinión en una ficha de cata de manera más o menos objetiva. Se entienden tres tipos de pruebas: descriptivas, discriminativas, y afectivas o de consumidor. A su vez, las pruebas afectivas se clasifican en pruebas de preferencia, medición del grado de satisfacción o de aceptación (Anzaldúa-Morales, 1994). Entre las pruebas de aceptación, la escala más utilizada es la **hedónica**, la cual se emplea para cuantificar la magnitud del grado de aceptación de un

producto. La escala hedónica más usada es la escala bipolar de nueve puntos (con un centro neutral y con cuatro categorías positivas y negativas a cada lado), en la que cada uno de ellos está marcado por un número asociado a una expresión descriptiva, que refleja la intensidad de la sensación de aceptación o rechazo provocada por el alimento (Costell y Duran, 2002; 2009).

Hay características, como la textura, que tienen un carácter multifactorial y de tal complejidad, que un equipo instrumental es incapaz de determinarla, siendo imprescindible el uso de humanos (Szczesniak, 2002), ya que no existen receptores específicos para la textura, y aunque cierta evaluación de la misma se lleva a cabo visual y manualmente, la principal valoración se produce en boca (Rohm., 1990; Varela, 2007).

Concretamente, en este trabajo mediante ensayos estandarizados UNE-ISO 6658 (AENOR, 2019) se realizan análisis sensoriales en términos de sabor (TAA) y textura (TEA), de los reestructurados de GM-pescado con y sin ingredientes adicionales (aceite, sal), y sometidos a tratamientos de conservación (congelación, pasteurización).

REFERENCIAS

AENOR. (2019). Análisis sensorial de alimentos. Metodología. Guía general. UNE-ISO.

Ajinomoto, K. K. (1994). Trans-glutaminase to bulk raw cod roe, forming mixt. In mould, etc. Patente Japonesa. JP6153869-A/JP3042226-B2.

Al-Ghazzewi, F. H., Khanna, S., Tester, R. F., y Piggott, J. (2007). The potential use of hydrolysed konjac glucomannan as a prebiotic. Journal of the Science of Food and Agriculture, 87(9), 1758-1766.

Alimentarius, C. (2003). CAC/RCP 1-1969, Rev. 4, 2003. Recommended International Code of Practice General Principles of Food Hygiene, 3.

An, H., Peters, M. Y., y Seymour, T. A. (1996). Roles of endogenous enzymes in surimi gelation. Trends in Food Science & Technology, 7(10), 321-327.

Anderson, J. W., Baird, P., Davis, R. H., Ferreri, S., Knudtson, M., Koraym, A., … Williams, C. L. (2009). Health benefits of dietary fiber. Nutrition reviews, 67(4), 188-205.

Annable, P., Williams, P. A., y Nishinari, K. (1994). Interaction in Xanthan-Glucomannan Mixtures and the Influence of Electrolyte. Macromolecules. 27(15), 4204-4211.

Anzaldúa-Morales, A. (1994). La evaluación sensorial de los alimentos en la teoría y la práctica. Ed. Acribia. Zaragoza, España.

Appleton, K. M., Rogers, P. J., y Ness, A. R. (2010). Updated systematic review and meta-analysis of the effects of n-3 long-chain polyunsaturated fatty acids on depressed mood. American Journal of Clinical Nutrition. 91(3), 757-770.

Astiasarán, I., y Martínez, A. (2000). Alimentos: Composición Y Propiedades. McGrow Hill Interamericana.

Badui, S., Guerrero, I., López, E., y Armenta, R. . (2006). Química de los alimentos. En Ed. Pearson educación.

Behera, S. S., y Ray, R. C. (2016). Konjac glucomannan, a promising polysaccharide of Amorphophallus konjac K. Koch in health care. International journal of biological macromolecules, 92, 942-956.

Behera, S. S., y Ray, R. C. (2017). Nutritional and potential health benefits of konjac glucomannan, a promising polysaccharide of elephant foot yam, Amorphophallus konjac K. Koch: A review. Food Reviews International, 33(1), 22-43.

Boles, J. A., y Shand, P. J. (1999). Effects of raw binder system, meat cut and prior freezing on restructured beef. Meat science, 53(4), 233-239.

Borderías, A. J., Sánchez-Alonso, I., y Pérez-Mateos, M. (2005). New applications of fibres in foods: Addition to fishery products. Trends in Food Science and Technology. 16(10), 458-465.

Borderías, A Javier, Carballo, J., y Moreno Conde, H. M. (2008). Conformación de miotomos o mioseptos en productos pesqueros reestructurados.

Borderías, A Javier, Pérez-Mateos, M., Solas, M., y Montero, P. (1997). Frozen storage of high-pressure-and heat-induced gels of blue whiting (Micromesistius poutassou) muscle: rheological, chemical and ultrastructure studies. Zeitschrift für Lebensmitteluntersuchung und-Forschung A, 205(5), 335-342.

Borderías, A Javier, y Pérez Mateos, M. (1996). Productos pesqueros reestructurados.

Campo-Deaño, L., y Tovar, C. (2009). The effect of egg albumen on the viscoelasticity of crab sticks made from Alaska Pollock and Pacific Whiting surimi. Food Hydrocolloids, 23(7), 1641-1646.

Cando, D., Borderías, A. J., y Moreno, H. M. (2016). Combined effect of aminoacids and microbial transglutaminase on gelation of low salt surimi content under high pressure processing. Innovative food science & emerging technologies, 36, 10-17.

Carballo, J., Ayo, J., y Colmenero, F. J. (2006). Microbial transglutaminase and caseinate as cold set binders: Influence of meat species and chilling storage. LWT - Food Science and Technology. 39(6), 692-699.

Cederholm, T., y Palmblad, J. (2010). Are omega-3 fatty acids options for prevention and treatment of cognitive decline and dementia? Current Opinion in Clinical Nutrition and Metabolic Care. 39(6), 692-699. https://doi.org/10.1097/MCO.0b013e328335c40b

Chan, A. P. N. (2009). Konjac Part I. Cultivation to Commercialization of Components.

Chen, H.-L., Fan, Y.-H., Chen, M.-E., y Chan, Y. (2005). Unhydrolyzed and hydrolyzed konjac glucomannans modulated cecal and fecal microflora in Balb/c mice. Nutrition, 21(10), 1059-1064.

Chen, H.-L., Sheu, W. H.-H., Tai, T.-S., Liaw, Y.-P., y Chen, Y.-C. (2003). Konjac supplement alleviated hypercholesterolemia and hyperglycemia in type 2 diabetic subjects—a randomized double-blind trial. Journal of the American College of Nutrition, 22(1), 36-42.

Chen, H., Lan, G., Ran, L., Xiao, Y., Yu, K., Lu, B., … Lu, F. (2018). A novel wound dressing based on a Konjac glucomannan/silver nanoparticle composite sponge effectively kills bacteria and accelerates wound healing. Carbohydrate polymers, 183, 70-80.

Chen, Jian, Li, J., y Li, B. (2011). Identification of molecular driving forces involved in the gelation of konjac glucomannan: Effect of degree of deacetylation on hydrophobic association. Carbohydrate Polymers. 86(2), 865-871.

Chen, Jianshe, y Stokes, J. R. (2012). Rheology and tribology: Two distinctive regimes of food texture sensation. Trends in Food Science & Technology, 25(1), 4-12.

Chen, L.-G., Liu, Z.-L., y Zhuo, R.-X. (2005). Synthesis and properties of degradable hydrogels of konjac glucomannan grafted acrylic acid for colon-specific drug delivery. Polymer, 46(16), 6274-6281.

Chen, L., y Opara, U. L. (2013). Texture measurement approaches in fresh and processed foods - A review. Food Research International. 51(2), 823-835.

Chiu, Y.-T., y Stewart, M. (2012). Comparison of konjac glucomannan digestibility and fermentability with other dietary fibers in vitro. Journal of medicinal food, 15(2), 120-125.

Chua, M., Baldwin, T. C., Hocking, T. J., y Chan, K. (2010). Traditional uses and potential health benefits of Amorphophallus konjac K. Koch ex NE Br. Journal of ethnopharmacology, 128(2), 268-278.

Cohen, J. T., Bellinger, D. C., Connor, W. E., Kris-Etherton, P. M., Lawrence, R. S., Savitz, D. A., … Gray, G. M. (2005). A quantitative risk–benefit analysis of changes in population fish consumption. American journal of preventive medicine, 29(4), 325-334.

Colmenero, F. J. (2002). Muscle protein gelation by combined use of high pressure/temperature. Trends in Food Science & Technology, 13(1), 22-30.

Comisión Europea. (2016). La Política Pesquera Común en datos y cifras - Edición 2016. En Información estadística bàsica. https://doi.org/10.1007/s13398-014-0173-7.2

Commission, E. U. (2011). Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by establishing a Union list of food additives. Official Journal of the European Union L, 295(4), 11-12.

Costell, E., y Duran, L. (2009). Food texture: sensory evaluation. En Food Engineering: Vol. II (pp. 238-255).

Costell, E., y Durán, L. (1981). El análisis sensorial en el control de calidad de los alimentos. I. Introducción. Revista de Agroquímica y Tecnología de los Alimentos, 21(1), 1-10.

Damodaran, S. (2017). Protein-stabilized foams and emulsions. En Food Proteins and their Applications. 15(2), 155-203.

Dea, I. C. M., y Morrison, A. (1975). Chemistry and interactions of seed galactomannans. En Advances in carbohydrate chemistry and biochemistry (Vol. 31, pp. 241-312). Elsevier.

Devaraj, R. D., Reddy, C. K., y Xu, B. (2018). Health-promoting effects of konjac glucomannan and its practical applications: A critical review. International journal of biological macromolecules.

Documentos COTEC. (1995). Documentos COTEC.Productos pesqueros reestructurados.

Doi, K. (1995). Effect of konjac fibre (glucomannan) on glucose and lipids. European journal of clinical nutrition, 49, S190-7.

Domingo, J. L. (2016). Nutrients and Chemical Pollutants in Fish and Shellfish. Balancing Health Benefits and Risks of Regular Fish Consumption. Critical Reviews in Food Science and Nutrition, 56(6), 979-988.

Du, X., Li, J., Chen, J., y Li, B. (2012). Effect of degree of deacetylation on physicochemical and gelation properties of konjac glucomannan. Food Research International. 46(1), 270-278.

EFSA, Panel on Food Additives and Nutrient Sources added to Food, (ANS). (2017). Re-evaluation of konjac gum (E 425 i) and konjac glucomannan (E 425 ii) as food additives. EFSA Journal, 15(6), e04864.

EFSA Panel on Dietetic Products, N. and A. (NDA). (2010). Scientific Opinion on the substantiation of health claims related to konjac mannan (glucomannan) and reduction of body weight (ID 854, 1556, 3725), reduction of post-prandial glycaemic responses (ID 1559), maintenance of normal blood glucose concentration. EFSA Journal, 8(10), 1798.

Europea, U. (2006). Reglamento (CE) no 1924/2006 del Parlamento Europeo y del Consejo, de 20 de diciembre de 2006, relativo a las declaraciones nutricionales y de propiedades saludables en los alimentos. Diario Oficial de la Unión Europea, 30, 9-25.

Europea, U. (2012). Reglamento (CE) no 432/2012 de la Comisión, de 16 de mayo de 2012, por el que se establece una lista de declaraciones autorizadas de propiedades saludables de los alimentos distintas a las relativas a la reducción del riesgo de enfermedad y al desarrollo . Diario Oficial de la Unión Europea, 25, 1-40.

European Food Safety Authority. (2007). Opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment. EFSA Journal, 5(1), 438.

Fan, L., Yi, J., Tong, J., Zhou, X., Ge, H., Zou, S., … Nie, M. (2016). Preparation and characterization of oxidized konjac glucomannan/carboxymethyl chitosan/graphene oxide hydrogel. International Journal of Biological Macromolecules. 91, 358-367.

Fang, W., y Wu, P. (2004). Variations of konjac glucomannan (KGM) from Amorphophallus konjac and its refined powder in China. Food Hydrocolloids, 18(1), 167-170.

FAO. (2016). El estado mundial de la pesca y la acuicultura.Contribución a la seguridad alimentaria y la nutrición para todos. Roma.

FAO. (2018). FAOSTAT Data. https://doi.org/10.1016/j.jad.2018.09.064

Fernández de Simón, B., CADAHia, E., Sanz, M., Poveda, P., Perez-Magariño, S., Ortega-Heras, M., y González-Huerta, C. (2008). Volatile compounds and sensorial characterization of wines from four Spanish denominations of origin, aged in Spanish Rebollo (Quercus pyrenaica Willd.) oak wood barrels. Journal of agricultural and food chemistry, 56(19), 9046-9055.

Food and Agriculture Organization of the United Nations/World Health Organization. (2010). Report of the joint FAO/WHO expert consultation on the risks and benefits of fish consumption. En FAO Fisheries and Acquaculture report n.o 978.

Friedman, H. H., Whitney, J. E., y Szczesniak, A. S. (1963). The texturometer—a new instrument for objective texture measurement. Journal of Food science, 28(4), 390-396.

Gao, S., Guo, J., y Nishinari, K. (2008). Thermoreversible konjac glucomannan gel crosslinked by

borax. Carbohydrate Polymers, 72(2), 315-325..

Gao, S., Guo, J., Wu, L., y Wang, S. (2008). Gelation of konjac glucomannan crosslinked by organic borate. Carbohydrate polymers, 73(3), 498-505.

Gao, S., y Nishinari, K. (2004, enero). Effect of degree of acetylation on gelation of konjac glucomannan. Biomacromolecules, Vol. 5, pp. 175-185.

Geng, S., Lin, R., Chen, M., Liu, S., y Wang, Y. (2009). Investigation of nanostructure of konjac-based water absorbents with atomic force microscopy. Frontiers of Chemical Engineering in China, 3(4), 357.

Gil, H. Á. (2010). Tratado de Nutrición, de Ángel Gil Hernández, 2a edic. 4 volúmenes. Edit. Médica Panamericana, 2010. *. 2da edición.

Gómez, B., Míguez, B., Yáñez, R., y Alonso, J. L. (2017). Manufacture and Properties of Glucomannans and Glucomannooligosaccharides Derived from Konjac and Other Sources. Journal of Agricultural and Food Chemistry, 65(10), 2019-2031.

Gonçalves, A. A., y Passos, M. G. (2010). Restructured fish product from white croacker (Micropogonias furnieri) mince using microbial transglutaminas. Brazilian Archives of Biology and Technology, 53(4), 987-995.

González Canga, A., Fernández Martínez, N., Sahagún, A. M., García Vieitez, J. J., Díez Liébana, M., Calle Pardo, A. P., … Sierra Vega, M. (2004). Glucomanano: propiedades y aplicaciones terapéuticas. Nutrición Hospitalaria, 19(1), 45-50.

Gopinath, B., Buyken, A. E., Flood, V. M., Empson, M., Rochtchina, E., y Mitchell, P. (2011). Consumption of polyunsaturated fatty acids, fish, and nuts and risk of inflammatory disease mortality. American Journal of Clinical Nutrition, 93(5), 1073-1079.

Gram, L., y Huss, H. H. (1996). Microbiological spoilage of fish and fish products. International journal of food microbiology, 33(1), 121-137.

Guerreiro, F., Pontes, J. F., da Costa, A. M. R., y Grenha, A. (2019). Spray-drying of konjac glucomannan to produce microparticles for an application as antitubercular drug carriers. Powder Technology, 342, 246-252.

Hamann, D. D., Amato, P. M., Wu, M. C., y Foegeding, E. A. (1990). Inhibition of modori (gel weakening) in surimi by plasma hydrolysate and egg white. Journal of Food Science, 55(3), 665-669.

Harris, W. S. (2004). Fish oil supplementation: Evidence for health benefits. Cleveland Clinic Journal of Medicine, 71(3), 208-221.

Herranz, B., Moreno, H. M., Borderías, A. J., y Tovar, C. A. (2017). Structural and rheological properties of weakly deacetylated glucomannan gels after high-pressure treatment. International Journal of Food Properties, 20(sup2), 2034-2042.

Herranz, B., Piñeiro, L., Borderías, A. J., y Tovar, C. A. (2017). Effect of long-term frozen storage on the rheological properties of pressurized glucomannan gels. Food Hydrocolloids, 67, 224-228.

Ho, H. V. T., Jovanovski, E., Zurbau, A., Blanco Mejia, S., Sievenpiper, J. L., Au-Yeung, F., … Vuksan, V. (2017). A systematic review and meta-analysis of randomized controlled trials of the effect of konjac glucomannan, a viscous soluble fiber, on LDL cholesterol and the new lipid targets non-HDL cholesterol and apolipoprotein B. The American journal of clinical nutrition, 105(5), 1239-1247.

Hoekstra, J., Hart, A., Owen, H., Zeilmaker, M., Bokkers, B., Thorgilsson, B., y Gunnlaugsdottir, H. (2013). Fish, contaminants and human health: Quantifying and weighing benefits and risks. Food and Chemical Toxicology, 54, 18-29.

Hosomi, R., Yoshida, M., y Fukunaga, K. (2012). Seafood Consumption and Components for Health. Global Journal of Health Science, 4(3), 72-86.

Hu, Y., Tian, J., Zou, J., Yuan, X., Li, J., Liang, H., … Li, B. (2019). Partial removal of acetyl groups in konjac glucomannan significantly improved the rheological properties and texture of konjac glucomannan and κ-carrageenan blends. International Journal of Biological Macromolecules, 123, 1165-1171.

Huang, L., Takahashi, R., Kobayashi, S., Kawase, T., y Nishinari, K. (2002). Gelation behavior of native and acetylated konjac glucomannan. Biomacromolecules, 3(6), 1296-1303.

Hurtado, J. L., Montero, P., y Borderias, A. J. (2000). Extension of shelf life of chilled hake (Merluccius capensis) by high pressure/Prolongación de la vida útil de merluza (Merluccius capensis) sometida a altas presiones conservada en refrigeración. Food Science and Technology International, 6(3), 243-249.

Iglesias-Otero, M. A., Borderías, J., y Tovar, C. A. (2010). Use of Konjac glucomannan as additive to reinforce the gels from low-quality squid surimi. Journal of food Engineering, 101(3), 281-288.

James, D., y Bligh, E. G. (1992). Seafood technology in the 90s: the needs of developing countries.

Ji, L., Xue, Y., Zhang, T., Li, Z., y Xue, C. (2017). The effects of microwave processing on the structure and various quality parameters of Alaska pollock surimi protein-polysaccharide gels. Food Hydrocolloids, 63, 77-84.

Jiang, M., Li, H., Shi, J., y Xu, Z. (2018). Depolymerized konjac glucomannan: Preparation and application in health care. Journal of Zhejiang University-SCIENCE B, 19(7), 505-514.

Jiménez-Colmenero, F., Carballo, J., y Cofrades, S. (2001). Healthier meat and meat products: their role as functional foods. Meat science, 59(1), 5-13.

Jiménez-Colmenero, F., Triki, M., Herrero, A. M., Rodríguez-Salas, L., y Ruiz-Capillas, C. (2013). Healthy oil combination stabilized in a konjac matrix as pork fat replacement in low-fat, PUFA-enriched, dry fermented sausages. LWT-Food Science and Technology, 51(1), 158-163.

Kato, K., y Matsuda, K. (1969). Studies on the chemical structure of konjac mannan: part I. Isolation and characterization of oligosaccharides from the partial acid hydrolyzate of the mannan. Agricultural and Biological Chemistry, 33(10), 1446-1453.

Kök, M. S., Abdelhameed, A. S., Ang, S., Morris, G. A., y Harding, S. E. (2009). A novel global hydrodynamic analysis of the molecular flexibility of the dietary fibre polysaccharide konjac glucomannan. Food Hydrocolloids, 23(7), 1910-1917.

Koroskenyi, B., y McCarthy, S. P. (2001). Synthesis of acetylated konjac glucomannan and effect of degree of acetylation on water absorbency. Biomacromolecules, 2(3), 824-826.

Kuraishi, C., Sakamoto, J., Yamazaki, K., SUSA, Y., KUHARA, C., y SOEDA, T. (1997). Production of restructured meat using microbial transglutaminase without salt or cooking. Journal of Food Science, 62(3), 488-490.

Lanier, T. C., y Lee, C. M. (1992). Surimi technology. Marcel Dekker New York.

Lapasin, R., y Pricl, S. (2013). Rheology of Industrial Polysaccharides Theory and Applications. Springer Verlag.

Lee, T., y Dugoua, J.-J. (2011). Nutritional supplements and their effect on glucose control. Current diabetes reports, 11(2), 142-148.

Li, B., Xia, J., Wang, Y., y Xie, B. (2005). Grain-size effect on the structure and antiobesity activity of konjac flour. Journal of Agricultural and Food Chemistry, 53(19), 7404-7407.

Li, B., y Xie, B. J. (2006). Single molecular chain geometry of konjac glucomannan as a high quality dietary fiber in East Asia. Food research international, 39(2), 127-132.

Li, Z., Su, Y., Haq, M. A., Xie, B., y Wang, D. (2016). Konjac glucomannan/polyacrylamide bicomponent hydrogels: Self-healing originating from semi-interpenetrating network. Polymer, 103, 146-151.

Liu, P. (1998). Research and utilization of Amorphophallus in China. Acta Bot. Yunn. Suppl., 10, 48-61.

LIU, T., WANG, Y., XIA, J., y LI, B. (2005). Influence Of Purification Method On The Structure And Properties Of Konjac Glucomannan [J]. Chemistry & Industry of Forest Products, 3, 19.

Mackie, I. M. (1993). The effects of freezing on flesh proteins. Food Reviews International, 9(4), 575-610.

Maekaji, K. (1978). A method for measurement and kinetic analysis of the gelation process of konjac mannan. J. Agric. Food Chem., 52(6), 251-257.

Maekaji, Kenji. (1974). The mechanism of gelation of konjac mannan. Agricultural and Biological Chemistry, 38(2), 315-321.

Malkin, A. Y., y Isayev, A. I. (2017). Rheology: concepts, methods, and applications. Elsevier.

Martino, F., Puddu, P. E., Pannarale, G., Colantoni, C., Martino, E., Niglio, T., Barillà, F. (2013). Low dose chromium-polynicotinate or policosanol is effective in hypercholesterolemic children only in combination with glucomannan. Atherosclerosis, 228(1), 198-202.

McGuire, R. G. (1992). Reporting of objective color measurements. HortScience, 27(12), 1254-1255.

Means, W. J., y Schmidt, G. R. (1986). Algin/calcium gel as a raw and cooked binder in structured beef steaks. Journal of Food Science, 51(1), 60-65.

Means, W. J., y Schmidt, G. R. (1987). Restructuring fresh meat without the use of salt or phosphate. Advances in meat research (USA).

Melgarejo, I., y Maury, M. (2002). Elaboración de hamburguesa a partir de Prochylodus nigricans boquichico. Revista amazónica de investigación alimentaria, 2(1), 79-87.

Menéndez, O., Rawel, H., Schwarzenbolz, U., y Henle, T. (2006). Structural changes of microbial transglutaminase during thermal and high-pressure treatment. Journal of agricultural and food chemistry, 54(5), 1716-1721.

Mezger, T. G. (2006). The rheology handbook: for users of rotational and oscillatory rheometers. Vincentz Network GmbH & Co KG.

Milani, J., y Maleki, G. (2012). Hydrocolloids in food industry. En Food industrial processes-methods and equipment. IntechOpen.

Min, B., y Green, B. W. (2008). Use of microbial transglutaminase and nonmeat proteins to improve functional properties of low NaCl, phosphate-free patties made from channel catfish (Ictalurus punctatus) belly flap meat. Journal of food science, 73(5), E218-E226.

Miyoshi, E., Takaya, T., y Nishinari, K. (1996). Rheological and thermal studies of gel-sol transition in gellan gum aqueous solutions. Carbohydrate Polymers, 30(2-3), 109-119.

Moore, W. J. (1978). Química Física (U. S. A. De Ediciones, Ed.). Bilbao.

Moreno Conde, H. M., Herranz, B., Borderías, A. J., y Tovar, C. A. (2016). Effect of high pressure treatment on the structural, mechanical and rheological properties of glucomannan gels. Food Hydrocolloids, 60, 437-444.

Moreno, H. M., Bargiela, V., Tovar, C. A., Cando, D., Borderias, A. J., y Herranz, B. (2015). High pressure applied to frozen flying fish (Parexocoetus brachyterus) surimi: Effect on physicochemical and rheological properties of gels. Food Hydrocolloids, 48, 127-134.

Moreno, H. M, Carballo, J., y Borderías, J. (2010). Gelation of fish muscle using microbial transglutaminase and the effect of sodium chloride and pH levels. Journal of muscle foods, 21(3), 433-450.

Moreno, H.M., Herranz, B., Pérez-Mateos, M., Sánchez-Alonso, I., y Borderías, A. J. (2016). New Alternatives in Seafood Restructured Products. Critical Reviews in Food Science and Nutrition,

56(2), 237-248.

Moreno, H.M, Carballo, J., y Borderías, A. J. (2008). Influence of alginate and microbial transglutaminase as binding ingredients on restructured fish muscle processed at low temperature. Journal of the Science of Food and Agriculture, 88(9), 1529-1536.

Moreno, H.M, Carballo, J., y Javier Borderias, A. (2009). Study of two different cold restructuring processes using two different qualities of hake (Merluccius capensis) muscle, with addition of microbial transglutaminase. Journal of the Science of Food and Agriculture, 89(8), 1346-1351.

Moreno, H.M, Cardoso, C., Solas, M. T., y Borderías, A. J. (2009). Improvement of cold and thermally induced gelation of giant squid (Dosidicus gigas) surimi. Journal of Aquatic Food Product Technology, 18(4), 312-330.

Mozaffarian, D., y Rimm, E. B. (2006). Fish intake, contaminants, and human health evaluating the risks and the benefits. Journal of the American Medical Association, 296(15), 1885-1899.

Nie, H., Shen, X., Zhou, Z., Jiang, Q., Chen, Y., Xie, A., … Han, C. C. (2011). Electrospinning and characterization of konjac glucomannan/chitosan nanofibrous scaffolds favoring the growth of bone mesenchymal stem cells. Carbohydrate polymers, 85(3), 681-686.

Nishinari, K., Williams, P. A., y Phillips, G. O. (1992). Review of the physico-chemical characteristics and properties of konjac mannan. Food hydrocolloids, 6(2), 199-222.

Nishinari, K., y Zhang, H. (2004). Recent advances in the understanding of heat set gelling polysaccharides. Trends in Food Science & Technology, 15(6), 305-312.

Niwa, T., Etoh, H., Shimizu, A., y SHIMIZU, Y. (2000). Cis-N-(p-coumaroyl) serotonin from konnyaku, Amorphophallus konjac K. Koch. Bioscience, biotechnology, and biochemistry, 64(10), 2269-2271.

Nussinovitch, A. (2004, enero 20). Encapsulating liquid with hydrocolloid membrane stable from about-20 to 90 degrees C without bursting. Google Patents.

Nussinovitch, A. (2009). Biopolymer films and composite coatings. En Modern biopolymer science (pp. 295-326). Elsevier.

Onitake, T., Ueno, Y., Tanaka, S., Sagami, S., Hayashi, R., Nagai, K., … Chayama, K. (2015). Pulverized konjac glucomannan ameliorates oxazolone-induced colitis in mice. European journal of nutrition, 54(6), 959-969.

Ordoñez, P. J., & De la Hoz, P. L. (1999). Carnes, pescados y huevos. Tratado de nutrición. Ed. Díaz de Santos.

Ordóñez, J. A., Cambero, M. I., Fernández, L., García, M. L., García de Fernando, G., De la Oz, L., y Selgas, M. D. (1998). Productos derivados de la pesca. Tecnologías de los alimentos, 2, 304-340.

Park, C. K., Noh, M. H., y Park, T. H. (2005). Rheological properties of cementitious materials containing mineral admixtures. Cement and concrete research, 35(5), 842-849.

Park, J. W. (1994). Functional Protein Additives in Surimi Gels. Journal of Food Science, 59(3), 525-527.

Park, J., y Morrissey, M. T. (1994). The need for developing uniform surimi standards. Quality control amd quality assurance for seafood, Newport, OR(USA), 16-18 May 1993, 1994, 169.

Park, J. W. (2000). Ingredient technology and formulation development. Food science and technology-new york-marcel dekker-, 343-392.

Peet, M., y Stokes, C. (2005). Omega-3 fatty acids in the treatment of psychiatric disorders. Drugs, 65(8), 1051-1059.

Pereira, M. A., O'Reilly, E., Augustsson, K., Fraser, G. E., Goldbourt, U., Heitmann, B. L., Ascherio, A. (2004). Dietary Fiber and Risk of Coronary Heart Disease: A Pooled Analysis of Cohort Studies. Archives of Internal Medicine, 164(4), 370-376.

Pérez-Mateos, M., Lourenço, H., Montero, P., y Borderías, A. J. (2002). Rheological and Biochemical Characteristics of High-Pressure- and Heat-Induced Gels from Blue Whiting (Micromesistius poutassou) Muscle Proteins . Journal of Agricultural and Food Chemistry, 45(1), 44-49.

Pérez-Mateos, Miriam. (1998). Estudio de hidrocoloides en la gelificación del músculo de bacaladilla (Micromesistius poutassou, Risso) inducida térmicamente y por alta presión.

Pieniak, Z., Verbeke, W., Scholderer, J., Brunsø, K., y Olsen, S. O. (2007). European consumers' use of and trust in information sources about fish. Food Quality and Preference, 18(8), 1050-1063.

Pilkington, S. M., Watson, R. E. B., Nicolaou, A., y Rhodes, L. E. (2011). Omega-3 polyunsaturated fatty acids: Photoprotective macronutrients. Experimental Dermatology, 20(7), 537-543.

Pons, M., y Fiszman, S. M. (1996). Instrumental texture profile analysis with particular reference to gelled systems. Journal of Texture Studies, 27(6), 597-624.

Ramirez, J., Uresti, R., Téllez, S., y Vázquez, M. (2002). Using salt and microbial transglutaminase as binding agents in restructured fish products resembling hams. Journal of Food Science, 67(5), 1778-1784.

Ramírez, J A, García-Carreño, E. L., Morales, O. G., y Sánchez, A. (2002). Inhibition of modori-associated proteinases by legume seed extracts in surimi production. Journal of Food Science, 67(2), 578-581.

Ramírez, José A., Uresti, R. M., Velazquez, G., y Vazquez, M. (2011). Food hydrocolloids as additives to improve the mechanical and functional properties of fish products: A review. Food Hydrocolloids, 25(8), 1842-1852.

Rao, M. A. (2014). Rheological behavior of processed fluid and semisolid foods. En Rheology of Fluid, Semisolid, and Solid Foods (pp. 231-329). Springer.

Rao, M. A. A. (2010). Rheology of fluid and semisolid foods: principles and applications. Springer Science & Business Media.

Ren, W., Zhang, A., Qin, S., y Li, Z. (2016). Synthesis and evaluation of a novel cationic konjac glucomannan-based flocculant. Carbohydrate polymers, 144, 238-244.

Resurreccion, A. V. A. (2004). Sensory aspects of consumer choices for meat and meat products. Meat Science, 66(1), 11-20.

Rohm, H. (1990). Consumer awareness of food texture in Austria. Journal of Texture Studies, 21(3), 363-374.

Rosell, M., Wesley, A. M., Rydin, K., Klareskog, L., y Alfredsson, L. (2009). Dietary fish and fish oil and the risk of rheumatoid arthritis. Epidemiology, 896-901.

Ruxton, C. H. S., Reed, S. C., Simpson, M. J. A., y Millington, K. J. (2004). The health benefits of omega-3 polyunsaturated fatty acids: A review of the evidence. Journal of Human Nutrition and Dietetics, 17(5), 449-459.

Sahin, S., y Sumnu, S. G. (2006). Physical properties of foods. Springer Science & Business Media.

Sánchez-Alonso, I., y Borderías, A. J. (2008). Technological effect of red grape antioxidant dietary fibre added to minced fish muscle. International Journal of Food Science and Technology, 43(6), 1009-1018.

Sánchez-Alonso, I., Haji-Maleki, R., y Borderias, A. J. (2007). Wheat fiber as a functional ingredient in restructured fish products. Food Chemistry, 100(3), 1037-1043..

Sanchez-Gonzalez, I., Carmona, P., Moreno, P., Borderías, J., Sanchez-Alonso, I., Rodríguez-Casado, A., y Careche, M. (2008). Protein and water structural changes in fish surimi during gelation as revealed by isotopic H/D exchange and Raman spectroscopy. Food Chemistry, 106(1), 56-64.

Sánchez Alonso, I., Pérez Mateos, M., y Borderías, A. J. (2004). Incorporación de fibra dietética a productos pesqueros reestructurados: una posibilidad.

Sandoval, J. L. S. (2015). Agente de carga a base de konjac y partículas de hidrogel como nuevos sistemas de incorporación de aceites en productos cárnicos. Universidad Complutense de Madrid.

Schärer, M. R., y Fenton, A. (1998). Food and Material Culture: Proceedings of the Fourth Symposium of the International Commission for Research Into European Food History. John Donald.

Seki, N., Nozawa, H., y Ni, S. (1998). Effect of transglutaminase on the gelation of heat-denatured surimi. Fisheries science, 64(6), 959-963.

Serrano, A., Cofrades, S., y Jiménez Colmenero, F. (2004). Transglutaminase as binding agent in fresh restructured beef steak with added walnuts. Food Chemistry, 85(3), 423-429.

Sevag, M. G. (1934). Eine neue physikalische Enteiweißungsmethode zur Darstellung biologisch wirksamer Substanzen. Biochem. z, 273(419), 102.

Shah, B. R., Li, B., Wang, L., Liu, S., Li, Y., Wei, X., … Zhenshun, L. (2015). Health benefits of konjac glucomannan with special focus on diabetes. Bioactive Carbohydrates and Dietary Fibre, 5(2), 179-187.

Shahidi, F., y Ambigaipalan, P. (2015). Novel functional food ingredients from marine sources. Current Opinion in Food Science, 2, 123-129..

Shimahara, H., Suzuki, H., Sugiyama, N., y Nisizawa, K. (1975a). Isolation and characterization of oligosaccharides from an enzymic hydrolysate of konjac glucomannan. Agricultural and Biological Chemistry, 39(2), 293-299.

Shimahara, H., Suzuki, H., Sugiyama, N., y Nisizawa, K. (1975b). Partial purification of β-mannanases from the konjac tubers and their substrate specificity in relation to the structure of konjac glucomannan. Agricultural and biological Chemistry, 39(2), 301-312.

Shinzato, C., Broussalis, A. M., y Ferraro, G. E. (1996). Glucomanano: Un aporte a su control de calidad. Revista de la Asociación Argentina de Farmacia y Bioquímica Industrial (SAFYB), 35, 26-31.

Sidhu, K. S. (2003). Health benefits and potential risks related to consumption of fish or fish oil. Regulatory Toxicology and Pharmacology, 38(3), 336-344.

Siro, I., Kápolna, E., Kápolna, B., y Lugasi, A. (2008). Functional food. Product development, marketing and consumer acceptance—A review. Appetite, 51(3), 456-467.

Smith, K. M., y Sahyoun, N. R. (2005). Fish consumption: Recommendations versus advisories, can they be reconciled? Nutrition Reviews, 63(2), 39-46.

Steffe, J. F. (1996). Rheological methods in food process engineering. Freeman press.

Sun, X. D. (2009). Utilization of restructuring technology in the production of meat products: a review. CyTA - Journal of Food, 7(2), 153-162

Suzuki, T. (1987). Tecnologia de las proteínas de pescado y krill. Acribia Zaragoza.

Szczesniak, A. S. (2002). Texture is a sensory property. Food quality and preference, 13(4), 215-225.

Szymanski, K. M., Wheeler, D. C., y Mucci, L. A. (2010). Fish consumption and prostate cancer risk: A review and meta-analysis. American Journal of Clinical Nutrition, 92(5), 1223-1233..

Tabilo-Munizaga, G., y Barbosa-Cánovas, G. V. (2005). Rheology for the food industry. Journal of food engineering, 67(1-2), 147-156.

Takigami, S. (2009). Konjac mannan. En Handbook of hydrocolloids (pp. 889-901). Elsevier.

Tarrant, P. V. (1998). Some recent advances and future priorities in research for the meat industry. Meat science, 49, S1-S16.

Tester, R. F., y Al-Ghazzewi, F. H. (2016). Beneficial health characteristics of native and hydrolysed konjac (Amorphophallus konjac) glucomannan. Journal of the Science of Food and Agriculture, 96(10), 3283-3291.

Tolstoguzov, V. B. (1998). Functional properties of protein-polysaccharide. Functional properties of food macromolecules, 252-275.

Torres, J. D., González-Morelo, K., y Acevedo, D. (2015). Análisis del perfil de textura en frutas, productos cárnicos y quesos. Revista RECITEIA: Revisiones de la Ciencia, Tecnología e Ingeniería de los Alimentos, 14(2), 63-75.

Tovar, C. A., Piñeiro, L., y Herranz, B. (2017). Comparative study of pH and high pressure treatment on the viscoelastic properties of glucomannan gels after long-term frozen storage. Food hydrocolloids, 72, 346-349.

Triki, M., Herrero, A. M., Jiménez-Colmenero, F., y Ruiz-Capillas, C. (2013). Effect of preformed konjac gels, with and without olive oil, on the technological attributes and storage stability of merguez sausage. Meat science, 93(3), 351-360.

Tunick, M. H. (2000). Rheology of dairy foods that gel, stretch, and fracture. Journal of Dairy Science, 83(8), 1892-1898.

Tur, J. A., Bibiloni, M. M., Sureda, A., y Pons, A. (2012). Dietary sources of omega 3 fatty acids: Public health risks and benefits. British Journal of Nutrition, 107(SUPPL. 2).

Tye, R. J. (1991). Konjac flour: properties and applications. Food technology (USA).

Uresti, R. M., Velazquez, G., Ramírez, J. A., Vázquez, M., y Torres, J. A. (2004). Effect of high-pressure treatments on mechanical and functional properties of restructured products from arrowtooth flounder (Atheresthes stomias). Journal of the Science of Food and Agriculture, 84(13), 1741-1749.

Usda, N. (2004). The PLANTS database, version 3.5. National Plant Data Center, Baton Rouge, LA, 70874-74490.

Varela, P. (2007). Desarrollos metodológicos para determinar el carácter crujiente como factor primario de calidad en alimentos de distintos orígenes. Universidad Politécnica de Valencia. Departamento de Tecnología de Alimentos.

Velankar, N. K., y Govindan, T. K. (1958). A preliminary study of the distribution of non-protein nitrogen in some marine fishes and invertebrates. Proceedings of the Indian Academy of Sciences-Section B, 47(4), 202-209. Springer.

Wang, B., Liao, L., Huang, Q., y Cheng, Y. (2012). Adsorption behaviors of benzonic acid by carboxyl methyl konjac glucomannan gel micropheres cross-linked with Fe 3+. Journal of Chemical and Engineering Data, 57(1), 72-77.

Wang, L., Jiang, Y., Lin, Y., Pang, J., y Liu, X. Y. (2016). Rheological properties and formation mechanism of DC electric fields induced konjac glucomannan-tungsten gels. Carbohydrate Polymers , 142, 293-299.

Wang, L., Zhuang, Y., Li, J., Pang, J., y Liu, X. (2016). The textural properties and microstructure of konjac glucomannan - Tungsten gels induced by DC electric fields. Food Chemistry, 212, 256-263..

Weichselbaum, E., Coe, S., Buttriss, J., y Stanner, S. (2013). Fish in the diet: A review. Nutrition Bulletin, 38(2), 128-177.

Wierbicki, E., Kunkle, L. E., y Deatherage, F. E. (1957). Changes in the water-holding capacity and cationic shifts during the heating and freezing and thawing of meat as revealed by a simple centrifugal method for measuring shrinkage. Food Technology, 11(2), 69-73.

Williams, M. A. K., Foster, T. J., Martin, D. R., Norton, I. T., Yoshimura, M., y Nishinari, K. (2000). A molecular description of the gelation mechanism of konjac mannan. Biomacromolecules, 1(3), 440-450.

Williams, P. A., Clegg, S. M., Day, D. H., Phillips, G. O., y Nishinari, K. (2010). Mixed Gels Formed with Konjac Mannan and Xanthan Gum. En Food Polymers, Gels and Colloids. pp. 339-348.

Wismer-Pedersen, J. (1994). Química de los tejidos animales. Ciencia de la carne y los productos cárnicos, 125-149.

Wu, L., Lin, X., Wu, J., Zhou, X., y Luo, X. (2016). Adsorption behavior of carboxymethyl konjac glucomannan microspheres for fluoride from aqueous solution. RSC Advances, 6(92), 89417-89429.

Xiao, C., Gao, S., Wang, H., y Zhang, L. (2000). Blend films from chitosan and konjac glucomannan solutions. Journal of Applied Polymer Science, 76(4), 509-515.

Yang, D., Yuan, Y., Wang, L., Wang, X., Mu, R., Pang, J., ... Zheng, Y. (2017). A review on konjac glucomannan gels: microstructure and application. International journal of molecular sciences, 18(11), 2250.

Yang, G., Huang, Q., Zhang, L., Zhou, J., y Gao, S. (2004). Miscibility and properties of blend materials from waterborne polyurethane and carboxymethyl konjac glucomannan. Journal of applied polymer science, 92(1), 77-83.

Yin, W., Zhang, H., Huang, L., y Nishinari, K. (2008). Effects of the lyotropic series salts on the gelation of konjac glucomannan in aqueous solutions. Carbohydrate polymers, 74(1), 68-78.

Yoshimura, M., y Nishinari, K. (1999). Dynamic viscoelastic study on the gelation of konjac glucomannan with different molecular weights. Food Hydrocolloids, 13(3), 227-233.

Zhang, C., y Yang, F. (2014). Konjac glucomannan, a promising polysaccharide for OCDDS. Carbohydrate polymers, 104, 175-181.

Zhang, T., Li, Z., Wang, Y., Xue, Y., y Xue, C. (2016). Effects of konjac glucomannan on heat-induced changes of physicochemical and structural properties of surimi gels. Food Research International, 83, 152-161.

Zhang, Y., Xie, B., y Gan, X. (2005). Advance in the applications of konjac glucomannan and its derivatives. Carbohydrate Polymers, 60(1), 27-31.

Zhu, F. (2018). Modifications of konjac glucomannan for diverse applications. Food chemistry, 256, 419-426.

Zia, F., Zia, K. M., Zuber, M., Ahmad, H. B., y Muneer, M. (2016). Glucomannan based polyurethanes: a critical short review of recent advances and future perspectives. International journal of biological macromolecules, 87, 229-236.

II. OBJETIVOS

El objetivo principal de esta tesis es el **diseño y la obtención de productos reestructurados a base de músculo de pescado picado, con prácticamente nula funcionalidad tecnológica, utilizando glucomanano (GM) procedente de *Amorphophallus konjac* como agente gelificante**.

Para la consecución de este objetivo general, se establecieron los siguientes **objetivos específicos**:

- Obtener geles de glucomanano partiendo de dispersiones coloidales agua-glucomanano por desacetilación del glucomanano en medio básico, optimizando los parámetros tecnológicos.

- Incorporar al sistema agua-glucomanano el músculo de pescado picado, y estudiar los parámetros tecnológicos anteriormente optimizados en estos sistemas mixtos, para elaborar prototipos de reestructurados de pescado con textura, sabor y apariencia similares a productos pesqueros.

- Estudiar la influencia de métodos de conservación tradicionales (pasteurización, refrigeración y congelación) sobre los parámetros fisicoquímicos, reológicos y sensoriales de los prototipos de reestructurado de pescado.

- Desarrollar nuevos productos reestructurados a partir de subproductos de pescado picado y glucomanano.

III. ESTRUCTURA DE LA TESIS

La presente tesis doctoral se enmarca dentro del proyecto del Ministerio de Ciencia, Innovación y Universidades, titulado "*Aplicación de konjac glucomanano en procesos de reestructuración de músculo de pescado*" (AGL2008-04892-C03-C3). La tesis se estructura en tres capítulos. El primero se centra principalmente en el estudio estructural y caracterización reológica de los geles de glucomanano. El segundo, se basa en la obtención de un prototipo de reestructurado de pescado a partir de músculo de pescado picado, utilizando la capacidad de gelificación del glucomanano, y además se evalúan los métodos tradicionales de conservación: refrigeración, congelación y pasteurización, en las propiedades físico-químicas y estructurales del reestructurado. El tercer capítulo se corresponde con el desarrollo de nuevos productos pesqueros reestructurados, elaborados en base a la propiedad de gelificación termoestable del glucomanano. Se citan los artículos de investigación generados en la temática de los capítulos, y la patente obtenida como consecuencia del desarrollo tecnológico de los reestructurados de pescado con glucomanano. Seguidamente, se presentan los contenidos íntegros de dichas publicaciones y patente, y a continuación, el resumen y la discusión, donde se analizan los aspectos más destacados de la investigación. Por último, se enumeran las conclusiones de la tesis doctoral.

CAPÍTULO 1:

Solo-de-Zaldívar, B., Herranz, B. and Borderias, J. A. (2012). ***First steps in using glucomannan to make thermostable gels for potencial use in mince fish restructuration.*** International Journal of Food Engineering, 8 (1), 1556-3758. (DOI: 10.1515/1556-3758.2407).

Herranz, B., Tovar, C. A., Solo-de-Zaldívar, B. and Borderias, A. J. (2012). ***Effect of alkalis on konjac glucomannan gels for use as potential gelling agents in restructured seafood products***. Food Hydrocolloids 27, 145-153. (DOI: 10.1016/j.foodhyd.2011.08.003).

Solo-de-Zaldívar, B., Tovar, C. A., Borderías, A. J. and Herranz, B. (2014*). **Effect of deacetylation on the glucomannan gelation process for making restructured seafood products***. Food Hydrocolloids, 35, 59-68. (DOI:10.1016/j.foodhyd.2013.04.009).

Herranz, B., Borderias, A. J., Solo-de-Zaldívar, B., Solas, M. T. and Tovar, C. A., (2012). ***Thermostability analyses of glucomannan gels. Concentration influence.*** Food Hydrocolloids, 29, 85-92. (DOI: 10.1016/j.foodhyd.2012.02.011).

Herranz, B., Borderias, A. J., Solo-de-Zaldívar, B., and Tovar, C. A. (2013). ***Influence of alkali and temperature on glucomannan gels at high concentration.*** LWT-Food Science and Technology, 51, 500-506. (DOI: 10.1016/j.lwt.2012.11.023).

CAPÍTULO 2:

Herranz, B., Solo-de-Zaldívar, B. and Borderias, J. A. (2013). ***Obtaining a restructured seafood product from non- functional fish muscle by glucomannan addition: first steps.*** Journal of Aquatic Food Product Technology, 22(2), 201-208. (DOI: 10.1080/10498850.2011.632114).

Solo-de-Zaldívar, B., Herranz, B., Borderías, A. J. and Tovar, C. A., (2014). ***Effect of freezing and frozen storage on restructured fish prototypes made with glucomannan and fish mince.*** Food Hydrocolloids, 41, 233-240. (DOI: 10.1016/j.foodhyd.2014.04.019).

Solo-de-Zaldívar, Tovar, C. A., B., Borderías, A. J., and Herranz, B. (2015*). **Pasteurization and chilled storage of restructured fish muscle products based on glucomannan gelation***. Food Hydrocolloids, 43, 418-426. (DOI: 10.1016/j.foodhyd.2014.06.016).

CAPÍTULO 3:

CSIC (2011). Borderıas, A. J., Solo de Zaldívar, B., and Herranz, B. ***Producto alimenticio a base de pescado y glucomanano, y procedimiento de obtención***. Patente. ES 2 363 291 (28/07/2011).

***Nuevos productos reestructurados pesqueros a partir de músculo de pescado picado y glucomanano. Obtención de análogos de pescado*.**

CAPÍTULO 1

OPTIMIZACIÓN DE LA METODOLOGÍA PARA LA OBTENCION DE LOS GELES DE GLUCOMANANO. CARACTERIZACIÓN REOLÓGICA Y ESTRUCTURAL

RESEARCH NOTE

FIRST STEPS IN USING GLUCOMANNAN TO MAKE THERMOSTABLE GELS FOR POTENCIAL USE IN MINCE FISH RESTRUCTURATION

Solo-de-Zaldívar, B., Herranz, B. and Borderias, J. A.

Instituto de Ciencia y Tecnología de los Alimentos y Nutrición (ICTAN-CSIC), calle José Antonio Nováis, 10, 28040 Madrid, Spain

International Journal of Food Engineering, 8(1), 1556-3758.
(DOI: 10.1515/1556-3758.2407).

ABSTRACT

A methodology for making thermostable konjac glucomannan (KGM) g
examined with a view to eventual use with fish mince to make restructured pro
gelation method was developed with 3 and 6 % (w/v) aqueous glucomannan dis
(AGD) by adding alkali v/v (NaOH or KOH) up to pH 11.8- 12 and then allowing it to set (1 h at 30 °C and 4 h at 5 °C). Both 3 and 6 % are suitable concentrations and KOH and NaOH (at 0.6 and 1M) are the most suitable alkalis for deacetylation of the glucomannan at pH 11.8- 12.0.

KEYWORDS: Glucomannan; Thermostable gels; Deacetylation

1. INTRODUCTION

When fish mince is used as a raw material it sometimes lacks functionality as a result of prior processing or because it contains too much fat. It is not possible to make fibre- or myotome-like structures using the conventional restructuring procedure. To avoid these problems, the technological properties of Konjac glucomannan (KGM) offer an interesting, new and unexplored possibility for restructuring this type of products. One way to achieve this would be to make KGM gels that can retain the unfunctional minced muscle. This is an unexploited technology and only a very few papers have been published reporting addition of KGM in powder form as an ingredient (Park, 1996; Xiong et al. , 2009; Iglesias-Otero, Borderias & Tovar, 2010).

KGM is a neutral polysaccharide derived from the tuber of *Amorphophallus konjac* C. (Nishinari, Williams & Phillips, 1992) that has long been used as a thickener and gelling agent in traditional Asian foods and is generally recognized as safe (GRAS) (Nishinari et al., 1992; Thomas, 1997; Chua, et al., 2010). It is composed of β-(1 - 4) linked D-mannose and D-glucose in a molar ratio of 1.6:1 (Kato and Matsuda, 1969; Maeda et al., 1980; Katsuraya, et al., 2003) and some branches linked to this backbone, although the exact branched position is still debatable (Nishinari et al., 1992; Katsuraya et al., 2003). KGM backbone has a low proportion of acetyl groups (approximately 1 acetyl group per 19 residues) (Kato & Matsuda, 1969; Maekaji, 1974; Maeda et al., 1980), which is assumed to be an important factor promoting the solubility of KGM in water. It is well known that KGM generally forms a thermally irreversible gel in the presence of an alkaline coagulant (Jacon et al., 1993;

Williams et al., 1999; Zhang et al., 2001; Huang et al., 2002). The effect of the addition of alkali is to facilitate deacetylation of the KGM chains, although the mechanism is not fully understood (Jacon et al., 1993; Yoshimura and Nishinari, 1999;Williams et al., 2000; Huang et al., 2002; Zhang et al., 2010).

The aim of this paper is to study the initial steps in the use of KGM to make thermo-irreversible gels strong enough to be used eventually in minced fish restructuring. The paper also examines solubilization conditions, concentrations and types of alkalis used for the deacetylation step in order to make suitable gels.

2. MATERIAL AND METHODS

Purified glucomannan from konjac (KGM ≥ 99%) was purchased from Guinama, Valencia. All chemical reagents were supplied by Panreac, Química S.A., Barcelona and were of reagent grade.

2.1 Preparation of glucomannan gels

Aqueous glucomannan dispersions (AGD) were prepared at 1, 3 and 6% (w/v) total polysaccharide concentration using distilled warm water at 60 °C in a Stephan vacuum cutter machine (Stephan, Universal Machine UC12, Stephan u. Söhne GmbH & Co., Hameln, Germany) for 30 min and then left to cool at room temperature. Then, different alkali aqueous solutions were added to these dispersions for the deacetylation step: NaOH, KOH or Na_2CO_3 (Panreac Química, S. A., Barcelona, Spain) at three concentrations: 0.2M, 0.6M and 1M each one until the pH reached 11.8- 12.0 (in the present work the pH was determined by FT-IR as described below). The samples were placed in Petri dishes (1.5 cm thick and 90 cm diameter), then set at 30 °C for 1 h and then at 5°C for 4 h. The gels were neutralized by placing them in a 0.2M citrate-phosphate buffer at pH 5 (gel: buffer proportion 1:10) for 20 h. In this way, heat-stable gels were produced with an approximate pH of 6.5-7.

2.2 Analyses

FT-IR spectroscopy analysis:

AGD (3%) was mixed with different amounts of a solution of NaOH 1M to achieve different pHs (6.3, 7.5, 8.2, 10, 11.2 and 12) and analysed by FT-IR. The FT-IR

spectrophotometer used to record spectra was a FT-IR / FT-NIR, Spectrum-400, from Perkin-Elmer (USA). Samples were freeze-dried and dispersed in Fluorolube, which only fits absorption bands in IR below 1500 cm^{-1} and therefore does not interfere in the observation of the acetyl bands. The dispersion was carried out in the agate mortar. Once a very fine powder had been obtained, a small quantity was mixed with CaF_2 crystals, they were mounted on the supports and transmission measured in the FTIR. In all cases, IR spectrum were recorded by accumulation of at least 32 scans, with a resolution of 2 cm^{-1} in the 4000 - 500 cm^{-1} range. Measurements were carried out in triplicate. The spectrum was normalized to the acetyl band area. In this way the pH of the sample was associated with the relative intensity of the acetyl bands and hence to the degree of acetylation of the glucomannan. The spectral data were processed with the Grams /AI (Thermo Electron Corporation, Waltham, MA) software, which includes baseline correction, smoothing (with a nine-point Savitsky-Golay function) to reduce the noise, and band area measurement.

pH:

The pH was determined in quadruplicate using a pH meter (Thermo-Orion 720, Spain) with a specific electrode for direct measuring on the mixture (Thermo-Orion Sure-flow 9165BNWP, Spain).

Water Binding Capacity (WBC):

Samples were cut into small pieces, weighed (approx. 2 g) and then placed in a centrifuge tube (10 mm diameter) with filter paper as absorber (Whatman nº 1). Samples were then centrifuged in a Heraeus Multifuge 3 Plus centrifuge (USA) for 10 min at 3000×g at room temperature. Water Binding Capacity (WBC) was expressed as % water retained per 100 g water present in the sample prior to centrifuging. Measurements were carried out in triplicate.

Colour:

The surface colour of the samples was evaluated on a colorimeter (Minolta. CR-400 Konica-Minolta, Japan) (D65/2°), using CIELab scale (Lightness, L*; redness, a* and yellowness, b*). Whiteness index was determined using the following formula: 100 -[(100-

$L^*)^2 + a^{*2}b^{*2}]^{1/2}$ (Park, 1995). Colour determinations were carried out after neutralizing time. Determinations were performed on five points per formulation.

Puncture test:

Gels (cylindrical samples of diameter 3 cm x height 3.5 cm) were penetrated to breaking-point using a TA-XT plus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) equipped with a rod with spherical tip (5 mm diameter (P/5S)). Cross-head speed was 1mm/s and a 5 Kg load-cell connected to the crosshead of the Texture Analyzer was used. Breaking force [N] and breaking deformation [mm] were determined in the force-deformation curves. Puncture tests were carried out on gels kept at room temperature. All determinations were carried out at least in quadruplicate.

Statistical analysis:

Analysis of variance (One–way ANOVA) was carried out to evaluate the statistical significance ($p < 0.05$). Statistical analysis was performed using Statgraphics Plus version 5.0.

3. RESULTS AND DISCUSSION

3.1 KGM dispersion conditions

First at all it is very important to get a homogeneous solution of glucomannan. Glucomannan forms highly viscous solutions when dissolved in water (Shinzato et al., 1996; Kök et al., 2009) but it is not always easy to obtain a homogeneous dispersion. Hence, different concentrations of glucomannan (1-6%) were studied at various temperatures within the range reported in the literature (50-100 °C) and times (30- 90 min) in order to obtain a homogeneous solution without lump formation at the lowest temperature and in the shortest time possible. The best dispersion conditions were 30 min at 60 °C with continuous stirring in a homogenizer.

After that, the next step was to determine the pH level that would assure complete deacetylation so as to convert the glucomannan solution into a thermo-stable gel.

3.2 Determining the deacetylation pH by analysis of the acetyl bands measured by FTIR

To determine the pH level that would assure deacetylation of the KGM, FTIR analysis was performed in AGD at different pHs. A solution of 1M NaOH was added to different 3% AGD aliquots to obtain a range of diverse pHs (6.3, 7.5, 8.2, 10, 11.2 and 12). This range of pH values was based on the one described by Thomas (1997), who reported pH values of 9-10, and Kohyama and Nishinari (1990), who reported 11.3- 12.6 for KGM gelation. In this case other lower pHs were checked to observe the gradual disappearance of acetyl groups as a function of pH.

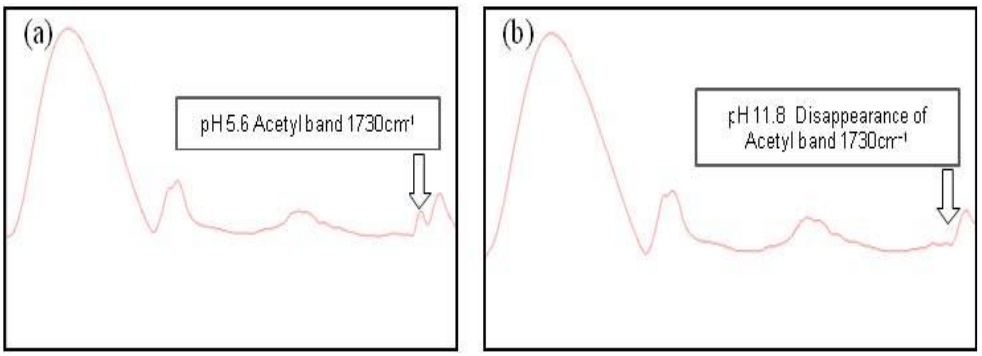

Fig. 1. FTIR spectra. FT-IR spectrum of glucomannan at: (a) pH native (pH= 5.6) with acetyl band at 1730 cm^{-1} and (b) pH of gelation (pH= 11.8) without acetyl band.

As shown in the spectrum in ***Figure 1a,*** the band at 1730 cm^{-1} is visible in glucomannan dispersions at pH = 5.6 (before alkali addition) and practically disappears when the dispersion reaches a pH value of 11.8 (***Figure 1b***). These results agree with the findings of other authors (Maekaji, 1974; Jacon et al. 1993; Zhang et al, 2001), who assigned this peak to the C=O group. One may therefore assume that this peak corresponds to the acetyl group and its removal from the KGM backbone initiates the polymer chain interactions to form a gel (Zhang et al., 2001). ***Figure 2*** shows the regresion line of sample pH in relation to the normalized area of the above-mentioned band at 1730 cm^{-1}. This figure shows that the area of the mentioned band is nil from pH 11.8.

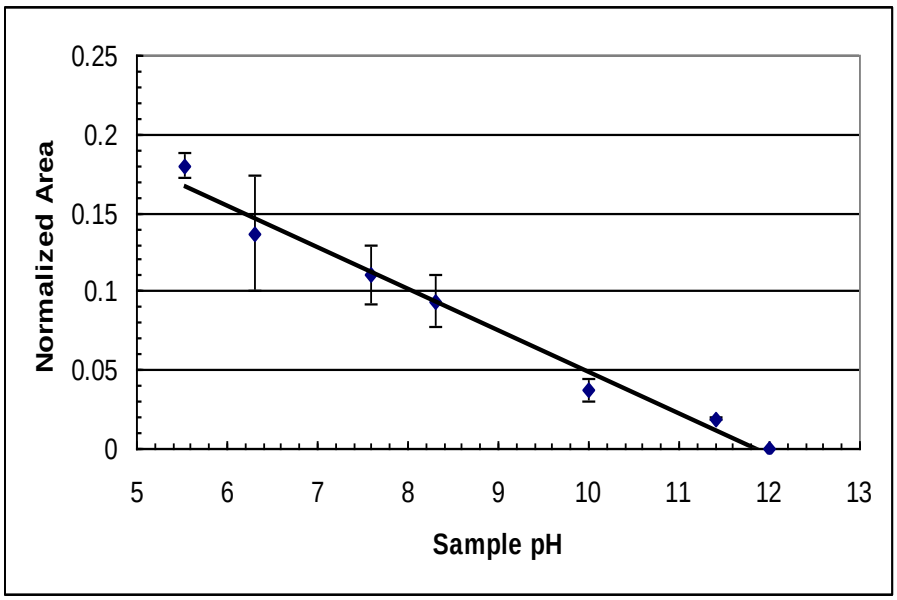

Figure 2. Lineal regression between pH and relative intensity of the acetyl bands.

3.3 Selection of type and concentration of alkali added for deacetylation

Various types of alkaline solutions at different concentrations (0.2, 0.6 and 1M) were added at 1, 3 and 6% (w/v) AGD until the pH reached 11.8- 12.0, to obtain a gel of a firmness and texture similar to that of whole fish muscle. As a guideline, various alkalis were selected (KOH, NaOH and Na_2CO_3) from Thomas (1997), which describes the relative gel strength of different glucomannan gels with different alkalis added at different pHs.

Table 1 shows the breaking force (N) and breaking deformation (mm) of these gels at 1 day of chilled storage. Glucomannan gels were more rigid when made at a concentration of 6% than 3% as shown by the significant differences ($p<0.05$) in breaking force values (*Table 1A*), but breaking deformation was similar in all gels (*Table 1B*). On the other hand, the concentration of alkali did not significantly influence the breaking force of gels with 6% glucomannan. Breaking force values were higher in glucomannan gels made at a concentration of 3% with NaOH at 1M than at 0.6M. Breaking deformation, was only affected by alkali concentration ($p<0.05$) at both glucomannan concentrations in gels made with KOH. Breaking deformation was higher at 0.6M than at 1M KOH concentration.

All gels were translucent with high whitness values except the ones made with Na_2CO_3, which had a light brown colour (*Table 2*). This high whiteness value is important because it can be transmited to the mince. European consumers consider whiteness a quality factor for white fish products (Ang, 1993).

Table 1. Breaking force (N) and breaking deformation (mm) in gels at 1, 3 and 6% of glucomannan (w/v) deacetylated with NaOH, KOH and Na_2CO_3 at different concentrations.

A.

	Breaking force (N)		
Concentration of AGD*	**1%**	**3%**	**6%**
Type of alkali & Concentration			
0.2M NaOH	NG**	NG**	NG**
0.6M NaOH	NG**	0.86 ± 0.20 d	4.1 ± 1.0 a
1M NaOH	NG**	1.37 ± 0.39 c	4.11 ± 0.73 a
0.2M KOH	NG**	NG	NG**
0.6M KOH	NG**	1.04 ± 0.16 c,d	3.50 ± 0.55 b
1M KOH	NG**	0.94 ± 0.10 c,d	3.7 ± 1.1 a,b
0.2M Na_2CO_3	NG**	NG**	NG**
0.6M Na_2CO_3	NG**	NG**	0.44 ± 0.03 d
1M Na_2CO_3	NG**	NG**	1.12 ± 0.29 c,d

*AGD: Aqueous glucomannan dispersion.
**NG: Not gel formation.
a-d Different letters indicate significant differences ($p < 0.05$) among gels.

B.

	Breaking deformation (mm)		
Concentration of AGD	**1%**	**3%**	**6%**
Type of alkali & concentration			
0.2M NaOH	NG**	NG**	NG**
0.6M NaOH	NG**	8.68 ± 0.81 e,f	9.4 ± 1.3 c,d,e
1M NaOH	NG**	8.9 ± 1.8 d,e,f	9.85 ± 0.79 b,c
0.2M KOH	NG**	NG**	NG**
0.6 KOH	NG**	9.49 ± 0.48 b,c,d	9.49 ± 0.70 b,c,d
1M KOH	NG**	8.33 ± 0.57 f	8.49 ± 0.34 f
0.2M Na_2CO_3	NG**	NG**	NG**
0.6M Na_2CO_3	NG**	NG**	10.80 ± 0.76 a,b
1M Na_2CO_3	NG**	NG**	11.3 ± 1.4 a

*AGD: Aqueous glucomannan dispersion.
**NG: Not gel formation.
a-f Different letters indicate significant differences ($p < 0.05$) among gels.

Table 2. Whiteness index of gels at 1, 3 and 6% of glucomannan (w/v) deacetylated with NaOH, KOH and Na_2CO_3 at different concentrations.

Concentration of AGD*	Whitenes Index		
Type of alkali & Concentration	**1%**	**3%**	**6%**
0.2M NaOH	NG**	NG**	NG**
0.6M NaOH	NG**	48.0 ± 1.6 c	52.13 ± 0.66 a
1M NaOH	NG**	44.95 ± 0.48 d	50.5 ± 1.3 a,b
0.2M KOH	NG**	NG**	NG**
0.6 M KOH	NG**	40.4 ± 1.7 f	49.2 ± 1.2 b,c
1M KOH	NG**	42.8 ± 1.6 e	50.89 ± 0.72 a
0.2M Na_2CO_3	NG**	NG**	NG**
0.6M Na_2CO_3	NG**	NG**	31.33 ± 0.95 g
1M Na_2CO_3	NG**	NG**	32.27 ± 0.81 g

*AGD: Aqueous glucomannan dispersion.
**NG: Not gel formation.
a-g Different letters indicate significant differences ($p < 0.05$) among gels.

Gels made with 3 and 6 % glucomannan deacetylated by 0.6 and 1 M solutions of NaOH and KOH were therefore chosen on the basis of their mechanical properties and colour. Gels made with Na_2CO_3 as the alkali were not suitable for our purpose because they were very soft at the same glucomannan concentration and also sticky, and their colour was too dark.

The water binding capacity (WBC) measured in gels made by alkalinization with KOH and NaOH is shown in ***Figure 3***. WBC values were around 70, except for gels containing 1M NaOH, where the value was almost 80%, and 1M KOH gels, whose values were slightly lower; however, there were no significant differences ($p<0.05$) among any of the gels. The extraordinarily high WBC of KGM has already been reported by some authors. One gram of KGM is reportedly able to absorb up to 200 ml of water (Maeda et al., 1980; Wen et al., 2008).

Briefly then, based on the power of different added alkalis to deacetylate glucomannan, KOH and NaOH are suitable gelling agents at concentrations of 0.6M and 1M to be used.

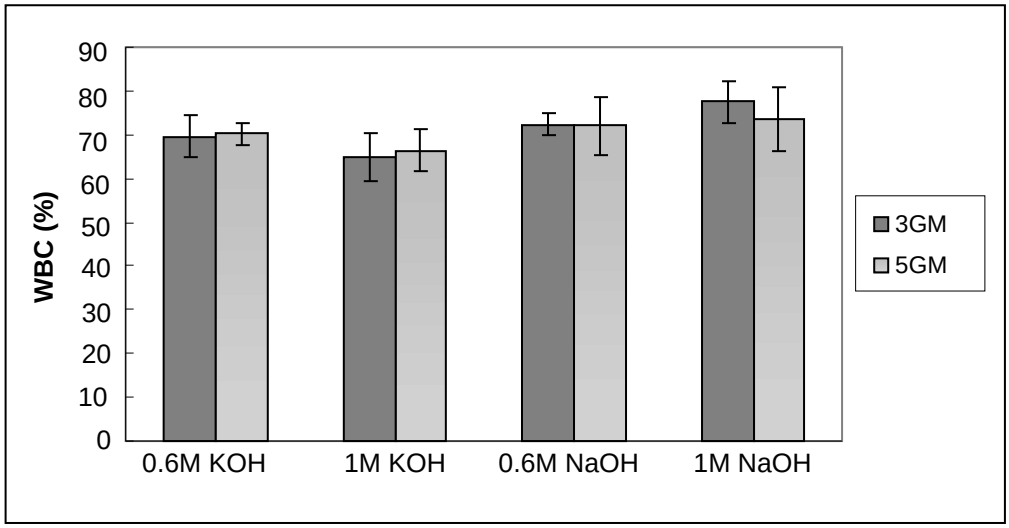

Figure 3. WBC (%) of the different gels elaborated with NaOH and KOH at 0.6N and 1N. 3GM= 3% glucomannan gels, 5GM= 5% glucomannan gels.

4. CONCLUSION

Aqueous dispersions of glucomannan at concentrations of 3 and 6 % made following the procedure reported above are suitable for deacetylation with 0.6M KOH and 1M NaOH at pH 11.8- 12.0. This produces thermostable gels with an appropiate texture for structural formation when used in combination with fish mince.

REFERENCES

Ang, J. F. (1993). Reduction of fat in fried batter coatings with powdered cellulose. Journal of the American Oil Chemists' Society, 70, 619-622.

Chua, M., Baldwin, T.C., Hocking, T. J., & Chan, K. (2010). Traditional uses and potential health of Amorphophallus konjac K. Koch ax N.E.Br. Journal of Ethnopharmacology, 12, 268-278.

Huang, L.,Takahashi, R., Kobayashi, S., Kawase, T., & Nishinari, K. (2002). Gelation behavior of native and acetylated konjac glucomannan. Biomacromolecules, 3,, 1296-1303.

Iglesias-Otero, M. A., Borderias, J., & Tovar, C. A. (2010). Use of konjac glucomannan as additive to reinforce the gels from low quality squid surimi. Journal of Food Engeenering, 101, 281- 288.

Jacon, S. A., Rao M. A., Cooley, H. J., & Walter, R. H. (1993). The isolation and characterization of a water extract of konjac flour gum. Carbohydrate Polymers, 20, 35-41.

Kato, K., & Matsuda, K. (1969). Studies on the chemical structure of konjac mannan. Agricultural and Biological Chemistry, 33, 1446-1453.

Katsuraya, K., Okuyama, K., Hatanaka, K., Oshima, R., Sato, T., & Matsuzaki K. (2003). Constitution of konjac glucomannan: chemical analysis and 13C NMR spectroscopy. Carbohydrate Polymers,

53, 183-189.

Kohyama, K., & Nishinari, K. (1990). Dependence of the specific volume of konjac glucomannan on pH. In G. O. Phillips and D. J. Wedlock & P. A. Williams (Eds.), Gums and Stabilisers for the Food Industry, 5, (p. 459). Oxford: IRL Press.

Kök, M. S., Abdelhameed, A. S., Ang, S., Morris, G. S., & Harding, S. E. (2009). A nobel global hydrodynamic analysis of the molecular flexibility of the dietary fibre polysaccharide konjac glucomannan. Food Hydrocolloids, 23, 1910–1917.

Maeda, M., Shimahara, H., & Sugiyama, N. (1980). Studies of mannan and related-compounds .5. detailed examination of the branched structure of konjac glucomannan. Agricultural and Biological Chemistry, 44, 245-252.

Maekaji, K. (1974). Mechanism of gelation of konjac mannan. Agricultural and Biological Chemistry, 38, 315-321.

Nishinari, K., Williams, P. A., & Phillips, G. O. (1992). Review of the physico-chemical characteristics and properties of konjac mannan. Food Hydrocolloids, 6, 199-222.

Park, J. W. (1995). Surimi gel colors as affected by moisture-content and physical conditions. Journal of Food Science, 60, 15-18.

Park, J. W. (1996). Temperature-tolerant fish protein gels using konjak flour. Journal of Muscle Food, 7, 165- 174.

Shinzato, C., Broussalis, A. M., & Ferraro, G. E. (1996). Glucomanano: Un aporte a su control de calidad. Revista de la Asociación Argentina de Farmacia y Bioquímica Industrial (SAFYB) 35, 26-31.

Thomas, W. R. (1997). Konjac gum. In Imeson A, (Ed.), Thickening and gelling agents for foods (pp.169-179). London: Blackie Academic & Professional.

Wen, X., Wang, T., Wang, Z. Y., Li L., & Zhao, C. S. (2008). Preparation of konjac glucomannan hydrogels as DNA-controlled release matrix. International Journal of Biological Macromolecules, 42, 256-263.

Williams, M. A., Foster, T. J., Martin, D. R., Norton, I. T., Yoshimura, M., & Nishinari, K. (2000). A molecular description of the gelation mechanism of konjac mannan. Biomacromolecules, 1, 440-450.

Xiong, G., Cheng, W., Ye, L., Du, X., Zhou, M., Lin, R., Geng, S., Geng, S., Chen, M., Corke, H., & Cai, Y. Z. (2009). Effect of konjac glucomannan on physicochemical properties of myofibrillar protein and surimi gels from grass carp (Ctenopharyngodon idella). Food chemistry, 116, 413- 418.

Yoshimura, M., & Nishinari, K. (1999). Dynamic viscoelastic study on the gelation of konjac glucomannan with different molecular weights. Food Hydrocolloids, 13, 227-233.

Zhang, F.Y., Zhou Y.M., Sun, Y.-Q., Ye, X.Y., & Huang, J.Y. (2010). Preparation and characterization of Chitosan/Konjac glucomannan/CdS nanocomposite film with low infrared emissivity. Materials Research Bulletin, 45, 859-862.

Zhang, H., Yoshimura, M., Nishinari, K., Williams, M. A. K., Foster, T. J., & Norton, I. T. (2001). Gelation behaviour of konjac glucomannan with different molecular weights. Biopolymers, 59, 38-50.

EFFECT OF ALKALIS ON KONJAC GLUCOMANNAN GELS FOR USE AS POTENTIAL GELLING AGENTS IN RESTRUCTURED SEAFOOD PRODUCTS

Beatriz Herranz[a], Clara A. Tovar[b], Beatriz Solo-de-Zaldívar[a] and A. Javier Borderías[a]

[a]Department of Products, Instituto de Ciencia y Tecnología de los Alimentos y Nutrición (ICTAN), Consejo Superior de Investigaciones Científicas (CSIC), José Antonio Nováis 10, 28040 Madrid, Spain

[b]Department of Applied Physics, Faculty of Sciences, University of Vigo, As Lagoas, 32004 Ourense, Spain

Food Hydrocolloids 27, 145-153.
(DOI: 10.1016/j.foodhyd.2011.08.003).

ABSTRACT

Four dispersions of 3% glucomannan in water, deacetylated with 5% 0.6N and 1N KOH (lots L1 and L2) and 0.6N and 1N NaOH (lots L3 and L4) as gelling agents, were evaluated for use in raw restructured seafood products. Several properties (pH, moisture content, water binding capacity, cooking loss and lightness) together with puncture data (breaking force and breaking deformation) were determined after 1 and 10 days of chilled storage at 5 °C. All these data were analysed together with different viscoelastic parameters obtained at small amplitude oscillatory strain (SAOS) after 1 day of chilled storage, showing that L1 and L4 samples were the most suitable gels for incorporation in raw restructured fish products. In both cases the highest stress (σ_{max}) and strain (γ_{max}) amplitude values were found in the linear viscoelastic (LVE) range; however, L1 showed both high strain amplitude and breaking deformation values. Moreover, creep and recovery (transient) data showed that L1 was the most-time stable gel with the highest elasticity and the lowest relaxation exponent (n). L4 gel showed strong rigidity, i.e. the highest values of breaking force and storage moduli (G') and the highest n value, making it less gel-like. Both L1 and L4 gels became significantly less gel-like over 10 days of chilled storage due to the loss of gel strength (S) and a noticeable increase of n. These chilled storage effects were more intense in L4 than in L1.

KEYWORDS: Konjac glucomannan; Breaking deformation; Restructured seafood product; Alkaline coagulant; Deacetylation

1. INTRODUCTION

Restructured seafood products are processed from minced and/or chopped muscle, usually with added ingredients, to make products with a new appearance and texture. The last 30 years have seen the development of a new generation of seafood products called analogues or substitutes, most of which mimic seafood or other high-value products and are formulated essentially from surimi, or in some cases mince. The processing is the result of thermal gelation or in some cases cold gelation with the help of ingredients like alginates or transglutaminase (Moreno, Carballo, & Borderias, 2008). However, if muscle protein functionality has been lost in processing, for example heating, gel formation to produce

structures is not possible. One possibility in that case would be to find a substance, neutral in colour and flavour, that after being mixed with minced muscle could form a thermo-stable gel. Such a substance could be Konjac glucomannan (KGM) a neutral polysaccharide derived from the tuber of *Amorphophallus konjac* C. Koch (Nishinari, Williams, & Phillips, 1992) that has the property of making thermo-stable gels in certain conditions (Nishinari, 1987; Nishinari et al., 1992; Yoshimura & Nishinari, 1999; Zhang et al., 2001) and is generally recognized as safe (GRAS). It consists of a linear backbone of β-1—4-linked D-glucopyranose and D-mannopyranose sugars in a random order in a molecular ratio of 1:1.6 (Kato & Matsuda, 1969) and possesses between 5-10% of acetyl substituted residues (Dea et al., 1977; Maekaji, 1978a), which makes it soluble in aqueous solution and improves chain flexibility. Eliminating acetyl groups produces deacetylated konjac glucomannan, which is able to build junction zones through hydrogen bonding, Coulombic (between solvated (Na^+, K^+) cations and KGM deacetylated anions ($R\text{-}CH_2O^-$) being R the framework of carbohydrate, dipole-dipole (among water molecules and OH groups of deacetylated KGM), van der Waals, charge transfer and hydrophobic interactions (Lapasin & Pricl, 1999). However, it is not yet fully understood how this mechanism works under different conditions yet (Yin, Hongbin, Huang, & Nishinari, 2008; Yoshimura & Nishinari, 1999). The type of binding in the junction zone and the amount of molecules forming the junction zones are the most important factors determining the rheological and thermal properties of a gel (Williams & Phillips, 2004). Acidic deacetylation is possible, but alkaline reactions are preferred to limit hydrolysis (Imeson, 2010). The importance of KGM gels in rheological terms is firstly its capacity to extensively modify the rheology of aqueous media to which they are added, even at fairly low concentrations, and that is the basis of their functional properties as thickening and gelling agents. Secondly they are also involved in many other types of applications, such as encapsulation, controlled release, etc. (Lefebvre & Doublier, 2005).

Therefore, small- and large-deformation mechanical tests need to be monitored in various different alkaline conditions to obtain information about the structure and mechanisms of gelation in different gels at a constant polysaccharide concentration.

The rate of gel formation is dependent on various factors: concentration and molecular weight of glucomannan, processing temperature, degree of acetylation, alkaline concentration (Nishinari & Zhang, 2004), and also pH (Huang, Takahashi, Kobayashi, Kawase, & Nishinari, 2002). In general, at a fixed alkaline concentration the gelation process

accelerates with increasing molecular weight, KGM concentration or heating temperature (Yoshimura & Nishinari, 1999; Huang et al., 2002) but it is delayed with an increasing degree of acetylation (Huang et al., 2002). As regards pH, Kohyama and Nishinari (1990) reported that the gelation of KGM occurs from pH 11.3 to 12.6, suggesting that a change in molecular structure is necessary for gelation. However, Thomas (1997) indicated that the pH range necessary for gelation is 9 to 10. It seems that the gelation rate also depends on the alkaline concentration and is lower at higher concentrations, but there is a critical alkaline concentration below which gelation does not occur (Huang et al., 2002). In a previous work, Maekaji (1978a) suggested that the deacetylation produced by the addition of alkalis is governed by the concentration of hydroxide ion, irrespective of the kind of alkali, and the deacetylation ratio—i.e. acetyl groups removed/total acetyl groups—was practically independent of gelling temperature and increased with decreasing KGM concentration.

Very little scientific literature is available reporting studies of mixes of KGM and fish. Park (1996) reported that KGM has the ability to reinforce gel hardness 8-10 fold in both whiting and pollock surimi. Thomas (1997) also reported the use of KGM at a concentration of 1 % in surimi, but he did not elaborate. Iglesias-Otero, Borderias, and Tovar (2010) added glucomannan to reinforce squid surimi gelation.

Also, a recent paper verified the good cryoprotective effect of KGM on protein from grass carp (*Ctnophryngodon idella)* during frozen storage (Xiong et al., 2009). The present authors have studied different KGM solubilization conditions, different KGM concentrations and types of alkali at different concentrations with a view to producing homogeneous gels with a texture similar to that of fish fillets. As regards the type of alkali, the literature reports the use of various different alkali coagulants such as phosphate buffers (Penroj, Mitchell, Hill, & Ganjanagunchorn, 2005), Na_2CO_3 (Hata, Ono, & Toda,1951; Huang et al., 2002; Huang & Lin, 2004), K_2CO_3 (Case, Knopp, Hamman, & Schwartz, 1992; $Ca(OH)_2$ (Hata et al.,1951; Jiménez-Colmenero et al., 2010; Na_3PO_4 (Nishinari et al., 1992), NaOH or KOH (Maekaji, 1978b). Using this information, in preliminary studies for the work (not included in this paper), various types of alkaline solutions (KOH, NaOH, $Ca(OH)_2$ Na_2CO_3, K_2CO_3, Na_3PO_4 and K_3PO_4) at different concentrations (0.2, 0.6 and 1N) were added to make gels from KGM aqueous solutions. In these experiments gels firm enough to hold the mince as filler and with a suitable colour only were obtained using KOH and NaOH, the alkaline agents used in the present work, at concentrations of 0.6 and 1N. These levels translate as 0.073- 0.196

g/kg, which is very little for standard food products, and do not pose problems as regards flavour or health properties.

This work is the first step of a study whose objective is to produce restructured seafood products in which KGM aqueous solutions and non-functional minced fish muscle are mixed, in such a way that the glucomannan forms a gel structure and the mince acts as filler, with a view to forming structures of various kinds fibres, myotomes, etc. The aim of this paper is to study the influence of the type and concentration of the alkali on the viscoelastic, mechanical and water retention properties of KGM gels in order to choose the most suitable thermo-stable gel for making restructured seafood products with different textures.

2. MATERIALS AND METHODS

2.1. Preparation of glucomannan gels

Aqueous solutions (3% (w/v) of glucomannan from konjac (glucomannan purity 100%, Guinama, Valencia, Spain) were prepared by continuous stirring for 30 min at low speed in a homogenizer (Stephan UM5, Stephan u. Söhne GmbH & Co., Hameln, Germany) at 60 °C. Then 5% of NaOH or KOH (Panreac Química, S. A., Barcelona, Spain) was added at a concentration of 0.6 or 1N of alkali coagulant, mixing for 1 min at 50 rpm to induce gel formation. After that, Petri dishes were filled with these mixtures and immediately vacuum packed into plastic bags (Barrier bag®, Cryovac Air Corporation, Barcelona, Spain) to compact the samples. Subsequently the samples were set by heating at 30 °C for 1 h and then at 5 °C for 4 hours to obtain heat-stable gels. The last step was to reduce the high pH values by taking the gels out of the Petri dishes and placing them in a 0.2M citrate-phosphate buffer at pH 5 (gel:buffer proportion 1:10). After 20 hours at 5 °C, the pH of thermostable gels changed to neutral. They were then kept refrigerated (5±1 °C) for 10 days. Analyses were performed at day 1 after gel preparation and after 10 days of chilled storage.

The different lots were named L1, L2, for gels made with 0.6N and 1N of KOH respectively, and L3 and L4 for the gels made with 0.6N and 1N of NaOH respectively.

2.2. Analyses

2.2.1. Physicochemical analysis

The pH was measured using a model 9165BNWP pH probe (Analítica Instrumental, S.A., Barcelona) inserted in the gel. The pHmeter was an Orion model 720A (Analítica Instrumental, S.A., Barcelona).

Water content was determined by drying the sample to constant weight at 110°C and the results are expressed as a percentage (AOAC, 2000). Samples were analysed in triplicate on days 1 and 10 after gel preparation.

The pH was measured in triplicate on days 0, 1, 5 and 10 of chilled storage; day 0 was the day immediately prior to putting the samples into the pH 5 buffer.

2.2.2. Water binding capacity (WBC)

Gels were cut into small pieces (2 g) and placed in a centrifuge tube (diameter 10 mm) with enough filter paper (2 filters Whatman nº 1, diameter 90 mm). Then the samples were centrifuged in a Jouan MR1812 centrifuge (Saint Nazaire, France) for 10 min at 3000g at room temperature. WBC was expressed as per cent water retained per 100 g water present in the sample prior to centrifuging. Measurements were carried out in triplicate on days 1 and 10 of refrigeration storage.

2.2.3. Cooking loss determination

A sample (40 g) was cut into small pieces and placed in a plastic bag where small holes had been made to drain the drip. Then, this bag with the sample inside was put inside another bag, hung with the holes at the bottom and cooked in that position in an oven (Rational Combi-Master CM6) for 20 min at 100 °C. The sample was then cooled and weighed. Cooking loss was expressed as g/100 g by weight difference between uncooked and cooked samples.

2.2.4. Colour measurements

Lightness (L*), was analysed using a CIELab scale. Measurement was analysed using a colorimeter (Minolta Chroma Meter Cr-200, Japan). The colour coordinates were measured five times on the surface of the gel at three different analysis times (on days 0, 1 and 10 of chilled storage). Before use, the colorimeter was standardized using a white calibration plate.

2.2.5. Puncture tests

Cylindrical samples (diameter 3 cm x height 3.5 cm) were filled after gel preparation. After neutralization in the buffer probes they were removed from the cylindrical cells. Before the analyses, probes were tempered at room temperature (25 °C). They were then taken out of the bags and analysed. Gels were pierced to breaking point using a TA-XTplus Texture Analyser (Stable Micro System Ltd., Surrey, UK) with a 5 mm–diameter round–ended metal probe. Crosshead speed was 1 mm/s, and a 5 kg load cell was used. The load (as breaking force) and the depth of depression (as deformation) when the gel sample lost its strength and ruptured were recorded. All determinations were carried out at least in sextuplicate.

2.2.6. Dynamic rheometry measurements

Small deformation shear oscillatory testing was performed using a Bohlin CVO controlled stress rheometer (Bohlin Instruments, Inc. Cranbury, NJ). The measurements were carried out using parallel-plate geometry (20 mm in diameter and 1 mm gap). Definitive gels were cut into disk-shaped slices 20 mm in diameter and 1 mm thick on a 570 S.T.E slicer (Germany). Any excess sample protruding beyond the upper plate was carefully removed. Samples were allowed to rest for 15 min before analysis to ensure both thermal and mechanical equilibrium at the time of measurement. Samples were covered with a thin film of Vaseline oil (Codex purissimum) to limit evaporation. No evidence of specimen slippage at the bottom plate was detected in any case (disk-shaped slices remained intact at the same initial position). The temperature was controlled to within 0.1 °C by a Peltier element in the lower plate and was kept at 25.0 °C.

2.2.6.1. Stress sweep tests. To determine the linear viscoelastic (LVE) region, stress amplitude sweeps were run at 6.28 rad/s, and 25 °C. The amplitude sweeps were conducted by varying the shear stress (σ) of the input signal from 0.24 to 1000 Pa. 300 points in the continuous mode were used in all instances. Changes in storage modulus (G'), loss modulus (G'') and complex modulus (G^*) were recorded. The critical (maximum) values of the amplitude sweeps—shear stress (σ_{max}) and shear strain (γ_{max}) at which the G^* values are just beginning to show a noticeable deviation from the previously constant values—were determined from these data. The range of tolerable deviation (± 10%) was corroborated using creep and recovery tests (Mezger, 2006, chap. 8).

2.2.6.2. Creep and recovery tests. An instantaneous stress σ_0 (30 Pa) was applied for 600 s to each sample in the creep tests and the resulting change in strain over time was monitored. When the stress was released, some recovery was also observed for 600s. Creep measurements were made over the linear viscoelastic range on each sample (σ_0 corresponding to 0.5% shear strain). The creep and recovery results are described in terms of the shear compliance function, $J(t) = \gamma(t)/\sigma_0$. Compliance curves generated at different linear stress levels overlap, making it possible to examine and compare the structural properties of the different food gels on larger time scales (Steffe, 1996, chap. 5).

From *J(t)* data we obtained the relaxation modulus *G(t),* which was used to find the gel strength (*S*) and relaxation exponent (*n*) (Ferry, 1980).

All measurements were made at 25 °C.

2.2.6.3. Mechanical spectra. Samples were subjected to stress that varied harmonically with time at a variable frequency. The shear strain amplitude was fixed at 0.5 %; oscillatory frequency sweeps were run from 10 to 0.1 Hz, and measurements were made at 25 °C. The complex modulus (*G**), storage modulus (*G'*), loss modulus (*G''*), and loss factor, *tanδ*, were determined as functions of frequency. Data were obtained in such a way as to ensure that the resulting σ in the sample would always fall within the linear viscoelastic range.

2.2.7. Statistical analyses

At least five independent batches were tested for each experiment and data are presented as averages. Statistical analysis was carried out using Microsoft Excel software. Trends were considered significant when means of compared sets differed at $p < 0.05$ (Student's t-test).

Statistical correlations between the textural and viscoelastic parameters were determined by multiple regression with confidence intervals of 95% ($p < 0.05$) using the SPSS Statistics 17.0 software.

3. RESULTS AND DISCUSSION

3.1. Evolution of pH

At Table 1 shows, the evolution of pH was the same in all the gels. The values were around 12 just after addition of 5% alkali and gel setting (day 0). These values dropped drastically to around 6-7 after the samples had been kept for 20 hours in citrate-phosphate buffer 0.2M pH 5 and remained constant over 1, 5 and 10 days of chilled storage. There were no significant differences ($p < 0.05$) in the average values in the different lots.

Table 1. Evolution of pH of the glucomannan gels during chilled storage.

	0 day	1 days	5 days	10 days
L1	12.17 ± 0.06a	6.62 ± 0.43b	6.59 ± 0.19b	6.65 ± 0.31b
L2	12.23 ± 0.03a	6.79 ± 0.57b	6.81 ± 0.28b	6.85 ± 0.33b
L3	12.13 ± 0.10a	6.58 ± 0.17b	6.62 ± 0.21b	6.71 ± 0.35b
L4	12.31 ± 0.09a	6.88 ± 0.41b	7.03 ± 0.47b	6.85 ± 0.24b

a–b Different letters indicate significant differences ($p < 0.05$) among gels.

3.2. Moisture content, Water Binding Capacity (WBC) and cooking loss

Table 2 shows the moisture content, water binding capacity (WBC) and cooking loss of samples L1- L4.

All the samples showed very high moisture content (96-97%). There were no significant differences ($p < 0.05$) among the different lots at days 1 and 10. Teramoto & Fuchigami (2000) reported that konjac glucomannan gel as a food had high water content (approximately 97%) but he did not indicate the percentage of glucomannan and the type of alkali (sodium carbonate or calcium hydroxide) used in the formation of these gels. Some authors (Kök, Abdelhameed, Ang, Morris, & Harding, 2009; Shinzato, Broussalis, & Ferraro, 1996) have reported that KGM forms highly viscous solutions when dissolved in water, suggesting that KGM has the highest viscosity at lowest concentration of any known dietary fibre (Shinzato et al., 1996; Yassen, Herald, Aramouni, & Alavi, 2005) so that it can take up to 200 times its weight in water.

Table 2. Moisture content (%), WBC (%) and cooking loss (%) of neutralized glucomannan gels after 1 and 10 days of chilled storage.

	Moisture content		WBC (%)		Cooking loss (%)	
	1 day	10 days	1 day	10 days	1 day	10 days
L1	96.55 ± 0.04a	96.97 ± 0.90a	69.65 ± 4.7bc	72.20 ± 2.7bc	4.39 ± 0.01d	3.94 ± 0.10g
L2	97.26 ± 0.70a	97.01 ± 0.45a	64.84 ± 5.5bd	68.23 ± 1.5b	3.35 ± 0.07e	3.13 ± 0.17e
L3	97.16 ± 0.54a	96.86 ± 0.07a	72.41 ± 2.6cd	78.47 ± 4.3c	2.68 ± 0.55f	2.11 ± 0.09fh
L4	97.22 ± 0.63a	96.49 ± 0.20a	77.47 ± 5.0c	70.43 ± 4.2bc	2.37 ± 0.35fh	2.01 ± 0.10fh

a–h Different letters indicate significant differences ($p < 0.05$) among gels.

WBC values were between 64.8%-78.5%. At day 1 values seemed to tend to be higher in samples made with NaOH (L3 and L4), although none of the samples showed significant differences ($p < 0.05$). These WBC values were stable after 10 days of chilled storage. L3 (NaOH 0.6N) showed the highest value ($p < 0.05$) but was only significant respect L2 (KOH 1N). Extraordinarily high water binding capacity has been reported in KGM although no data were given (Kök et al., 2009). The values for cooking loss were slightly but significantly ($p < 0.05$) higher in samples treated with KOH (L1 and L2) than in samples with NaOH added (L3 and L4), both at day 1, just after making the gel, and after 10 days. Cooking loss values were higher ($p < 0.05$) in the sample with the lower alkaline concentration (0.6N KOH) (L1) than in the one with the higher concentration (1N KOH) L2. Water retention values of about 86% have been reported in restructured fish muscle products made with minced fresh horse mackerel muscle, and about 75% in frozen hake muscle (Sánchez-Alonso, Haji-Maleki, & Borderias, 2007).

In general, all these data seem to indicate that glucomannan could reinforce the ability of the final product to capture moisture during cooking and retain its texture. There also seem to be some small and non-significant differences depending on the alkali used for WBC and slightly higher for cooking drip retention when Na^+ ions are added as NaOH than when K^+ ions are added as KOH. One possible explanation is the difference in the radius of Na^+ and K^+ ions, which would contribute to the degree of hydration of the structures. Large ions, as in the case of K^+, possess a lower hydration number (N_w), i.e. they are surrounded by a larger number of water molecules (not cation-linked), which retain their translational degrees of freedom. K^+ ions show a smaller average hydration number ($N_w = 2$) compared to Na^+ ions ($N_w = 3$). The later one possesses a higher change density since the same positive charge is located on the smaller Na^+ ion. Therefore, it polarizes the negative electronic clouds of water molecules more effectively (Moore, 1978). Hence NaOH can bind the water more easily (Fennema, 1976). Moreover, Kragh (1977) reported that in the ordered Lipotropic Series of Hofmeister, a cation series from higher to lower hydratation ability, the Na^+ is classified with more hydration ability. Kragh (1977) reported that the mechanism in this series is not clear but is related to the polarity and size of the ion. As noted earlier, these could be the reasons why in general WBC and cooking drip retention are higher when Na^+ ions are added, since this causes greater hydratation.

3.3. Colour

All the gels obtained were translucent, showing high values of lightness (L*). At day 1, L* was higher in gels made with NaOH than those made with KOH ($p < 0.05$) (Table 3), but after 10 days of chilled storage, L* did not differ significantly in any of the samples except L3 (KOH 1N). The other two colour parameters (a* and b*) were not affected by the type and/or concentration of alkali (data not shown). The lightness of whiting and pollock surimi with added glucomannan was reported by Park (1996), who found that L* was increased by the addition of glucomannan-rich konjac flour. We have found no more colour data for konjac gels in the literature.

Table 3. Lightness values (L*) of gel samples on days 1 and 10 of chilled storage.

	L* (1 day)	L* (10 days)
L1	41.0 ± 1.5a	46.79 ± 0.44d
L2	43.1 ± 1.5ab	46.1 ± 2.0bcd
L3	47.7 ± 2.0cd	43.59 ± 0.86b
L4	45.67 ± 0.46cd	45.14 ± 0.88cb

a–d Different letters indicate significant differences ($p < 0.05$) among gels.

In general, when gelation is performed at low ionic strength and in acidic or alkaline conditions, it produces fine-stranded gels which are translucent and have high water binding capacity (Rao, 2007). For that reason, in the light of their colour and the WBC data (Table 2) the four samples may be classified among the fine-stranded type physical gel networks.

3.4. Puncture test

Fig. 1 shows the Breaking force (1a) and Breaking deformation (1b) of samples L1-L4 at 0 and 10 days of chilled storage. There are significant differences ($p < 0.05$) in breaking force (1a) between L4 (1N NaOH) and the other samples. After 10 days, L4 still had the highest values for strength and L1 the lowest.

Although there was an increase in breaking force between day 1 and day 10 in all samples, this was much smaller and not significant in L1 than in the other samples, which could mean that the gel was more stable over chilled storage.

On the other hand, breaking deformation was very similar in all samples. The higher values were found in L1 and L4 at day 1, and L1 differed significantly ($p < 0.05$) from L2 and L3 (Fig. 1b). As in the case of breaking force, L1 showed practically no change between day 1 and day 10. L4 likewise showed no significant differences ($p < 0.05$) over the storage time, although these differences were slightly greater than in L1. Therefore, again it seems that the L1 network was the most time-stable of all the samples.

In short, these large-deformation rheological measurements made it possible to distinguish L1 and L4 samples, since when NaOH 1N was used as an alkaline agent, the resulting gel possessed similar breaking deformation but significantly higher breaking force, making the gel significantly more rigid. This mechanical behaviour is consistent with the fact that viscoelastic moduli values from mechanical spectra were higher for the respective samples, as will be discussed in the next paragraph. The trend was sustained after 10 days of chilled storage (Fig. 1a and 1b).

Although large-strain deformation mechanical tests, provide information that correlates more with the sensory perception and handling properties of food products (Bollaín, Angioloni, & Collar, 2005; van Vliet, 1995), small deformation oscillatory measurements have been considered a necessary and useful tool to study the network structure of foods, especially in food gels that present complex viscoelastic behaviour (Romero et al., 2009). Therefore, we considered it necessary to conduct a thorough rheological study to identify the properties of these gel networks.

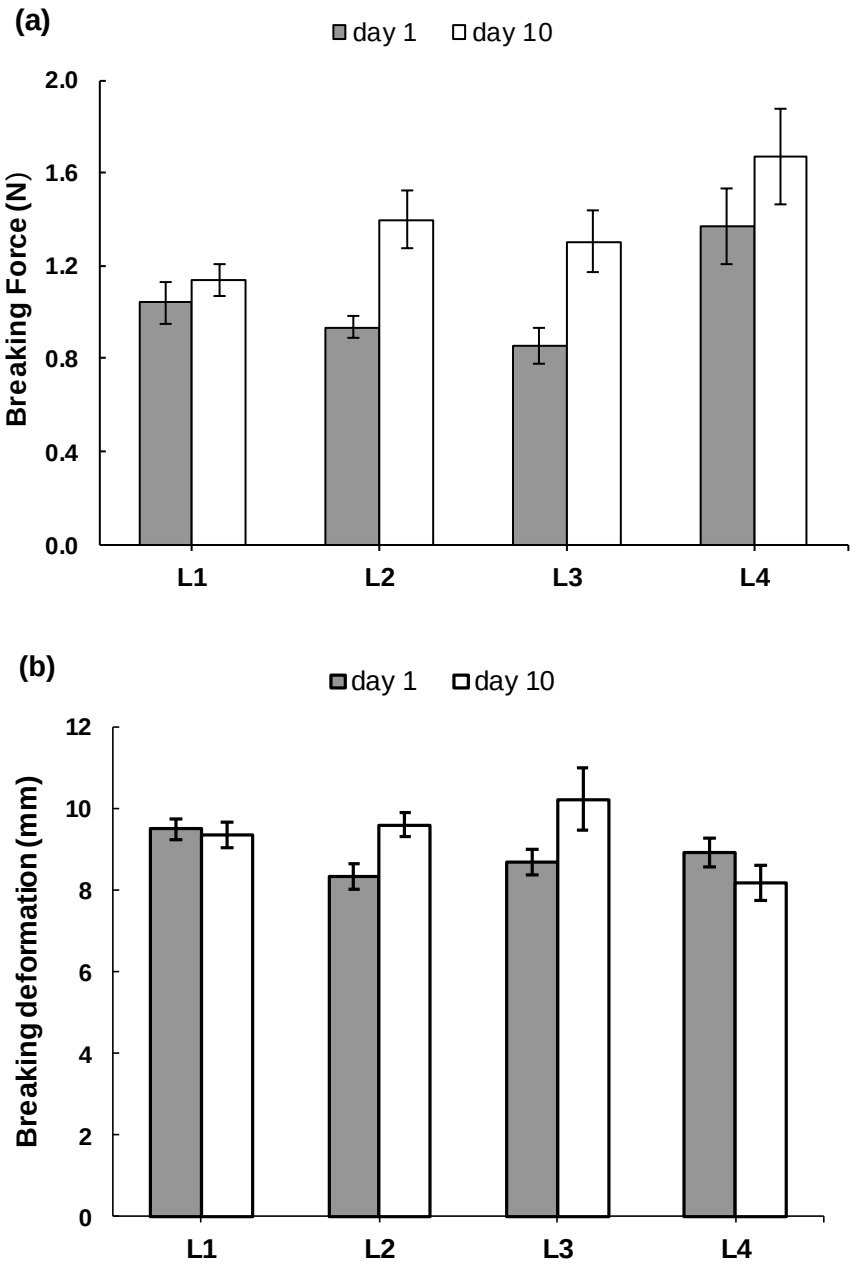

Fig. 1. The change of the breaking force (a) and breaking deformation (b) of glucomannan gels deacetylated with different alkali at 0.6N and 1N after 1 and 10 days of chilled storage.

3.5. Rheological measurements at small deformation. Influence of chilled storage

3.5.1. Overview of the small amplitude oscillatory (SAOS) results

The viscoelastic behaviour of the four samples was characterized at initial time in terms of several critical parameters which determine the linear viscoelastic range (LVE), together with their mechanical spectra. The information from the oscillatory tests was then supplemented by the results of transient tests, also at initial time, to discriminate which samples had better gel properties. Thereafter, we examined the influence of 10 days of chilled storage on the viscoelastic magnitudes, but only in the samples with better gel characteristics (higher stress and strain amplitudes from stress sweeps, low frequency-dependence on G', and lower viscous moduli from mechanical spectra).

3.5.2. Stress sweeps at initial time

The first step was to investigate the LVE range for all the polysaccharide gels when a different kind and concentration of alkali was used. Within this range the viscoelastic moduli are independent of the stress. As the applied stress increases, the bonds holding the network together begin to rupture. At critical values of stress (σ_{max}) and strain (γ_{max}), the network structure breaks down leading to a sharp decrease in the moduli. These critical values, which may serve as a measure of the stability of viscoelastic materials, were obtained from an automatic analysis (Campo-Deaño, Tovar, Pombo, Solas, & Borderias, 2009) and were corroborated using creep and recovery tests, since compliance curves generated at different stress levels overlap when data are collected in the LVE range (Steffe, 1996, chap. 5).

Table 4 shows the more representative magnitudes which characterize the amplitude of this LVE region. $Tan\delta = G''/G'$ values were similar (≈0.2), and significantly lower than 0.5 in the four samples, meaning that samples behave like a viscoelastic gel since G' is larger than G'' indicating the presence of a network structure (Mezger, 2006, chap. 8).

Table 4. Limit values of linear viscoelastic range (LVE): stress (σ_{max}) and strain (γ_{max}) amplitudes, complex modulus (G^*) and loss factor ($tan\delta$) of gels after 1 day of chilled storage, ν=1Hz ,T= 25 °C.

	L1	L2	L3	L4
σ_{max} (Pa)	87±8.7a	44 ±4.4b	54 ±5.4b	80 ±8a
γ_{max}(%)	1.72±0.26c	0.79b ± 0.16d	0.85b ± 0.20d	1.47± 0.26c
G* (kPa)	5.35±0.81e	5.76 ± 0.98e	6.7 ±1.4e	5.7 ± 1.2e
tan delta max	0.222±0.055f	0.203±0.091f	0.19±0.12f	0.170±0.078f

a-f Different letters in the same row indicate significant differences (p < 0.05).

There was no statistically appreciable difference in the overall rigidity of their networks (G^*) (Table 4), however L1 and L4 samples showed higher stress and strain amplitudes than L2 and L3, which is consistent with the fact that they showed the highest breaking force and breaking deformation at initial time, as confirmed by large deformation measurements (Fig. 1a and b).

3.5.3. Frequency sweeps at initial time

The frequency dependence of G' and G'' can provide valuable information about the structure of a gel. Mechanical spectra for selected alkali/concentration values of glucomannan gels are presented in Fig. 2. The mechanical spectra show little variation of G' and G'' over the entire frequency range studied. The G' values can be fitted to the power law (equation 1), but the G'' moduli remain practically constant from high to low frequencies.

$$G' = G_0' \cdot \omega^{n'} \qquad (1)$$

Table 5. Power law parameters G_0', n' from equation 1 and quality factor Q from equation 2 and average loss modulus after 1 day of chilled storage, T= 25°C.

	Go' (kPa)	*n'*	r^2	G'' (kPa)	*Q*
L1	4.810 ± 0.009a	0.063 ± 0.001h	0.96	0.496 ± 0.005e	68.41 ± 0.67l
L2	4.468 ± 0.010b	0.080 ± 0.002i	0.96	0.599 ± 0.005f	54.22 ± 0.49m
L3	5.951 ± 0.008c	0.080 ± 0.003j	0.97	0.611± 0.005f	67.73 ± 0.58l
L4	6.050 ± 0.006d	0.080 ± 0.004h	0.95	0.633 ± 0.014g	67.5 ± 1.5l

a-m Different letters in the same column indicate significant differences (p < 0.05).

Table 5 shows the power law parameters for G_0' and n' together with the mean viscous modulus between 0.63 and 63 rad/s. In L1-L4 samples, G' is practically frequency-independent over this time scale (n'<0.1). In addition G_0'>>G'' so samples, can be classified

as true gel systems (Kaur, Singh, Singh, & McCarthy, 2008). There was significant ($p < 0.01$) negative correlation of *n' (-0.998)* and *tanδ* (-0.970) with *WBC*, confirming that when a network gel approximates to a true gel (low *n'* and *tanδ*), the gel functionality is better and hence water binding capacity noticeably increases.

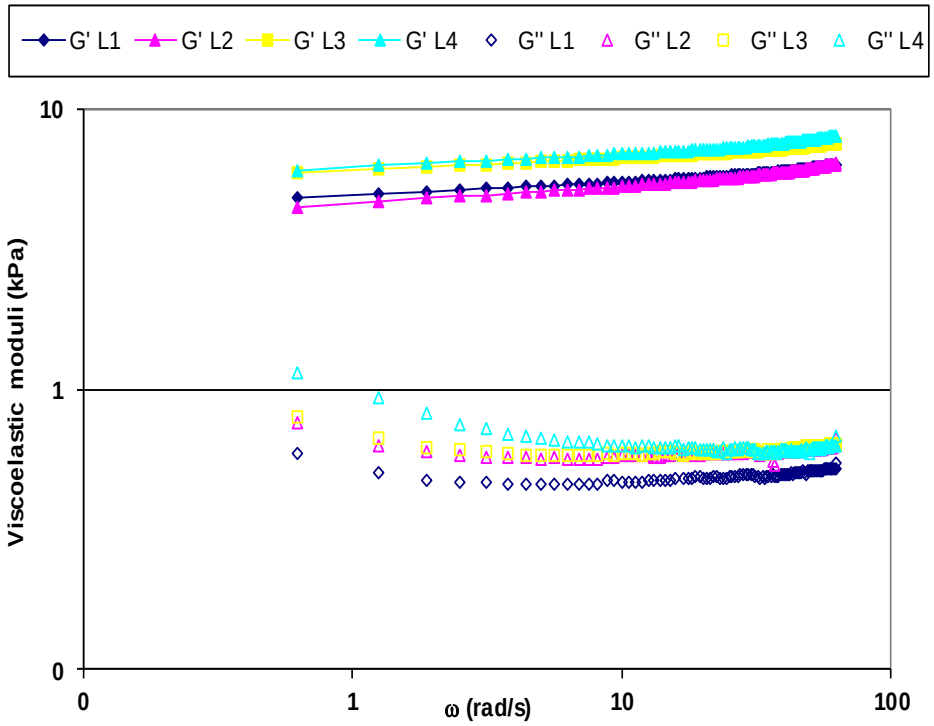

Fig. 2. Mechanical spectra data of glucomannan gels deacetylated with different alkali at 0.6 N and 1 N after 1 day of chilled storage. Closed symbols *G'*, open symbols *G''*. T = 25 °C.

This rheological behaviour is also quantifiable in terms of quality factor *Q* (Campo-Deaño, Tovar, & Borderias, 2010), a dimensionless quantity which represents the degree of damping of an oscillator. The *Q* factor is 2π times the ratio between the energy stored and the average energy loss per period (Arya, 1990). On the basis of the oscillatory character of frequency sweeps and the peculiar mechanical spectra data (Fig. 2 and Table 5), *Q* can be calculated from *G'* (eq. 1) and the mean viscous modulus in a sinusoidal strain (Table 5):

$$Q = 2\pi \frac{G_0^{'}}{G^{''}} \omega^{n'} \qquad (2)$$

Note that except for L2 gel, all the rest possess high Q values, which is a measure of structural stability in their networks on short time scales. The fact that the mean viscous moduli are lowest in L1 gel in particular indicates that the relevant polar interactions to cross-links are junction zones with finite energy, which act more cooperatively to ensure gel stability at short times. A more permanent three-dimensional network was formed, so that although the gels contained around 97% solvent (Table 2), they are macroscopically connective, generating a superstructure which can store more energy. Thus, a minimum amount of energy is required for the internal relative motion between molecular segments (low molecular friction) and hence Q increases, indicating higher viscoelastic stability in physical gels over short experimental times.

3.5.4. Creep and recovery tests at initial time

Creep analysis is a transient test which was done at constant stress within the LVE range. The results of these experiments were used to compare the different structural responses of the four gels. This type of analysis produces data on creep compliance, *J(t)*, the ratio of strain to stress over time. Thus, the time dependent properties connected with the viscoelastic characteristics of physical gels can be studied on longer time scales than those associated with oscillatory tests (Mezger, 2006, chap. 8). The rheological characterization was completed with transient experiments, which provide a means of trying regimes of t> 100s and can help to distinguish among noncovalent crosslinked gels. Long-term behaviour may be associated with the re-orientation of chain segments, and probably with the movement of whole molecules relative to one another, causing relatively strong crosslinks to break (Lapasin and Pricl, 1999, chap. 4). Transient tests, then, can cause the breakage of short-range interactions. Therefore information can be obtained about the relative long-range properties of these physical networks (Steffe, 1996, chap. 5). From J_{max} and J_{min} on the creep and recovery curves respectively, it is possible to quantify the percentage of elasticity in the networks according to equation 3:

$$Elasticity\ (\%) = \left(\frac{J_{\max} - J_{\min}}{J_{\max}} \right) \cdot 100 \qquad (3)$$

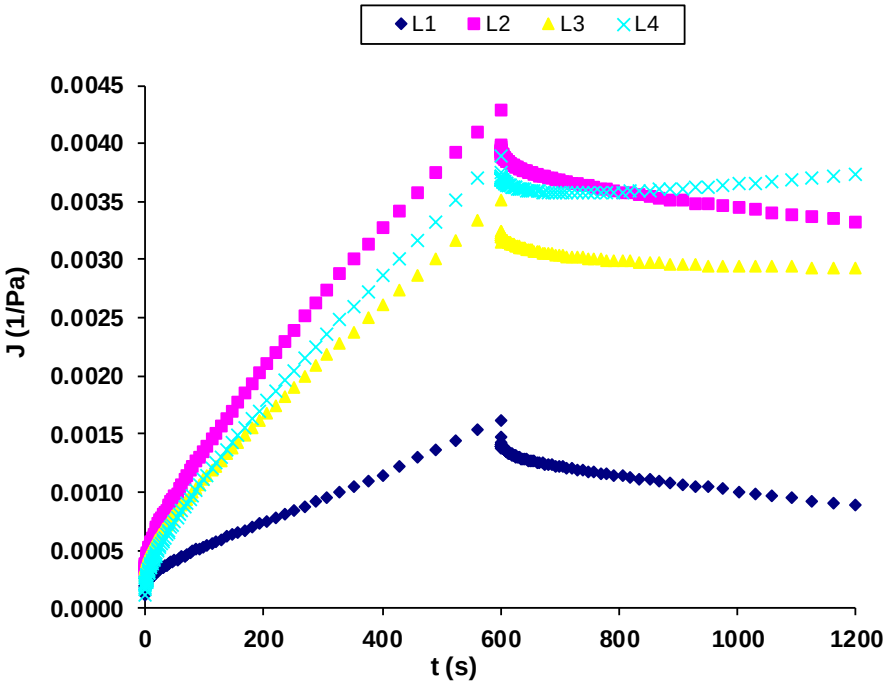

Fig. 3. Creep and recovery compliance *J(t)* data of glucomannan gels deacetylated with different alkali at 0.6 N and 1N at day 1 of elaboration. T= 25 °C

Fig. 3 shows creep-recovery compliances for glucomannan gels. L1 sample presents the lowest compliance data over the entire time interval. When the load was applied at time t=0, there was an instantaneous deformation, from which this sample showed the most complete recovery upon removal of the load, indicating that it was the most elastic (Table 6). Moreover, the fact that it presented the least permanent deformation indicated that for longer loading times there was less structural collapse and less irreversible breakage of interactions (Deman & Beers, 1987).

By means of an experiment based on a different physical principle, we can corroborate the viscoelastic stability of the physical network of L1 sample. This stability effect was deduced from stress sweeps (higher strain amplitude) and from mechanical spectra (lowest loss modulus) (Tables 4 and 5 respectively). We should note that the evaluation of the viscous component of gels from frequency sweeps presented the same trend as the irreversible compliance data obtained from the residual strain at the end of the recovery process. We can therefore state that the polymer network in L1 sample possesses similar connectivity to the covalently crosslinked networks, as was also reported by Case et al. (1992) for konjac gels.

For their part, the L2-L4 gels exhibited higher creep and recovery compliance values, with a significantly low degree of elasticity (Table 6). During creep time, break a lot of hydrogen bonds which origined certain degree of the irreversibly structural damage, thereafter when the load was removed the higher proportion of the structure collapsed gave *J(t)* on the recovery increased and elasticity diminished. This result is consistent with the higher viscous modulus values of L2-L4 samples (Table 5); therefore, if residual strain increases at the end of the recovery period, as in L2-L4 gels, can be attributed to rearrangement of the gel network (Williams & Phillips, 2004), then there would be more broken interactions, which would explain their higher *G''* values.

L4 gel was made with the alkali with the lowest cation diameter (Na^+) at maximum concentration, so that there were more Na^+ ions to link a larger number of water molecules, producing more layers of associated water molecules and hence a larger number of ion-dipole interactions. This could originate a *physical principle* based on the predominance of strong cation-dipole interaction, added to the corresponding anion-dipole (deacetylated KGM-water), which is less important since the anion is larger. This causes first an average preferential direction among molecular domains, which may generate a particular dipolar chain orientation (local order), and second a noticeable decrease of the available water, which cannot act as a lubricant between anionic polymer chains; this could explain the fact that the sample's physical network was more brittle (high breaking force and low breaking deformation) than L1 (Fig. 1a and b), as discussed in section 3.4.

Moreover, the *J(t)* from the creep values gives us the relaxation modulus *G(t),* since if we plot log *J(t)* versus log *t* over the entire time interval, the slope of the function m<<1 (data not shown here), *G(t)* becomes the reciprocal of *J(t)* (Ferry, 1980). Thus, the equation of Winter and Chambon (te Nijenhuis, 1997) can be used to calculate other parameters related to *gel strength (S)*, and also to the *relaxation exponent (n)*, by:

$$G(t) = S \cdot t^{-n} \qquad (4)$$

where *S* ($Pa \cdot s^n$) depends on the strength of the zone junctions between molecular domains and *n* is related to the density of these zone junctions, i.e. the degree of connectivity in the gel (Gabriele, de Cindio, & D'Antona, 2001). Table 6 shows that L1 gel presented the highest

S and the lowest *n* parameters. The lower the *n* values, the higher is the density of physical crosslinks, which increases the extension of the junction zones in noncovalently crosslinked networks (Lapasin & Pricl, 1999, chap. 4). L4 gel possessed a similar *S* value to L1, but *n* was the highest as a result of less connectivity and may reflect more levels of heterogeneity than in the other samples. Also, there was a significant ($p < 0.01$) positive correlation between *S* and the critical stress ($r^2 = 0.997$) and strain ($r^2 = 0.996$) (from stress sweeps). This is because strong gels may remain in the LVE range at greater strains level, reflecting higher gel strength values (Steffe, 1996, chap. 5).

Table 6. Percentage of elasticity from equation 3 and power law parameters from equation 4 of L1-L4 gels after 1 day of chilled storage, T= 25°C.

	S (kPa.s^n)	*n*	r^2	Elasticity (%)
L1	5.803 ± 0.087d	0.266 ± 0.009e	0.90	45
L2	2.948 ± 0.054a	0.319 ± 0.011f	0.89	22
L3	3.362 ± 0.001b	0.305 ± 0.011f	0.89	16
L4	5.234 ± 0.094c	0.395 ± 0.011g	0.93	4

a-g Different letters in the same column indicate significant differences ($p < 0.05$).

To summarize, from large and SAOS measurements we can deduce that sample L1, followed by L4, was the most stable, with the most ordered three dimensional network. Both had the highest maximum stress (σ_{max}) and strain (γ_{max}) values, as reflected by their high values of breaking force and breaking deformation respectively. However, L1 was the most elastic, presenting the lowest viscous moduli (*G''*), and recovery compliance (*J*) together with the lowest *relaxation exponent (n*), indicating a better-organized and better-ordered network as explained above. In addition, L1 showed the least variation in breaking force and breaking deformation over 10 days, which means that this gel was very stable, an important characteristic in a restructured fish product intended for chilled storage. All this means that L1 (0.6N KOH) offers the best properties for use as a gelling agent in restructured fish products. L4 (1N NaOH) could also be used although its network is less well- ordered and time-stable than L1's. It was therefore decided to study the influence of 10 days of chilled storage on viscoelastic properties of L1 an L4 gels.

The last step of processing is neutralization with a citrate-phosphate buffer. Although this should theoretically stimulate hydrogen bonding, the gel texture remains unchanged as unpublished previous analyses showed. As most of the bonds probably might already be

formed at the time of deacetylation and therefore contribute to a certain thermodynamical stability rearragements of bonds should be limited.

3.5.5. Effect of chilled storage time on the LVE interval and mechanical spectra

First, stress sweeps were used to evaluate the influence of 10 days of chilled storage on the σ_{max} and γ_{max} values in both L1 and L4 samples. There were practically no differences with respect to the corresponding values at initial time (Table 4): σ_{max} values after 10 days were: 90±9 and 70±7 Pa for L1 and L4 respectively. Although γ_{max}. values increased after the 10 days, 2.19 ± 0.59 and 1.62 ± 0.33 for L1 and L4 respectively, there were no significant differences ($p < 0.05$) with respect to the corresponding γ_{max} values at day 1 (Table 4).

However, the frequency sweeps showed considerable changes in the viscoelastic properties when compared to day 1. There was thus a general decrease in the gel-like character of both samples after 10 days: on the one hand the G_0' parameter (equation 1) decreased significantly in both samples, more so in L4 (29 % with respect to initial value) than in L1 (18%) (Fig. 4a). There was also a greater increase in frequency-dependence, similar in L1 and L4 (Fig. 4c), while G'' increased considerably in L1 (40% with respect to initial value) and decreased in L4 (Fig. 4b), making for a smaller gap between G' and G''. This meant a sharp decline in the elasticity of the networks.

These results show that in this kind of transient networks, chilled storage breaks the junction zones stabilized by thermolabile polar interactions such as ion-dipole and hydrogen bonds between polymer chains and with water. This effect was more pronounced in L1 sample given that the quality factor Q decreased by 39% while in L4 it decreased by 10% with respect to the corresponding values at day 1 (Table 5). This trend reflects a higher degree of connectivity by means of physical interactions in L4 sample, since Na^+ has more hydration ability (Kragh, 1977), and hence the loss of solidity was smaller than in L1 on short time scales.

3.5.6. Effect of chilled storage time on the creep and recovery data

Fig. 5 shows the influence of 10 days of chilled storage on the gel strength and relaxation exponent parameters from equation 4. In both L1 and L4 samples, these transient

data confirm that chilled storage time caused some structural damage. This effect can be seen in the noticeable loss of the gel strength (Fig. 5a) and the significant increase of the relaxation exponent (Fig. 5b), which is associated with a decrease of polymer molecular weight due to the rupturing of a sequence of physical crosslinks (Lapasin & Pricl, 1999). After 10 days this damage was somewhat greater in L4, which presented a slightly greater decrease in extent (17%) and strength of connectivity (45%) than L1 (12 % and 39% respectively). In general, the effect of chilled storage was slightly greater in gels made with NaOH than those made with KOH; in the former the number and distribution of anionic sites of KGM is altered by higher number of big hydrated ions that could generate, more dipolar fluctuations within the network (number and position of all these noncovalent cross-links), and hence the effect of experimental and storage time will be greater. On the other hand, as noted earlier, L1 (with minor quantity of small hydrated ions) was more time-stable in terms of breaking force and breaking deformation (Fig. 1), with hardly any difference between day 1 and day 10 for either parameter.

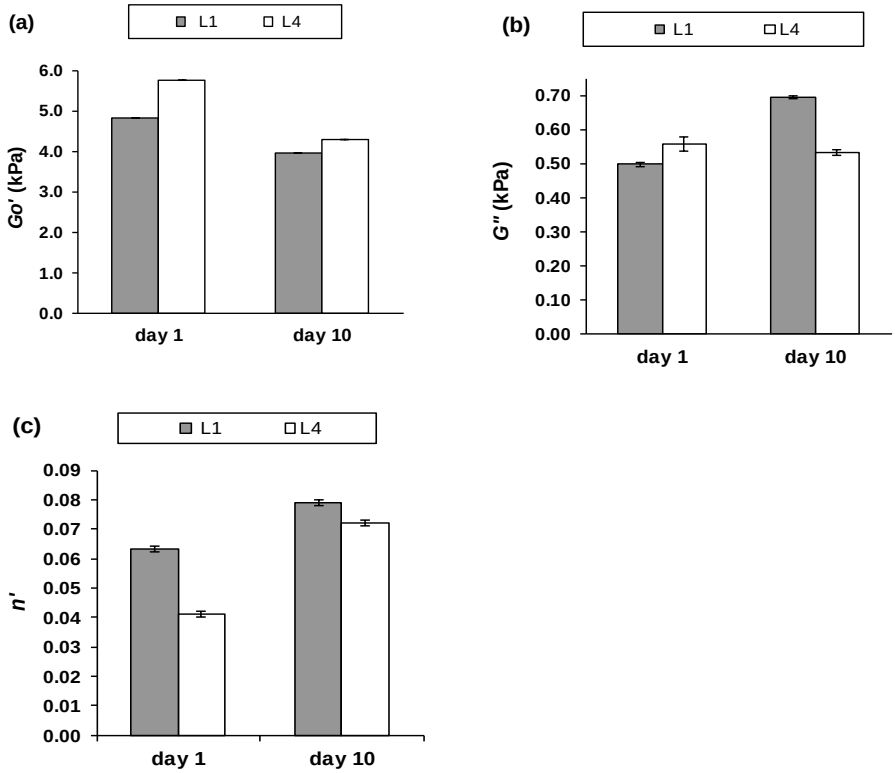

Fig. 4. Effect of refrigerated time at 5 °C on power law parameters from equation (1) G_0', n', and viscous modulus (G'') from frequency sweeps of L1 and L4. T = 25 °C.

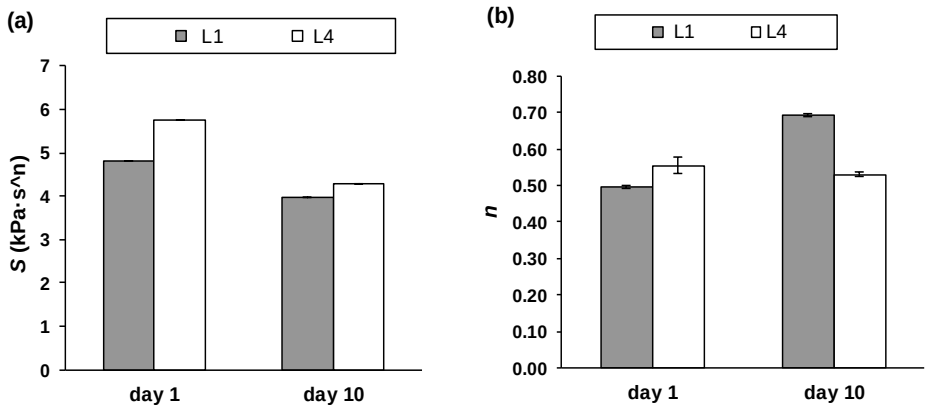

Fig. 5. Influence of refrigerated time at 5 °C of L1 and L4 gels, on gel strength (*S*) and relaxation exponent (*n*) from equation (4). T = 25 °C.

4. CONCLUSIONS

In this study various physicochemical measurements and various rheological techniques based on different physical principles such as large and SAOS deformations on different time scales (oscillatory and transient tests), were used to determine the influence of several alkalis at different concentrations on deacetylated glucomannan gels. Of the samples analyzed aqueous dispersions of 3% glucomannan deacetylated with 0.6N KOH and with 1N NaOH seem to possess well-structured gel properties and to be moderately stable over time, making them suitable for use in raw restructured seafood products that have to be stored chilled.

Of the samples assayed, the one with 0.6 N KOH performed best in terms of elasticity and time- and structural stability. Tests done over longer time scales, such as creep-recovery experiments, show low residual strain values and greater connectivity, which is consistent with results from experiments performed over shorter times, such as low viscous moduli values from mechanical spectra and high breaking deformation (from large deformations).

In order to be able to use them as gelling agents in restructured fish products, a thermal rheological study of these two gels (0.6N KOH and 1N NaOH) is required to demonstrate their thermo-stability.

ACKNOWLEDGEMENTS

This work has been supported by the Consejo Superior de Investigaciones Científicas for Postdoctoral Scholarship JAE-DOC-2008 under proyect AGL2008-04892-CO3-C3. The authors wish to thank the Xunta de Galicia for its financial support under the Consolidation and restructuring program of competitive research units: Strategic Research Partnerships (2009/060).

REFERENCES

AOAC. (2000). Official method of analysis. 17th ed. Maryland, USA: Association of Official Analytical Chemists.

Arya, A.P. (1990). Introduction to Classical Mechancis. Boston: Allyn and Bacon

Bollaín, C., Angioloni, A., & Collar, C. (2005). Bread staling assessment of enzyme supplemented pan breads by dynamic and static deformation measurements. European Food Research and Technology, 220, 83–89.

Campo-Deaño, L., Tovar, C., Pombo, M. J., Solas, M. T., & Borderias, J. (2009). Rheological study of giant squid surimi (Dosidicus gigas) made by two methods with different cryoprotectants added. Journal of Food Engineering, 94, 26–33.

Campo-Deaño, L., Tovar, C. A., & Borderias, J. (2010). Effect of several cryoprotectants on the physicochemical and rheological properties of suwari gels from frozen squid surimi made by two methods. Journal of Food Engineering 97, 457–464.

Case, S. E., Knopp, J. A., Hamann, D. D., & Schwartz, S. J. (1992). Characterization of gelation of konjac mannan using lyotropic salts and rheological measurements. In G. O. Philips, & P. A. Williams, & D. J. Wedlock (Eds.), Gums and stabilisers for the food industry, 6, (pp. 489). Oxford: Oxford University Press.

Dea, I. C. M., Morris, E. R., Rees, D. A., Welsh, E. J., Barnes, H. A., & Price, J. (1977). Associations of like and unlike polysaccharides-mechanisms and specificity in glucomannans, interacting bacterial polysaccharides and related systems. Carbohydrate Research, 57, 249-272.

Deman, J.M., & Beers, A.M. (1987). Fat crystal networks: structure and rheological properties. Journal of Texture Studies, 18, 303-318.

Fennema, C. (1976). Water and ice. In O. Fennema (Ed.), Principles of food science (pp. 13). New York: Dekker.

Ferry, J. D. (1980). Viscoelastic properties of polymers. (3rd ed.). New York: John Wiley and Sons, Inc.

Gabriele, D., de Cindio, B., & D'Antona, P. (2001). A weak gel model for foods. Rheologica Acta, 40, 120–127.

Hata, T., Ono, Y., & Toda, S. (1951). Kogyo Kagaku Zasshi, 54, 105.

Huang, H. Y., & Lin, K. W. (2004). Influence of pH and added gums on the properties of konjac flour gels. International Journal of Food Science and Technology, 39, 1009-1016.

Huang, L., Takahashi, R., Kobayashi, S., Kawase, T., & Nishinari, K. (2002). Gelation behaviour of native and acetylated konjac glucomannan. Biomacromolecules, 3, 1296-1303.

Iglesias-Otero, M. A., Borderias, J., & Tovar, C. A. (2010). Use of konjac glucomannan as additive to reinforce the gels from low quality squid surimi. Journal of Food Engeenering, 101, 281- 288.

Imeson, A. (2010). Food Stabilisers, Thickners and Gelling Agents. Iowa: John Wiley and Sons, Inc

Jiménez-Colmenero, F., Cofrades, S., López-López, I., Ruiz-Capillas, C., Pintado, T., & Solas, M. T. (2010). Technological and sensory characteristics of reduced/low-fat, low-salt frankfurters as affected by the addition of konjac and seaweed. Meat Science, 84, 356-363.

Kato, K., & Matsuda, K. (1969). Studies on the chemical structure of konjac mannan. Agricultural and Biological Chemistry, 33, 1446-1453.

Kaur, L., Singh, J., Singh, H., & McCarthy, O. J. (2008). Starch-cassia gum interactions: a microstructure-rheology study. Food Chemistry, 111, 1–10.

Kohyama, K., & Nishinari, K. (1990). Dependence of the specific volume of konjac glucomannan on pH. In G. O. Phillips and D. J. Wedlock & P. A. Williams (Eds.). Gums and Stabilisers for the Food Industry, 5, (p. 459). Oxford: IRL Press.

Kök, M. S., Abdelhameed, A. S., Ang, S., Morris, G. S., & Harding, S. E. (2009). A nobel global hydrodynamic analysis of the molecular flexibility of the dietary fibre polysaccharide konjac glucomannan. Food Hydrocolloids, 23, 1910–1917.

Kragh A. M. (1977). Swelling, adsorption and the photographic uses of gelatine. In A. G., Ward, & A. Courts (Eds), Science and Technology of gelatine, (p. 439). New York: Academic Press.

Lapasin, R., & Pricl S. (1999). Rheology of Industrial Polysaccharides: Theory and Applications. Gaithersburg: Aspen Publishers

Lefebvre, J., & Doublier, J.L. (2005). Rheological behaviour of polysaccharides aqueous systems. In S. Dumitriu (Ed.), Polysaccharides Structural Diversity and Functional Versatility. (pp. 357-394). New York: Marcel Dekker.

Maekaji, K. (1978a). Determination of acidic components of konjac mannan. Agricultural and Biological Chemistry, 42, 177-178.

Maekaji, K. (1978b). A method for measurement and kinetic analysis of the gelation process of konjac mannan (kinetic study on the gelation of konjac mannan). Nippon Nogeikagakukaishi, 52, 251-257 (in Japanese with summary and figure captions in English).

Mezger, T. G. (2006). The Rheology Handbook. (2nd ed). Hannover: Vincentz Network.

Moore W. J. (1978) Química Física (Tomo 1). Urmo, S.A. De Ediciones, Bilbao

Moreno, H. M., Carballo, J., & Borderías, J. (2008). Influence of alginate and microbial transglutaminase as binding ingredients on restructured fish muscle processed at low tempertature. Journal of the Science of Food and Agriculture, 88, 1529-1536.

Nishinari, K. (1987). In G.O. Phillips, D. J. Wedlock, & P. A. Williams (Eds), Gums and Stabilisers for the Food Industry 4, (P. 373). Oxford: Oxford University Press.

Nishinari, K., Williams, P. A., & Phillips, G. O. (1992). Review of the physico-chemical characteristics and properties of konjac mannan. Food Hydrocolloids, 6, 199-222.

Nishinari, K., & Zhang, H. (2004). Recent advances in the understanding of heat set gelling polysaccharides. Trends in Food Science and Technology, 15, 305-312.

Park, J. W. (1996). Temperature-tolerant fish protein gels using konjak flour. Journal of Muscle Food, 7, 165- 174.

Penroj, P., Mitchell, J. R., Hill, S. E., & Ganjanagunchorn, W. (2005). Effect of konjac glucomannan deacetylation on the properties of gels formed from mixtures of kappa carrageenan and konjac glucomannan. Carbohydrate Polymers, 59, 367-376.

Rao, M. A. (2007). Rheology of Fluid and Semisolid Foods. Principles and Applications (2nd ed.). New York: Springer,

Romero, A., Cordobés, F., Puppo, M C., Villanueva, A., Pedroche, J., & Guerrero, A. (2009). Linear viscoelasticity and microstructure of heat-induced crayfish protein isolate gels. Food Hydrocolloids, 23, 964–972.

Sánchez-Alonso, I., Haji-Maleki, R., & Borderías, A. J. (2007). Wheat fiber as a functional ingredient in restructured fish products. Food Chemistry, 100, 1037-1040.

Shinzato, C., Broussalis, A. M., & Ferraro, G. E. (1996). Glucomanano: Un aporte a su control de calidad. Revista de la Asociación Argentina de Farmacia y Bioquímica Industrial (SAFYB), 35, 26-31.

Steffe, J. F. (1996). Rheological Methods in Food Process Engineering. (2nd ed.). East Lansing: Freeman Press.

te Nijenhuis, K. (1997). Advances in Polymer Science 130. Thermoreversible Networks. Viscoelastic Properties and Structure of Gels. Berlin Springer-Verlag.

Teramoto, A., & Fuchigami, M. (2000). Changes in temperature, texture and structure of konnyaku (konjac glucomannan gel) during high-pressure-freezing. Journal of Food Science, 65, 491–497.

Thomas, W. R. (1997). Konjac gum. In Imeson A, (Ed.). Thickening and gelling agents for foods (p.169-179). London : Blackie Academic & Professional.

Van Vliet, T. (1995). Mechanical properties of concentrated food gels. In E. Dickinson & D. Lorient (Eds.), Food macromolecules and colloids (pp. 447-455). Cambridge: The Royal Society of Chemistry.

Williams, P. A., & Phillips, G. O. (2004). Gums and Stabilisers for the Food Industry 12. The Royal Society of Chemistry, Cambridge. U.K.

Xiong, G., Cheng, W., Ye, L., Du, X., Zhou, M., Lin, R., Geng, S., Geng, S., Chen, M., Corke, H., & Cai, Y. Z. (2009). Effect of konjac glucomannan on physicochemical properies of myofibrillar protein and surimi gels from grass carp (Ctenopharyngodon idella) . Food chemistry, 116, 413- 418.

Yassen, E. I., Herald, T. J., Aramouni, F. M., & Alavi, S. (2005). Rheological properties of selected gum solutions. Food Research International, 38, 111–119.

Yin, W., Hongbin, Z., Huang, L., & Nishinari, K. (2008). Effects of lyotropic series salts on the gelation of konjac glucomannan in aqueous solutions. Carbohydrate Polymers, 74, 68-78.

Yoshimura, M., & Nishinari, K. (1999). Dynamic viscoelatic study on the gelation of konjac glucomannan with different molecular weights. Food Hydrocolloids, 13, 227-233.

Zhang, H., Yoshimura, M., Nishinari, K., Williams, M. A. K., Foster, T. J., & Norton, I. T. (2001). Gelation behaviour of konjac glucommannan with different molecular weights. Biopolymers, 59, 38-50.

EFFECT OF DEACETYLATION ON THE GLUCOMANNAN GELATION PROCESS FOR MAKING RESTRUCTURED SEAFOOD PRODUCTS

Solo-de-Zaldívar, B.[a*], Tovar, C. A.[b], Borderías, A. J.[a] and Herranz, B.[a]

[a]Department of Products, Instituto de Ciencia y Tecnología de los Alimentos y Nutrición (ICTAN-CSIC), José Antonio Nováis 10, 28040 Madrid, Spain

[b]Department of Applied Physics, Faculty of Sciences, University of Vigo, As Lagoas, 32004 Ourense, Spain

Food Hydrocolloids, 35, 59-68.

(DOI: 10.1016/j.foodhyd.2013.04.009)

ABSTRACT

This paper focuses on the relationship between network structure and physicochemical and rheological properties of aqueous glucomannan dispersions (AGD) as a function of pH, to establish optimal conditions of glucomannan gelation for making restructured seafood products. Various lots of AGD were prepared from 3% (L1) and 5% (L2) konjac glucomannan adding different amounts (0.5-5%) of 0.6N KOH to obtain samples with successively increasing degrees of alkalinity, from pH= 8.9 to =11.9 (samples L1.1-L1.6 for 3% AGD) and from pH= 7 to 11.4 (samples L2.1-L2.6 for 5% AGD). The spectra of the different AGDs were obtained by Fourier Transform Infrared spectroscopy (FT-IR) to quantify the deacetylation ratio, whose effect on the physicochemical, mechanical (puncture), viscoelastic (at both small and large time scales) and structural characteristics (scanning electron microscopy (SEM)) was analysed. A linear dependence was found between the relative area of acetyl bands of AGD and pH, showing a discontinuous region in the function or gap zone between pHs 9.3 (L1.2) and 9.8 (L1.3) for 3% AGD and between 9.2 (L2.3) and 10.7 (L2.4) for 5 % AGD. Samples before the gap zone (L1.2 and L2.3) were gels of varying degrees of weakness, becoming strong gels thereafter. The gelation conditions were best at pH~10.7 for both 3 and 5% AGDs, corresponding to high and moderate deacetylation ratio, respectively. The resulting gels possessed elastic, cohesive and time-stable networks and thus formed basic structures able to contain several ingredients for making restructured seafood products. The SEM photographs corroborated the physicochemical and rheological characteristics.

KEYWORDS FT-IR; Deacetylation ratio; Restructured seafood products; Glucomannan gels

1. INTRODUCTION

Restructured seafood products offer a means of using muscle by-products and developing new products that have different textures and can also contain functional ingredients. These structures are usually formed by thermostable protein gelation, but such gelation is not possible when the muscle has been previously processed and the protein is degraded. In previous works (Herranz, Borderias, Solo-de-Zaldívar, Solas, & Tovar, 2012;

Herranz, Tovar, Solo-de-Zaldívar, & Borderias, 2012; 2013), the authors proposed the use of Konjac glucomannan (KGM) as a thermostable gelling agent for restructuring. For that purpose an aqueous glucomannan dispersion (AGD) could be mixed with minced muscle after alkaline deacetylation to obtain thermostable gels with a texture and flavour similar to those of seafood muscle. This kind of thermostable gel is a three dimensional network, with different physical gel characteristics depending on the kind of processing. For instance, it is accepted (Maekaji, 1974; Williams, et al., 2000) that the addition of alkali strongly diminishes steric hindrance caused by the acetyl groups, causing the formation of non covalent cross-links among junction zones. The number, size and position of junctions can fluctuate with time and temperature, producing transient networks (Herranz, Borderias, Solas, & Tovar, 2012; Ross-Murphy, 1995) involving non-covalent bonds such as hydrogen bonds, hydrophobic interactions, ionic bonds, etc., which under specific conditions may behave as permanent cross-links (Lapasin & Pricl, 1999, chap. 4). Hydrogen bonds in particular are considered the main interactions responsible for gel formation, although hydrophobic interactions, which gain in importance with increasing deacetylation, also seem to play an important role (Chen, Li, & Li, 2011). Moreover, when pH increases, the resulting anionic groups can form KGM chains that can change the structural function of water within the network, modifying the final properties of KGM gels. Specifically, Herranz et al. (2012) reported differences among electrostatic interactions such as ion-dipole, depending on the type and concentration of alkali, e.g. among cations (Na^+ and K^+) with OH groups of KGM chains, and with water molecules, on mechanical and viscoelastic properties of KGM gels. The authors found that 0.6N KOH was the most suitable alkali to deacetylate KGM, thus producing KGM gels with more elastic and time-stable networks. However, the effect of pH on the deacetylation ratio and its influence on the physical properties of resulting samples has not yet been studied. This aspect is of paramount importance for designing gels with adequate textural characteristics for the particular technological purpose. In the present paper, the authors propose that strong gels capable of retaining a non-functional minced muscle as a filler and with a texture and appearance as similar as possible to real seafood muscle, which can also be heated, would be a real solution to upgrading many different muscle by-products remaining after processes that render the muscle remain non-functional.

The objective of this study was to evaluate the effect of the percentage of acetyl groups released from KGM chains, as measured by FT−IR, on the physicochemical, mechanical, viscoelastic and the microstructural characteristics of KGM gels. The practical aim was to optimize KGM gelation conditions in line with KGM concentration, for use in the manufacture of restructured seafood products.

2. MATERIALS AND METHODS

2.1. Preparation of samples

3 and 5 % (w/v) aqueous glucomannan dispersions (AGD) from konjac glucomannan (Glucomannan purity 100%, Guinama, Valencia, Spain) were prepared according to the methodology previously described in Herranz et al. (2012). For KGM gelification, 0.6N KOH (Panreac Química, S. A., Barcelona, Spain) was the alkali used to raise the pH of AGD from around 6 to around 11.9 (3% AGD) and 11.4 (5% AGD). After setting for 1 h at 30 °C and 5 hs at 5 °C, they were removed from cylindrical containers (diameter 3 cm x height 3.5 cm) and Petri dishes, and placed in citrate-phosphate buffer at pH 5 (the gel:buffer proportion was 1:10) for 20 h at 5 °C in order to bring the pH of the gels down to 6.5-6.8.

The different lots of gels were prepared with 3 and 5% AGD (L1 and L2 respectively) and increasing amounts of 0.6N KOH (from 0.5 to 6%) to reach a set of pH values for both lots, which were designated as shown in Table 1.

An aliquot of each sample was frozen (-80 °C) and freeze dried using a VirTis Benchtop-6KB freezer (Gardiner, NY,USA), for further analysis by *FT-IR* spectroscopy analysis.

2.2. Analyses

2.2.1. pH and moisture

The pH was measured using a model 9165BNWP pH probe (Analítica Instrumental, S.A., Barcelona) inserted in the gel. The pHmeter was an Orion model 720A (Analítica Instrumental, S.A., Barcelona). The pH was measured after homogenization of AGD for 3 min immediately after addition of the necessary amount of 0.6N KOH.

Water content was determined by drying the sample to constant weight at 110 °C. The results are expressed as a percentage (AOAC, 2000).

Both analyses were done in triplicate

Table 1. Nomenclature of samples containing 3 and 5% glucomannan at different pHs.

pH			
GM 3%		**GM 5%**	
L1.0	5.8	L2.0	5.5
L1.1	8.9	L2.1	7
L1.2	9.3	L2.2	8.1
L1.3	9.8	L2.3	9.2
L1.4	10.8	L2.4	10.7
L1.5	11.4	L2.5	11
L1.6	11.9	L2.6	11.4

2.2.2. Fourier Transform IR Spectra Measurement or FT-IR spectroscopy analysis

AGD (3% and 5%) was mixed with different proportions of a solution of 0.6N KOH (0, 0.5, 1, 2, 3 and 4(w/v)) to achieve different pHs and analysed by FT-IR. This range of pH values for KGM gelation was based on the reports by Thomas (1997) (pH values of 9-10), and Kohyama and Nishinari (1990) (pH values of 11.3- 12.6). In this case other lower pHs were assayed to observe the acetyl band in FT-IR spectra and its gradual disappearance with increasing pH (or alkaline addition). The freeze-dried samples were dispersed in the agate mortar. Fluorolube was used as a matrix for dispersion of samples; this only fits absorption bands in frequency range above 1360 cm^{-1} except at 2321.9 cm^{-1}, and therefore it does not interfere in the observation of the bands that were of relevance to this study. Once a very homogeneous paste had been obtained, a small quantity was placed betwen CaF_2 crystals; these were mounted on the supports and transmission measured in the FTIR. Prior to sample measurement, spectrophotometry was prepared by running a "background" air absorption spectrum and a Fluorolube IR spectrum, which were later subtracted from the samples to avoid potential interference.

In all cases, IR spectra were recorded by accumulation of at least 32 scans, with a resolution of 2 cm^{-1} in a frequency range of about 4000 to 100 cm^{-1} (mid-infrared spectroscopy). Measurements were carried out in triplicate. The spectral data were processed with the Grams/AI (Thermo Electron Corporation, Waltham, MA) software, which includes baseline correction, smoothing (with a nine-point Savitsky-Golay function) to reduce the noise, and band area measurement.

Because the intensity of the absorption is proportional to the concentration of the adsorbing species, quantitative analysis is possible by FT-IR spectroscopy (Wilson, Slack, Appleton, Sun, & Belton 1993). The area under the acetyl band was measured relative to the area of the CH band. In this way, the pH of the sample was associated with the relative intensity of the acetyl bands and hence with the percentage of acetyl in the KGM chains.

Three independent spectra were taken from each sample and the area of interest was measured. The spectral data were processed with the Grams /AI (Thermo Electron Corporation, Waltham, MA) software and smoothed using the Savitsky-Golay algorithm with nine points to reduce the noise. Baseline corrections were done with a non-automatic function (multipoint correction) and smoothing with a nine-point Savitsky-Golay function to reduce the noise. Carbonyl stretching vibration (C=O) in Acetyl groups at ~1730 cm^{-1}, and –CH stretching vibration at ~2920 cm^{-1} bands area, were measured for each spectrum. The average relative areas and the mean value were then obtained and plotted versus pH by linear regression to each AGD concentration.

2.2.3. Water binding capacity (WBC)

Neutralized gels were cut into small pieces (2 g) and placed in a centrifuge tube (diameter 10 mm) with a filter paper (2 filters Whatman nº 1, diameter 90 mm). The samples were then centrifuged in a Heraeus Multifuge 3L-R (Kendro Laboratory Products, Germany) for 10 min at 3000g and room temperature. WBC was expressed as percent water retained per 100 g water in the sample prior to centrifuging. Measurements were carried out in triplicate.

2.2.4. Colour measurement

The lightness parameter (L^*) was measured five times on the surface of the gel using a colorimeter (Minolta Chroma Meter Cr-200, Japan). The colorimeter was standardized using a white calibration plate. Colour determinations were performed. Determinations were carried out on five surface points of each sample.

2.2.5. Puncture tests

Cylindrical samples (diameter 3 cm x height 3.5 cm) were pierced to breaking point using a TA-XTplus Texture Analyser (Stable Micro System Ltd., Surrey, UK) with a 5 mm–diameter round–ended metal probe. Crosshead speed was 1 mm/s, and a 5 kg load cell was used. The load as breaking force (BF) and the depth of depression as breaking deformation (BD) when the gel sample lost its strength and ruptured were recorded. The measurements were carried out at least in sextuplicate.

2.2.6. Dynamic rheometry measurements

SAOS tests were performed using a Bohlin CVO controlled stress rheometer (Bohlin Instruments, Inc. Cranbury, NJ). The measurements were obtained using parallel-plate geometry (20 mm diameter and 1 mm gap). Before measurement, the gels were tempered at ambient temperature and cut from Petri dishes into disk-shaped slices 20 mm in diameter and 1 mm thick with a 570 S.T.E slicing machine (Germany). They were then put on the lower plate of the rheometer for measurement at 25 °C. Any excess sample protruding beyond the upper plate was carefully removed. Samples were allowed to rest for 15 min before analyses to ensure both thermal and mechanical equilibrium at the time of measurement. Samples were covered with a thin film of Vaseline oil (Codex purissimum) to avoid evaporation. The temperature was controlled to within 0.1°C by a Peltier element in the lower plate which was kept at 25.0 °C.

2.2.6.1. Stress sweep tests. To determine the linear viscoelastic (LVE) region, stress sweeps were run at 6.28 rad/s at 25 °C with the shear stress (σ) of the input signal varying from 0.24 to 1000 Pa. 300 points on the continuous mode were used in all instances. Changes in storage modulus (G'), loss modulus (G'') and loss tangent ($tan\delta$) were recorded. The critical

(maximum) values of shear strain (γ_{max}), and shear stress (σ_{max}) on the limit of LVE range were derived by the method previously described in Campo-Deaño and Tovar, (2009).

2.2.6.2. Frequency sweep tests. Samples were subjected to stress that varied harmonically with time at variable frequencies from 10 to 0.1 Hz. The strain amplitude was set at γ= 0.5 % within the LVE range.

2.2.6.2. Creep and recovery tests. An instantaneous stress σ_0 (30 Pa) corresponding to 0.5% shear strain within the LVE range was applied for 600 s in the creep tests and the resulting change in strain over time γ(t) was monitored. When the stress was released, some recovery was also observed for 600s. The creep and recovery results were described in terms of the shear compliance function, $J(t) = \gamma(t)/\sigma_0$. Compliance curves generated at different linear stress levels overlap, making it possible to examine and compare the structural properties of the different food gels on larger time scales (Steffe, 1996, chap. 5).

All viscoelastic measurements were carried out at least in quintuplicate.

2.2.7. Scanning electron microscopy (SEM)

The 3% KGM gels, pH 9.8 (L1.3) and 10.8 (L1.4) of 3% KGM gels, and the 5% KGM gels, pH 10.7 (L2.4) and 11 (L2.5) were first subjected to a frequency sweep, then 2-3 mm cubes were cut from them for microscopic examination. The samples were then fixed (1:1 v/v) in formaldehyde (4%) and glutaraldehyde (0.2%) in 0.1M phosphate buffer (pH 7.3) and post-fixed with OsO_4, ashed and dried in increasing concentrations of acetone, and critical-point dried as described by Moreno, Cardoso, Solas, and Borderias (2009). They were then sputter-coated (Balzer, SCD004) with gold/palladium and examined in a Jeol Scanning Microscope (Jeol, JSC 6400, Akishima, Tokyo, Japan), at 20 kV.

2.2.8. Statistical analyses

Statistical analysis was carried out using Microsoft Excel software. Data are presented as mean values of at least five independent batches and were tested for each experiment with expanded uncertainty limit (EUL) data as the maximum and minimum deviation from the respective mean value. Trends were considered significant when means of compared

sets differed at $p<0.05$ (Student's t-test).

3. RESULTS AND DISCUSSION

3.1 FT-IR spectroscopy analysis

The *FTIR* spectra of 3 and 5% AGD with and without different amounts of added alkali solution (0.6N KOH) are shown in Fig. 1.

In the IR spectra of samples, the broad absorption band was around 3415cm^{-1} and was attributed to the stretching vibration of O–H groups (Clark and Hester 1978, chap. 3). This absorption band broadened and shifted to a lower wave number with increasing alkali, indicating a gradual increase of intermolecular hydrogen bonding between KGM chains.

The strong bands at about 2920 cm^{-1} and 2850 cm^{-1} correspond to the asymmetric and symmetric -CH_2 stretching modes respectively and the -CH_2 bend band that appears at approximately 1420 cm^{-1} (Vasconcelos, Nunes, & Vasconcelos, 2012). The band at 1730cm^{-1} is due to the stretching of C=O of the carbonyl of acetyl groups in pure KGM. The band at 1645 cm^{-1}, which is attributed to in-plane deformation of the water molecule (Zhang, et al., 2001), reveals water molecule clusters with moderately strong hydrogen bonding with the network (Shen & Wu, 2003; Zhang, et al., 2001)). This band could therefore indicate the presence of intermolecular hydrogen bonds (Damodaran & Paraf, 1997, chap.16) established at the time of hydration.

In FTIR spectra from 3% AGD without alkali (L1.0) and treated with small quantities of alkali (L1.1 and L1.2), medium-high intensity acetyl bands in the range of 1730 cm^{-1} were found (Fig.1-a); these gradually disappeared, indicating a considerable decrease of the acetyl groups when the percentage of alkali increased (L1.3-L1.5). The deacetylation ratio was estimated as a ratio: (acetyl groups removed: total acetyl groups) x100. In the case of 3% KGM, the ratios were: sample with 1% alkali (L1.2), 26% deacetylation; 2% (L1.3), almost 75%; 3% (L1.4), 95%; and sample with 4% alkali the AGD was totally deacetylated.

The 5% AGD obviously needed more 0.6N KOH to achieve more than 90% deacetylation. For 5% AGD, the ratios were: sample with 2% alkali (L2.3), 23%; 3% (L2.4);

58%; 4% (L2.5), 91%. 6% alkali (L2.6) was needed achieve total deacetylation of AGD.

The acetyl band in FTIR spectra was barely perceptible within the pH range 10.8–11.4 in the case of 3 % AGD and 11.0-11.4 in the case of 5 % AGD. Within these pH ranges the percentage of acetyl released by KGM reached the point where structural changes take place, producing a gel suitable for our practical purposes. These are analysed in subsequent sections. The acetyl band was imperceptible and the area was not measurable within the spectrum of 3% AGD at pH 11.9 (L1.6).

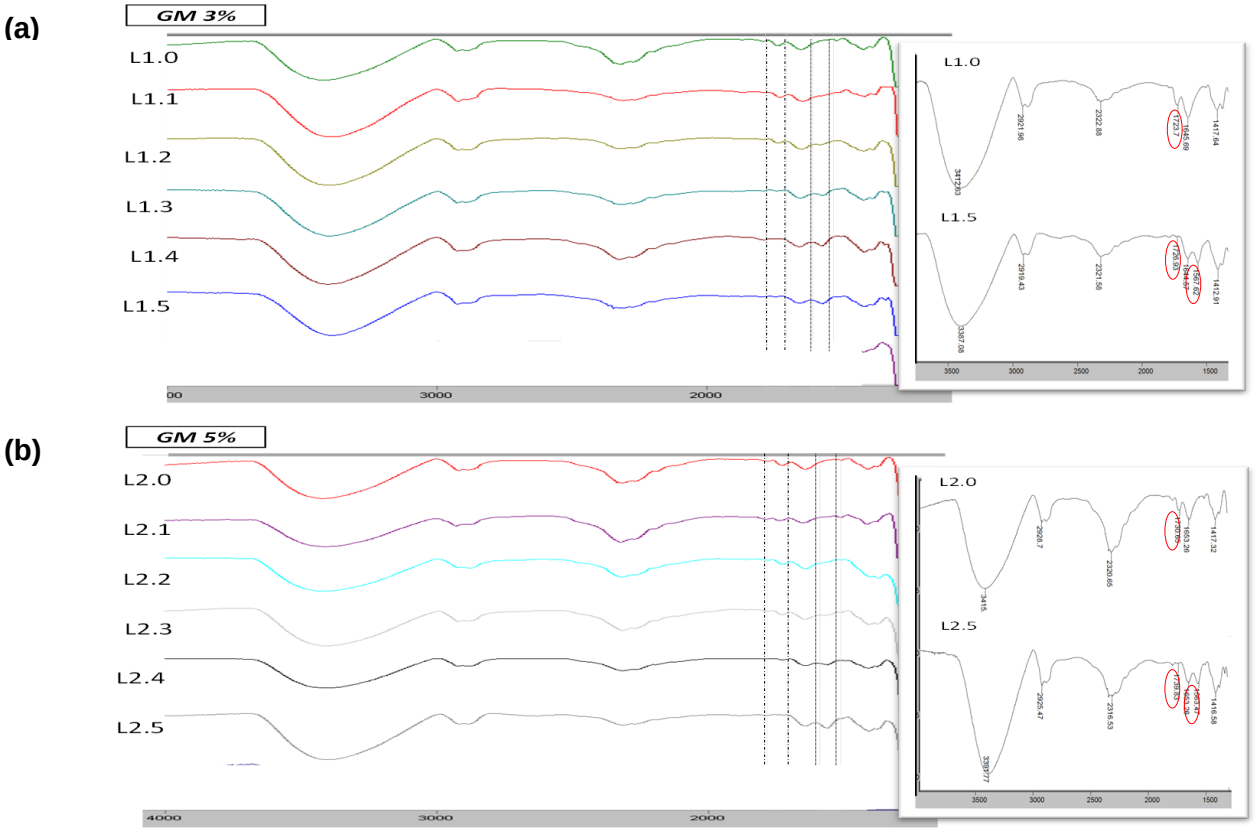

Fig. 1. FTIR spectra of two series of konjac glucomannan with different deacetylation ratio for (a) 3% (w/v) and (b) 5% (w/v) glucomannan different pHs.

As a consequence of the alkali addition there was also a significant increase in the intensity of the band in the region ~1560cm^{-1} (Figure 1), which may be attributed to the acetate anion (CH_3-COO^-) since this band was not found in L1.0 and L2.0 (AGD without alkali) at concentrations of either 3 or 5% AGD, respectively. The intensity of this characteristic band (1560 cm^{-1}) increases with increasing pH, i.e. when KGM chains are being deacetylated. The CH_3-COO^- anion has two resonance forms which are both equivalent, providing more energy stabilization for the acetate group (Allinger, 1988). The energy of this resonance hybrid is less, so that the acetate group can be recognized by its vibration frequency (1560 cm^{-1}), which is lower than that of carbonyl (1730 cm^{-1}) in the acetyl group prior to deacetylation.

The increase of KGM concentration did not influence the characteristic position of different FTIR bands, as the IR spectra were similar at 3% and 5% KGM.

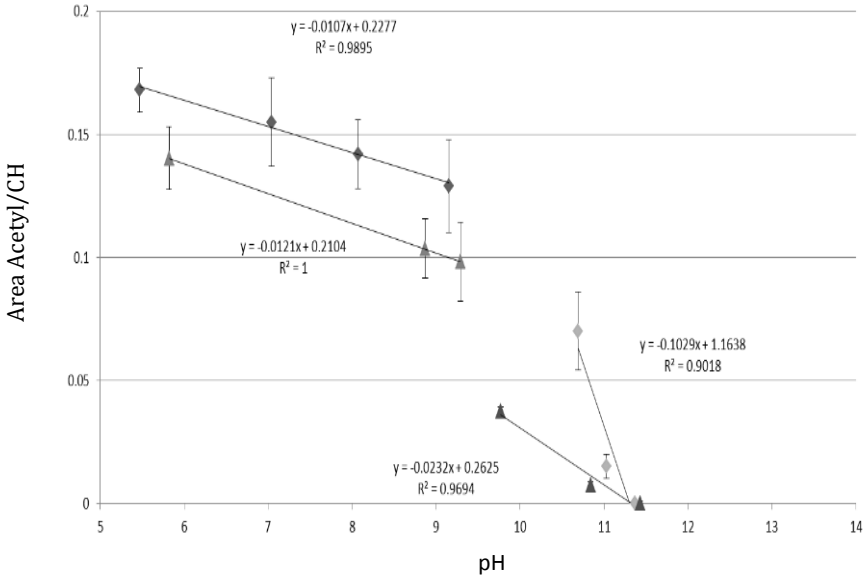

Fig. 2. Acetyl standardized band (FTIR) area of 3% w/v (▲) and 5% w/v (◆) glucomannan samples at various pH. Bars represent expanded uncertainty limit (EUL) for five replications.

Fig. 2 shows the linear dependence between the relative area of acetyl bands of AGD and pH. There was a visible zone of sharp discontinuity (gap zone) in the linear trend between pHs 9.3 (L1.2) and 9.8 (L1.3) in the case of 3% AGD and, 9.2 (L2.3) and 10.7 (L2.4) in the case of 5 % AGD.

These gap zones showed the pH ranges that induced faster alkaline deacetylation and separated the samples in terms of the physical, rheological and structural properties of interest in this study. The increase in the absolute value of the slope at both KGM concentrations indicates that per cent deacetylation was rheological level since the gel was already formed.

3.2. Influence of the deacetylation ratio on moisture, WBC and colour

All KGM gel samples showed very high moisture content (96-97%) indicating the ability of KGM to capture up to 100 g of water per g of sample (Koroskenyi & McCarthy, 2001).

Given that the purpose of this gel is to retain the texture in a network containing the muscle, and given that the WBC of this muscle is very low, these high WBC values of deacetylated KGM are essential to bind enough water to preserve juiciness during any heating process, e.g. cooking.

Table 2 shows the water binding capacity (WBC) of samples L1.3-L1.5 and L2.3-L2.5 together with lightness (L*) values of samples L1.1-L1.5 and L2.1-L2.5.

WBC values were all greater than or equal to 71% in weight (Table 2). In samples L1.4, L1.5 and L2.5, where the deacetylation ratio exceeded 90%, WBC rose significantly. When pH increased, so did the quantity of K^+ cations and OH^- anions in the KGM network, thus raising the density of the electrostatic charge and strengthening the ion-dipole interactions. This electrostatic charge reduced the mobility of the water molecules and facilitated the formation of clusters by hydrogen bonding among water molecules themselves and with OHs of KGM.

Table 2.

WBC (%) and lightness values (L*) of 3 and 5% glucomannan gels at different pHs.

WBC(%)				L*			
GM 3%		GM 5%		GM 3%		GM 5%	
L1.1	n.a. *	**L2.1**	n.a. *	**L1.1**	44.6 ± 3.9 abA	**L2.1**	46.8 ± 2.1d
L1.2	n.a. *	**L2.2**	n.a. *	**L1.2**	45.0 ± 0.9 aB	**L2.2**	45.7 ± 4.1dA
L1.3	71.9 ± 1.1 aA	**L2.3**	71.4 ± 1.5 cA	**L1.3**	48.9 ± 0.7 bcC	**L2.3**	45.9 ± 2.5 dBC
L1.4	76.2 ± 2.5 bB	**L2.4**	72.1 ± 1.9 cB	**L1.4**	49.2 ± 1.0 bcD	**L2.4**	48.0 ± 0.4dD
L1.5	77.3 ± 1.9 bD	**L2.5**	79.4 ± 1.9 dD	**L1.5**	49.9 ± 0.3cE	**L2.5**	54.0 ± 0.7eF

Values are given as mean ± expanded uncertainty limit (EUL)

a-e Different small letters in the same column indicate significant differences among different pHs at fixed glucomannan concentration ($p < 0.05$)

A-F Different capital letters in the same row indicate significant differences between 3 and 5% glucomannan concentration ($p < 0.05$) at similar pH.

(*) n.a. Sample not analysed

The Lightness (L*) values of gels were greater than 44 in all cases. This is similar to the value recorded in a white seafood muscle (Sánchez-Alonso, Careche, Moreno, González, & Medina, 2011), which is interesting if these gels are intended for use in seafood restructurates. The greater the deacetylation, the greater is L* (Herranz et al., 2012). L* values in samples L1.2 and L1.3 differed significantly ($p<0.05$) (Table 2). This is associated with a sharp change of the slope in the linear fit between the area values of the acetyl band and pH (Fig. 2). However, with a KGM concentration of 5 %, L* increased (although not significantly) between L2.3 and L2.4, but did increase significantly ($p<0.05$) between L2.4 and L2.5, with the highest value of L* in L2.5. This high L* value is consistent with the similarly high WBC value and may be related to the strong ion-dipole interaction reported above, so that the water was linked better and the light scattered through the different crystalline regions distributed locally through the network.

At a similar pH, L* did not differ significantly among gels with different concentrations of KGM; significant differences were found only at high pHs (L1.5 and L2.5). Gels with the higher KGM concentration (5%) had a high density of cross-links between chains with more numerous and extended junctions, resulting in a denser network that would scatter the light more intensively and thus increase L* values (Herranz, Borderias, Solo-de-Zaldívar, et al., 2012).

3.3. Influence of the deacetylation ratio on puncture data

The values of breaking force (BF) and breaking deformation (BD) measured at 25 °C are shown in Table 3.

The trend of pH dependence was similar in 3 and 5% KGM gels. There was a large, significant increase in BF between L1.2 and L1.3, and L2.3 and L2.4, in 3% and 5% KGM gels respectively. Levels were similar in L1.4 and L1.3, and slightly lower in L2.5 than in L2.4, indicating that where cross-link density increased most noticeably was immediately after the gap zone, mainly through hydrogen bonding between KGM chains, which improved mechanical properties of their networks like BF making them more rigid. However, neither pH nor KGM concentration caused any differences in BD values. This may be because fracture deformation is controlled by the shape of junctions and their ability to change form

or extend during applied force (Leksrisompong, Lanier, & Foegeding, 2012). The basic interactions within the network are electrostatic, principally hydrogen bonds, together with coulombic, van der Waals and hydrophobic interactions (before and after the gap zone). Thus, molecules associated by interactions of this kind possess a basic mechanism of inter-chain association based on the same physical principle (in both states: before and after gap zone), so the capacity to alter form during applied force is similar. This standardizes their mechanical response to applied stress, so that BD is practically constant in all samples (Table 3). Therefore, if we take the BF and BD data together, we find a considerable increase in the net rigidity of samples after the gap zone, due to enhancement of the number and size of the junction zones produced by more intense deacetylation.

With respect to increased KGM concentration, comparison of samples at similar pH shows that gels with 5% KGM were naturally firmer and stronger with higher breaking force (BF) and similar BD values, because inter-chain associations are more numerous at high polymer concentrations.

3.4. Influence of the deacetylation ratio on SAOS measurements

As in large-deformation measurements (section 3.3), it was decided to characterize the viscoelastic behaviour only of the samples located next to the gap zone (Figure 2), i.e. before (L1.2) and after the gap zone (L1.3 and L1.4) for 3% KGM concentration, and likewise for the 5% KGM concentration: before (L2.3) and after the gap zone (L2.4 and L2.5).

Table 3. Effect of pH, from the beginning to total deacetylation, on breaking force (BF) and breaking deformation (BD) values of glucomannan gels at 3% and 5% glucomannan concentration . T=25 °C.

	BF (N)			BD (mm)			
	GM 3%		GM 5%		GM 3%		GM 5%
L.1.2	0.21 ± 0.02 aA	**L2.3**	1.92 ± 0.24 dBC	**L1.2**	9.53 ± 0.56 fA	**L2.3**	9.84 ± 0.20 hAB
L1.3	1.62 ± 0.09 bC	**L2.4**	4.10 ± 0.18 eD	**L1.3**	9.50 ± 0.27fB	**L2.4**	10.00 ± 0.10 hB
L1.4	1.14 ± 0.14 cE	**L2.5**	3.56 ± 0.47 eF	**L1.4**	8.28 ± 0.47 gC	**L2.5**	8.87 ± 0.47 iC

Values are given as mean ± expanded uncertainty limit (EUL).
a-h Different small letters in the same column indicate significant differences among different pHs at fixed glucomannan concentration ($p < 0.05$).
A-F Different capital letters in the same row indicate significant differences between 3 and 5% glucomannan concentration ($p < 0.05$) at similar pH.

3.4.1. Stress sweeps

The effect of the deacetylation percentage at both KGM concentrations (3 and 5%) on stress and strain amplitudes (σ_{max}, γ_{max},), complex modulus (G^*) and loss factor ($tan\delta = G''/G'$) within the LVE range was examined (Table 4). In the case of 3% KGM, both amplitudes (σ_{max}, γ_{max}) significantly increased ($p<0.05$) between samples L1.2 (pH= 9.3) and L1.3 (pH=9.8), i.e. before and after the gap zone, respectively (Figure 2). This indicates that when there was a considerable increase in the percentage of deacetylation (from 26% to ~75%), there was a strong increase of interchain attractions among KGM molecules, so that numerous large junctions were formed, resulting in a denser and more flexible physical network with higher σ_{max}, and γ_{max} values (Mezger, 2006, chap. 8). Moreover, in these samples G^* increased and $tan\delta$ decreased, both significantly (Table 4), indicating that the increase of rigidity (higher G^* values) was caused by the increased elasticity of the samples ($G'>G''$), and hence $tan\delta$ diminished. For that reason, connectivity was noticeably enhanced after the gap region, so that the L1.3 network was more elastic and compact than the L1.2 network.

In the case of 5% KGM, the analogous gap zone which was found between pH values 9.2 (L2.3) and 10.7 (L2.4) was reflected in a different change in the internal structure of samples, transiting from a weak gel (high $tan\delta$) in L2.3 sample to a strong gel in L2.4 sample (low $tan\delta$ <0.1) (Table 4). The weak-gel character of L2.3 can also be seen in the high σ_{max}, γ_{max} values (Table 4), indicating that the network was more porous and weakly interconnected with smaller junctions; this produced some structural stabilization, basically through hydrogen bonding, enhancing the conformational flexibility of the L2.3 sample at pH= 9.2 and 5% KGM concentration. This is a first step in physical gelling. After the gap zone (L2.4), a strong gel was obtained, as evidenced by a considerable increase in G^* (268%) and a decrease in σ_{max}, more particularly in γ_{max} values between L2.3 and L2.4 samples. This result indicates that between 23% (L2.3) and 58% (L2.4) of deacetylation a continuous network was formed, having larger junctions produced by hydrogen bonds and hydrophobic interactions (Chen, et al., 2011), with a packing effect that increased at pH> 10.7 (L2.4 and L2.5 gels). Increased aggregation of KGM chains (5% KGM) made for tighter networks with high water-retaining ability (Herranz et al., 2012a) as reflected in the fact that the highest values of WBC were recorded in L2.5 gels (Table 2). Also, the network was highly compacted, making for a less ordered structure with more boundaries to scatter light

(Herranz, Borderias, Solo-de-Zaldívar, et al., 2012), as reflected in the fact that L* values were highest in L2.5 gel.

Table 4. Limit values of linear viscoelastic (LVE) range: stress (σ_{max})and strain (γ_{max}) amplitudes, complex modulus (G^*)and loss factor ($tan\delta$) of glucomannan gels among pHs from the beginning to total deacetylation at 3 and 5% glucomannan concentration ν=1Hz ,T= 25 °C.

	σ_{max} (Pa)	γ_{max} (%)	G* (kPa)	*tan δ*
GM 3%				
L1.2	20±2 aA	1.05±0.09 fG	1.99±0.16 iK	0.316±0.019 mQ
L1.3	78±7.8 bC	1.96±0.28 eI	4.08±0.54 jM	0.178±0.063 nS
L1.4	86±8.6 bE	2.42±0.57 eJ	3.77±0.90 jO	0.183±0.078 nU
GM 5%				
L2.3	352±35 dB	12.6±1.4 gH	2.85±0.35 kL	0.657±0.058 oR
L2.4	249±25 cD	2.44±0.46 hIJ	10.5±1.8 lN	0.072±0.006 pT
L2.5	261±26 cF	2.21±0.28 hJ	12.0±1.6 lP	0.087±0.021 pU

Values are given as mean ± expanded uncertainty limit (EUL)
a-p Different small letters in the same column indicate significant differences among different pHs at each glucomannan concentration (p <0.05)
A-U Different capital letters in the same column indicate significant differences between 3 and 5% glucomannan concentration (p <0.05) at similar pH.

3.4.2. Frequency sweeps

The mechanical spectra of the gels with 3% and 5% KGM are shown in Figure 3a and b respectively. In the case of 3% KGM gels, although all samples were more elastic than viscous, L1.2 differed considerably in this respect from L1.3 and L1.4 samples. In both L1.3 and L1.4 (after the gap zone) the relative difference between *G'* and *G''* was greater (*G'*>>*G''*) and there was minimum frequency dependence. These mechanical spectra are characteristic of "strong (true) gels" (Rao, 2007, chap. 8). In both L1.3 and L1.4, *G'* was practically frequency-independent and *G''* increased slightly, particularly at low frequencies (Figure 3a); this rheological response is characteristic of KGM gels made at 25 °C at the highest pH=12 (Herranz et al., 2012a) and stronger gels obtained at high temperature (Herranz et al., 2012c). L1.3 and L1.4 presented greater deacetylation, 75 and 95% respectively (section 3.1.), which promoted the formation of larger, more numerous junctions making for denser and more elastic networks, particularly in the case of L1.4 gel. However, the mechanical spectrum of L1.2 sample was different, firstly in that the relative difference between *G'* and *G''* values over the frequency range was smaller, and secondly in that *G'* and *G''* were considerably more frequency dependent than in L1.3 and L1.4 samples. This was particularly so in the case of *G''* modulus, in the low frequency range (Figure 3a),

where after a plateau region (from 62 to ~10 rad/s) *G''* values decreased continuously down to the lowest frequency (0.62 rad/s). Such behaviour may be summarized by simple power law relations (equation (1)):

$$G' \propto \omega^{p}, G'' \propto \omega^{q} \quad (1)$$

where the exponents *p* and *q* distinguish the different classes of weak gels, in this case at low frequency range (from 7 to 0.6 rad/s). Thus, the values of *p* (0.209) and *q* (0.178) in L1.2 sample are typical of weak-gels (Lapasin and Pricl, 1999). The difference between *p* and *q* was relatively small (15%), suggesting that the transience of the physical network (L1.2) cannot be determined precisely on the present low time scales. It is therefore analysed using creep and recovery tests (next section), which are more time-consuming. The reason for this rheological response is that less acetyls groups were released (25%) since L1.2 sample was an initial step in the 3% KGM gelling process. Thus, there were still enough acetyl groups in the KGM chains to prevent chain associations, making the L1.2 network weaker and less stable. This result is consistent with the lowest BF measured by puncture test (Table 3), and the lowest σ_{max} and γ_{max} and highest $tan\delta$ from stress sweeps (Table 4), as discussed earlier. Moreover, the fact that there were more acetyl groups in the KGM chains in L1.2 sample increased steric hindrance (Chen, et al., 2011), causing a decrease of WBC.

In the case of 5% KGM gels, the difference in the mechanical spectra before (L2.3) and after the gap zone (L2.4 and L2.5) was even more marked than in the case of 3% KGM. Thus, L2.4 and L2.5 were both strong (true) gels, with high elasticity (they were indistinguishable in that respect), while *G'* was approximately 1 or ~2 orders of magnitude larger than *G''*, and both moduli were practically frequency-independent over the frequency range analysed. L2.3 sample (23% deacetylation) presented a frequency dependence profile that was qualitatively similar to that of L1.2 (26% deacetylation), but in this case the decrease of both *G'* and *G''* with frequency was greater, particularly in the low frequency range, converging at the lowest frequency (0.1 Hz) (Figure 3b). Thus, the values of *p* and *q* were higher (*p*=0.462 and *q*=0.226) and the difference (50%) was greater than in 3% KGM gel, so that both elasticity and viscosity decreased with increasing oscillation time (lower frequencies). However, the loss of elasticity was noticeably faster than the loss of viscosity, indicating that the viscoelastic response of L2.3 shifted towards one typical of concentrated solutions, as

generally occurs when the system is close to the sol/gel limit (Lapasin & Pricl, 1999, chap. 4).

Therefore, in order to form a denser, packed network with a high degree of connectivity capable of holding seafood particles, higher values of pH are needed, regardless of KGM concentration. At these high pH values, a large number of acetyl groups may be released, forming junction zones that serve to build highly packed structures.

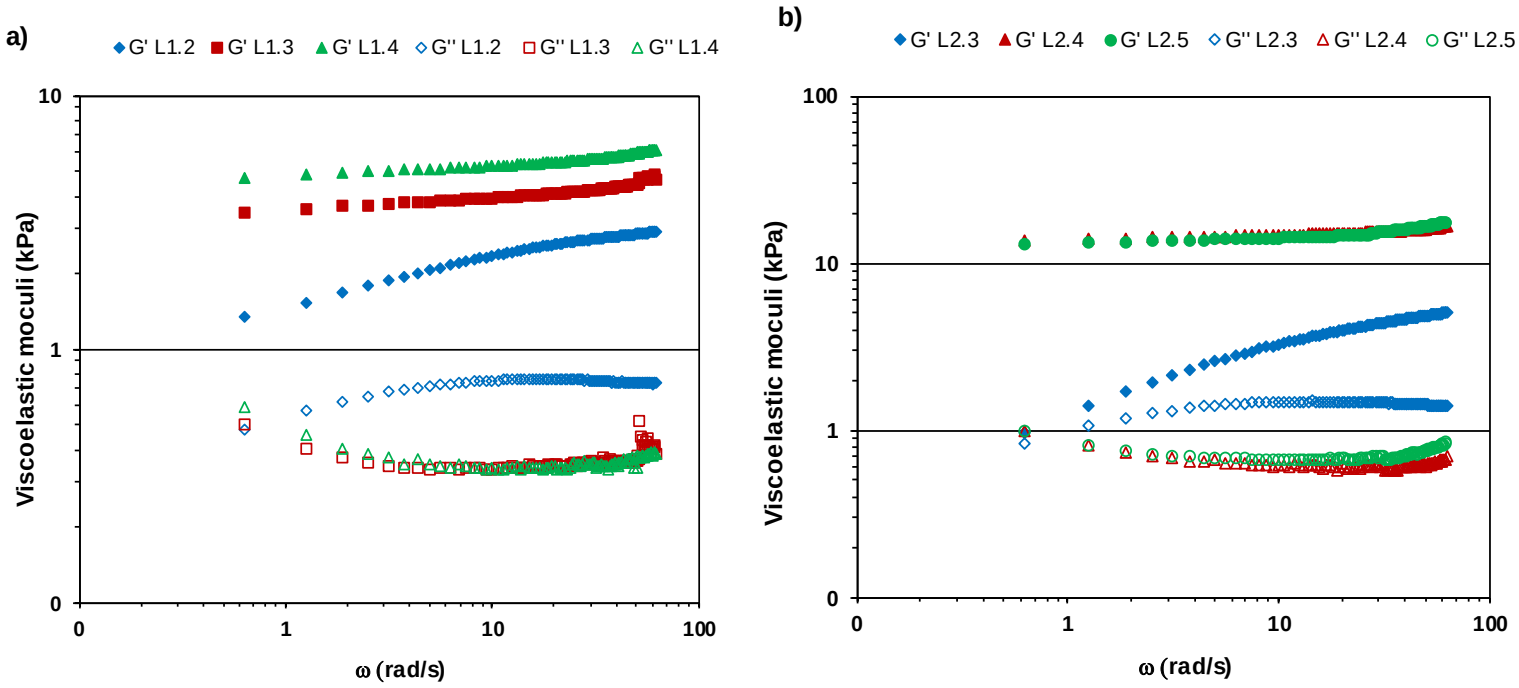

Fig. 3. Mechanical spectra data of (a) 3% (w/v) and (b) 5 %(w/v) glucomannan gels deacetylated with different pH. Open symbols *G''*, closed symbols *G'*. T=25 °C.

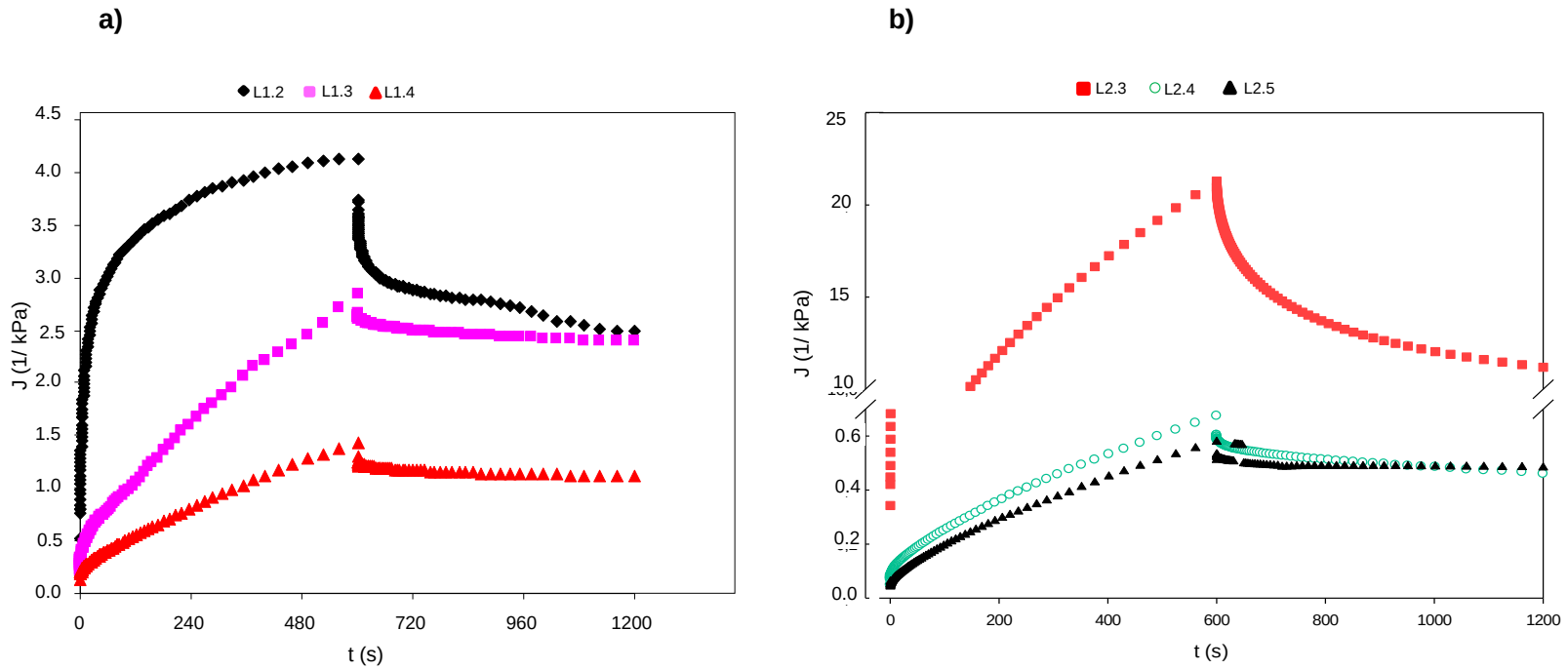

Fig. 4. Evolution of creep and recovery compliances of several aqueous glucomannan dispersions (a) 3% and (b) 5% glucomannan at different values of pH.

3.4.3. Creep and recovery tests.

The transient characteristics of KGM gels can be analysed on longer time scales than are associated with oscillatory tests, by means of creep-recovery experiments.

Fig. 4a and b show creep-recovery compliances *J(t)* for 3% and 5% KGM samples respectively. These data serve to derive the relaxation modulus *G(t)* (Ferry, 1980), which provides the gel strength (*S*) and relaxation exponent (*n*) by means of equation (2).

$$G(t) = S \cdot t^{-n} \qquad (2)$$

In the case of 3% KGM concentration, as occurred in mechanical spectra, the creep-recovery response of L1.2 sample was different from that of L1.3 and L1.4 gels since the *J(t)* values for L1.2 over the creep time were considerably higher than those of L1.3 and L1.4. However *J(t)* values (L1.2) decreased noticeably during the recovery stage and were similar to those of L1.3 gel at the end of the assay (Fig. 4a), indicating that the L1.2 network was softer (creep stage), with greater creep-recovery ability at the end of the test. This result may be explained by a low deacetylation percentage (26%) making for small junctions. This would reduce the inter-aggregate forces (low *S* values) within the network, permitting molecular rearrangements among the KGM chains after the load was removed (high elasticity) (Table 5).

Conversely, L1.4 gel had the lowest *J(t)* values during both creep and recovery stages, and its network had little resilience, being more rigid due to the high deacetylation ratio (95%). Thus, more KGM chains were associated into larger junctions (high *S* value) and permanent cross-linking increased (low *n* exponent), making the network less elastic (Table 5). The creep-recovery response of L1.3 gel was qualitatively similar to L1.4, with higher *J(t)* values (Figure 4a) during both creep and recovery times; this is because the deacetylation ratio was a little lower (75%) than that of L1.4, resulting in a rather less cohesive (low *S* values) and more time-dependent network (high *n* exponent) (Table 5).

In the case of L2.4 and L2.5 (58% and 91% deacetylation, respectively), the higher KGM concentration (5%) produced stronger gels so that *J(t)* values were considerably lower than in 3% KGM (L1.3 and L1.4) Figure 4b. Thus, when KGM concentration increased, resulting in more dense and rigid networks (Herranz et al., 2012d), it did so particularly in the case

of L2.5 because of the high deacetylation ratio, which produced larger junctions resulting in a less flexible network (low % elasticity). For instance, at longer experimental times, it can easily break into short chain fragments, which could explain why the *n* value was higher than in L2.4 gel (Table 5).

However, in the case of L2.3 (23% deacetylation), the *J(t)* values for both creep and recovery times were considerably higher than in L2.4 and L2.5 gels (under stresses within the LVE range). L2.3 is a peculiar sample in that two factors converge that contribute to the connectivity in the network in opposite ways: on the one hand it has less cross-links among chains due to its low deacetylation (low *S* value). And, on the other hand, it has more KGM chains (5% KGM), which naturally increase the number of polymer-polymer associations but contain enough acetyls to exert a considerable steric effect that tends to separate the KGM chains. Thus, the viscoelastic response of L2.3 is essentially time-dependent, as evidenced by the large increase of *J(t)* during creep time (Figure 4b), resulting in a more flexible network with high resilience, as reflected by high elasticity (Table 5).

In short, all the foregoing results indicate that both L1.2 and especially L2.3, are weak gels (more acetylated) that prevent the formation of the numerous large junction zones needed to make strong, time-stable networks, and so they are not suitable for our technological purposes.

Table 5. Power-law parameters of equation 2 for glucomannan gels among pHs from the beginning to total deacetylation at 3 and 5% glucomannan concentration, T= 25°C.

	S (kPa)	**n**	**r^2**	**% Elasticity**
GM 3%				
L1.2	0.85±0.01	0.219±0.004	0.972	40
L1.3	3.70±0.05	0.302±0.008	0.931	16
L1.4	5.77±0.10	0.252±0.01	0,852	22
GM 5%				
L2.3	1.20±0.01	0.498±0.003	0.996	47
L2.4	13.38±0.19	0.262±0.009	0.901	37
L2.5	21.30±0.40	0.320±0.012	0.888	16

3.5. *Scanning electron microscopy.*

Fig. 5 shows scanning electron microscopy (SEM) images at 3500x magnification of the 3% (L1.3 and L1.4) and 5% (L2.4 and L2.5) KGM gels after the gap zone. In general, the

samples showed well-formed and homogeneous networks containing little holes embedded and appeared like true gels under microscopy.

In the case of 3% KGM gels, sample L1.3 (Fig. 5a) showed some discontinuous networks, formed mainly by coarse particles with small "spongy zones" (circled in red). This lack of a continuous spongy matrix (Fig. 5a) is consistent with the fact that both *G'* (mechanical spectrum) and *S* (creep test) were lower than in L1.4 gel. Moreover, the coarse particles (L1.3) suggest less connectivity in the network and may explain why WBC was lower than in L1.4 since the irregular network is more time-dependent, as evidenced by the *n* parameter, which was higher than in L1.4. However, L1.4 gel (Fig. 5b) showed well-defined spongy networks with holes homogeneously distributed throughout the networks, allowing the water molecules to settle inside them (Herranz et al., 2012a). This makes for a more homogeneous structure (higher *S* values) which can better retain water molecules (high WBC), lending the network more elasticity (higher *G'* moduli in mechanical spectrum) and also more time-stability (lower *n* parameter) in creep tests. These results (L1.4) were the consequence of a higher percentage of acetyl release (95%), as discussed in previous sections.

In the case of 5% KGM gels, L2.4 and L2.5 (Fig. 5c and 5d respectively) showed denser, more compact spongy networks than L1.4 (Fig. 5b) because the higher KGM concentration naturally provided more numerous and larger junctions, much as reported previously for 5% KGM gels at pH=12 (Herranz et al., 2012d). Some differences were observed between the two samples (L2.4 and L2.5). L2.4 (Fig. 5c) showed a more "open" spongy matrix, while the L2.5 matrix (Fig. 5d) was slightly compacted. This increased aggregation of the L2.5 network due to the high deacetylation ratio (95%) is consistent with the very high values of *S* and *G**, together with the rigidity of the network indicated by the smaller force required to reach the failure point. This rigidity (L2.5) indicates good ability to transfer the breaking energy from one filament to another (Foegeding, González, Hamann & Case, 1994). It is also apparent in the higher *n*, and in the lower elasticity. This is consistent with the fact that the WBC value was the highest, since a tighter network can better retain the water molecules in the gel, thus reducing the rupture force.

Thus, from all the rheological and structural analyses, it seems that with 3% KGM concentration a very high ratio of acetyl release (95%) is needed to obtain more cohesive, elastic and time-stable networks. With a higher KGM concentration (5%), on the other hand, a moderate degree of deacetylation (58%) would be enough to obtain gels with acceptable rheological characteristics.

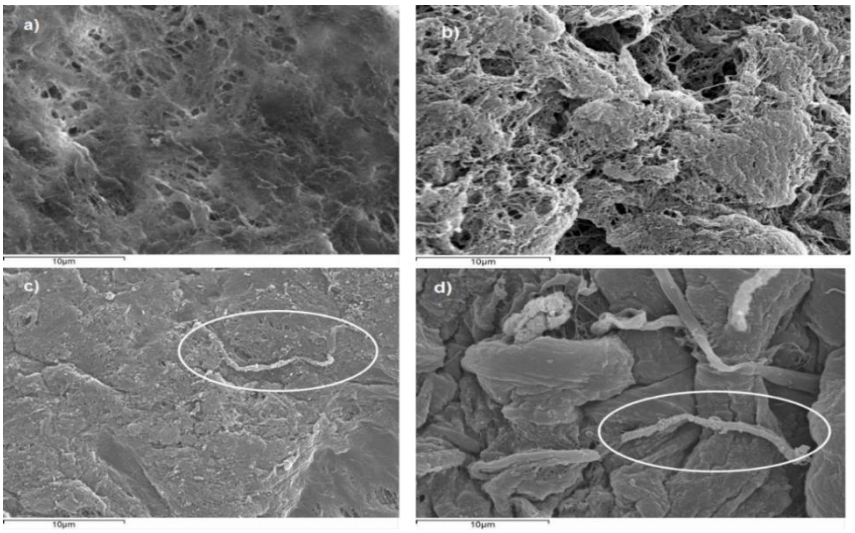

Fig. 5. Scanning electron micrograph (magnification: x3500) of L1.3 (a), L1.4 (b), L2.4 (c) and L2.5 (d) gels. L1.3 was KGM gel at pH = 9.3, L1.4 was KGM gel at pH = 10.8, L2.4 was KGM gel at pH = 10.7 y L2.5 was KGM gel at pH = 11.0.

4. CONCLUSIONS

The addition of different quantities of 0.6N KOH to 3 and 5% AGD was effective in enhancing the gelling ability of KGM in different ways. The difference was essentially dependent on the required deacetylation ratio.

Increased KGM concentration produced two essentially different rheological effects on the internal structure of the samples, depending on the gap found in the linear representation of the relative area of acetyl bands versus pH at either KGM concentration (3 and 5%). At low pH (~9.2) (before gap) the higher KGM concentration (5%) made physical networks less elastic (high $tan\delta$), much more flexible (high γ_{max}), more frequency-dependent (convergence between G' and G'' values at low frequencies in mechanical spectrum) and less time-stable (high n value) than samples with the lower KGM concentration (3%). Conversely, at high pH (~10.7) (after gap), samples containing either 3 or 5% KGM were all true gels, with little frequency dependence and with comparable

conformational flexibility (similar γ_{max}), although 5% samples were naturally more rigid (high values of BF, S and G^*). Therefore, it is reasonable to conclude that the gap zone separates the two basic behaviours of physical networks in terms of the possibility of transition from a gel to a sol state. This is greater in the case of 5% KGM, whose weaker network at pH ~9.2 would qualify as a reversible gel that may revert to a sol over longer times.

Therefore, at similar alkaline pH~10.7, a 3% KGM concentration produced suitable gels with a very high ratio of acetyl release (95%). On the other hand, with the higher KGM concentration (5%), moderate deacetylation (58%) was enough to obtain gels suitable for technological purposes.

ACKNOWLEDGEMENTS

This work has been supported by the Ministry of Economía y Competitividad under the Project AGL-24693 and by the Consejo Superior de Investigaciones Científicas for Predoctoral Scholarship BES-2009-018321. The authors wish to thank the Xunta de Galicia for its financial support under the Consolidation and restructuring program of competitive research units: Strategic Research Partnerships (2009/060).

REFERENCES

Allinger, N. L., Cava, M.P. de Jongh, D.C. Johnson, C.R. Lebel, N.A. and Stevens, C.L. (1988). (2a Ed.). Química Orgánica Vol. 1, Barcelona. Ed. Reverté.

AOAC. (2000). Official method of analysis (17th Ed.). Maryland, USA: Association of Official Analytical Chemists.

Campo-Deaño, L., & Tovar, C. (2009). The effect of egg albumen on the viscoelasticity of crab sticks made from Alaska pollock and Pacific whiting surimi. Food Hydrocolloids, 23, 1641–1646.

Chen, J., Li, J., & Li, B. (2011). Identification of molecular driving forces involved in the gelation of konjac glucomannan: Effect of degree of deacetylation on hydrophobic association. Carbohydrate Polymers, 86(2), 865–871.

Clark, R. J. & Hester, R.E. (1978). Advances in Infrared and Raman spectroscopy. Vol. 5, London: Heyden.

Damodaran, S. &. Paraf, A. (1997). Food Proteins and their Applications. Inc.New York.

Ferry, J. D. (1980). Viscoelastic properties of polymers. (3rd Ed.). New York: John Wiley and Sons, Inc.

Foegeding , E. A., González, C., Hamann, D. D. and Case, S. (1994). Polyacrylamide gels as elastic models for food gels. Food Hydrocolloids, 8(2), 125–134.

Herranz, B., Borderias, A. J., Solas, M. T., & Tovar, C. A. (2012). Influence of measurement temperature on the rheological and microstructural properties of glucomannan gels with different thermal

hisotries. Food Research International 48, 885–892.

Herranz, B., Borderias, A. J., Solo-de-Zaldívar, B., Solas, M. T., & Tovar, C. A. (2012). Thermostability analyses of glucomannan gels. Concentration influence. Food Hydrocolloids, 29(1), 85–92.

Herranz, B., Tovar, C. A., Solo-de-Zaldívar, B., & Borderias, A. J. (2012). Effect of alkalis on konjac glucomannan gels for use as potential gelling agents in restructured seafood products. Food Hydrocolloids, 27(1), 145–153.

Herranz, B., Tovar, C. A., Solo-de-Zaldívar, B. &. Borderias, A. J. (2012). Influence of alkali and temperature on glucomannan gels at high concentration. LWT-Food Science and Technology, 51(2), 500-506.

Kohyama, K., & Nishinari, K. (1990). Dependence of the specific volume of konjac glucomannan on pH. In G. O. Phillips, D. J. Wedlock, & P. A. Williams (Eds.), Gums and stabilisers for the food industry, 5 (pp. 459). Oxford: IRL Press.

Koroskenyi, B., & McCarthy, S. P. (2001). Synthesis of acetylated konjac glucomannan and effect of degree of acetylation on water absorbency. Biomacromolecules, 2(3), 824–826.

Lapasin, R., & Pricl S. (1999). Rheology of Industrial Polysaccharides: Theory and Applications. (1st Ed.). Gaithersburg: Aspen Publishers

Leksrisompong, P. N., Lanier, T. C., & Foegeding, E. A. (2012). Effects of Heating Rate and pH on Fracture and Water-Holding Properties of Globular Protein Gels as Explained by Micro-Phase Separation. Journal of Food Science, 77(2), E60–E67.

Maekaji, K. (1974). Mechanism of gelation of konjac mannan. Agricultural and Biological Chemistry, 38(2), 315–321.

Mezger, T. (2006). The Rheology Handbook. Hannover, Germany: Vincentz Network GmbH & Co. KG,

Moreno, H. M., Cardoso, C., Solas, M. T., & Borderias, A. J. (2009). Improvement of cold and thermally induced gelation of giant squid (Dosidicus gigas) surimi. Journal of Aquatic Food Product Technology, 18, 312–330.

Rao, M. A. (2007). Rheology of fluid and semisolid foods. Principles and applications (2nd Ed.). New York: Springer.

Ross-Murphy, S. B. (1995). Structure–property relationships in food biopolymer gelsand solutions. Journal of Rheology, 39, 1451–1463.

Sánchez-Alonso, I., Careche, M., Moreno, P., González, M. J., & Medina, I. (2011). Testing caffeic acid as a natural antioxidant in functional fish-fibre restructured products. LWT - Food Science and Technology, 44(4), 1149–1155.

Shen, Y., & Wu, P. Y. (2003). Two-dimensional ATR-FTIR spectroscopic investigation on water diffusion in polypropylene film: Water bending vibration. Journal of Physical Chemistry B, 107(18), 4224–4226.

Steffe, J. F. (1996). Rheological methods in food process engineering (2nd Ed) .East Lansing: Freeman Press.

Thomas, W. R. (1997). Konjac gum. In A. Imeson (Ed.), Thickening and gelling agentsfor foods (pp. 169 -179). London: Blackie Academic & Professional.

Vasconcelos, D. C. L., Nunes, E. H. M., & Vasconcelos, W. L. (2012). AES and MR characterization of sol-gel alumina films. Journal of Non-Crystalline Solids, 358(11), 1374–1379.

Williams, M. A. K., Foster, T. J., Martin, D. R., Norton, I. T., Yoshimura, M., & Nishinari, K. (2000). A molecular description of the gelation mechanism of konjac mannan. Biomacromolecules, 1(3), 440–450.

Wilson R. H., Slack, P. T., Appleton, G. P., Sun, L. & Belton, P. S. (1993). Determination of the fruit

content of jam using Fourier Transform Infrared Spectroscopy. Food Chemistry, 47, 303–308.

Zhang, H., Yoshimura, M., Nishinari, K., Williams, M. A. K., Foster, T. J., & Norton, I. T. (2001). Gelation behaviour of konjac glucomannan with different molecular weights. Biopolymers, 59(1), 38–50.

THERMOSTABILITY ANALYSES OF GLUCOMANNAN GELS. CONCENTRATION INFLUENCE

Beatriz Herranz[a*], A. Javier Borderías, Beatriz Solo-de-Zaldívar[a], M. Teresa Solas[c] and Clara A. Tovar[b]

[a]Department of Products, Instituto de Ciencia y Tecnología de los Alimentos y Nutrición (ICTAN-CSIC), José Antonio Nováis 10, 28040 Madrid, Spain

[b]Department of Applied Physics, Faculty of Sciences, University of Vigo, As Lagoas, 32004 Ourense, Spain

[c]Department of Celullar Biology, Faculty of Biological Sciences, Universidad Complutense de Madrid, Madrid, Spain.

Food Hydrocolloids, 29, 85-92.

(DOI: 10.1016/j.foodhyd.2012.02.011)

ABSTRACT

This paper examines the thermo-stability of glucommanan gels (3 and 5 %) deacetylated with 0.6N KOH (*A3, A5*) and 1N NaOH (*B3, B5*) at both concentrations. Gels were heated at heat treatment temperature (T_{ht}): 25, 50, 70 and 90 °C for 20 min. Different analyses: puncture, small amplitude oscillatory strain (SAOS) and scanning electron microscopy (SEM) were made at 25 °C. All gels (irrespective of alkali and glucomannan concentration) behaved as thermo-irreversible with permanently bonded networks. Some structural differences were found depending on the alkali and glucomannan concentration and T_{ht} range: increased T_{ht} produced stronger networks in A3 and B3, which were more orderly in A3. At high $T_{ht,}$ A3 had a more elastic network, with higher water binding capacity (WBC) than B3. At 5% glucomannan, gel networks (A5-B5) were tighter and were less reinforced by temperature. Mechanical and rheological analyses were consistent with the structures shown in SEM photoghaphs.

KEYWORDS: Konjac glucomannan; Alkaline agents; Gelation; Small amplitude oscillatory strain; Structural characterization.

1. INTRODUCTION

Restructured seafood products offer an alternative means of using muscle by-products and developing new products with different textures and/or containing functional ingredients. These structures are usually formed by thermostable gelation of their own protein, but such gelation is not possible when the muscle has previously been heated or the protein is seriously degraded for some reason. In previous work (Herranz, Tovar, Solo-de-Zaldívar, & Borderías, 2012), the authors proposed the use of konjac glucomannan (KGM) as a thermostable gelling agent in whom the minced muscle would act as a filler. That paper assessed glucomannan deacetylation at a constant temperature to make thermostable gels according to the type and concentration of alkali. The most suitable alkaline agents were found to be 0.6N KOH and 1N NaOH. However, the importance of heating for gel structure and rheological properties was not addressed. It was also reported that all the glucomannan gels were physically like most polysaccharide gels. This means these gels are formed basically by hydrogen bonds (Huang, Takahshi, Kobayshi, Kawase, & Nishinari, 2002) and hydrophobic interactions, which may play an important role with

increasing temperature and degree of deacetylation (Chen, Li, & Li, 2011). Moreover, there are strong ion-dipole interactions between cations Na^+ /K^+ and water molecules, which also contribute to the total stiffness of the physical network. These physically cross-linked networks are formed by elastically active or effective chains (EC), chains connecting junction zones, and dangling chains (DC)—chains with one end free and the other attached to any junction. These junction zones have a finite lifetime ("transient networks") due to the molecular reorganization within the network, which is macroscopically deformed under specific thermal conditions; chains under high tension snap off from the junctions and form dangling ends, while some DCs pick up sticky junctions in the course of thermal movement (Tanaka & Edwards, 1992). Temperature is thus the principal thermodynamic parameter since most gelation processes involving polysaccharides result from physical links that are transient however may act as "permanent" cross-links, stabilizing the network depending on the thermal level of the samples (Lapasin & Pricl, 1999, pp. 250-494). The effect of temperature on the microstructure, and hence on rheological properties, is therefore of paramount importance in the study of these physical gels, so that when used in restructured seafood products, these can be pasteurized at a high temperature (T_{ht}) prior to low-temperature storage. This T_{ht} (heat treatment temperature) was applied to the gels, which were deacetylated with 0.6N KOH and 1N NaOH prior to structural and rheological measurements at a constant moderate temperature (25 °C). These measurements help to better ascertain the effect of heating on structural integrity.

Therefore, the objective of this work was to assess the thermostability of glucomannan gels at two concentrations and deacetylated with 0.6N KOH and 1N NaOH, using a puncture test, small amplitude oscillatory shear (SAOS) and scanning electron microscopy (SEM).

2. MATERIALS AND METHODS

2.1. Preparation of samples

3% and 5% (w/v) aqueous solutions of glucomannan from konjac (glucomannan purity 100%, Guinama, Valencia, Spain) were prepared in a homogenizer (Stephan UM5, Stephan u. Söhne GmbH & Co., Hameln, Germany) at 60 °C for 30 min with continuous stirring at low speed. 5% of 0.6 N KOH or 1N NaOH (Panreac Química, S. A., Barcelona, Spain) was then added, followed by mixing for 1 min at 50 rpm to make the gels. Thereafter the methodology described in Herranz et al. (2012) was followed. Petri dishes and cylindrical containers

(diameter 3 cm x 3.5 cm height) were filled with these mixtures; all the samples were then set by heating first at 30 °C for 1 h and then at 5 °C for 4 h to obtain heat-stable gels with pH values between 12.2 and 11.9. These high pH values were reduced by placing samples in a 0.2M citrate-phosphate buffer at pH 5 (the gel:buffer proportion was 1:10) for 20 h at 5 °C. The resulting gels were thermostable with pH around 6.5-6.8. Finally, to mimic different thermal industrial processes, for instance heating to 50 °C – 90 °C, the gels were placed in thermostable plastic bags and heated in a water bath at different temperatures T_{ht} (25, 50 and 90 °C) for 20 min. After that, they were cooled and kept refrigerated (5 ±1 °C). All these gels had a very high moisture content, which was slightly lower (95 ±1%) in the samples with 5% glucomannan than in the samples with 3% (97 ±1%). Analyses were performed after 1 day of chilled storage. All samples were tempered to 25 °C before analysis.

The different lots were named A3, B3, for gels made with 3% glucomannan in water using 5% 0.6N KOH and 1N NaOH, respectively. A5 and B5 are gels containing 5% glucomannan plus 0.6N KOH or 1N NaOH, respectively.

2.2. Analyses

2.2.1. Water binding capacity (WBC)

Gels were cut into small pieces (2 g) and placed in a centrifuge tube (diameter 10 mm) with a filter paper (2 filters Whatman nº 1, diameter 90 mm). The samples were then centrifuged in a Jouan MR1812 centrifuge (Saint Nazaire, France) for 10 min at 3000g and room temperature. WBC was expressed as percent water retained per 100 g water in the sample prior to centrifuging.

2.2.2. Colour measurement

The lightness parameter (L^*) was measured five times on the surface of the gel using a colorimeter (Minolta Chroma Meter Cr-200, Japan). Measurements were analysed using a CIELab scale. The colorimeter was standardized using a white calibration plate. Both WBC and colour measurements were carried out in triplicate.

2.2.3. Puncture test

Cylindrical samples (diameter 3 cm x height 3.5 cm) were pierced to breaking point using a TA-XTplus Texture Analyser (Stable Micro System Ltd., Surrey, UK) with a 5 mm–diameter round–ended metal probe (Herranz et al., 2012). Crosshead speed was 1 mm/s, and a 5 kg load cell was used. The load as breaking force (BF) and the depth of depression as breaking deformation (BD) were recorded when the gel sample lost its strength and broke up. From these data we can calculate a ratio; this gives a new parameter, called the fracture constant (K_f) as it is analogous to the fracture modulus (Gf) from torsional data (Bowland & Foegeding, 1999; Case & Hamann, 1994). K_f was calculated as BF/BD and provided a measure of relative rigidity or firmness of gels at the failure point. All determinations were carried out at least in six replicates, at 25 °C.

2.2.4. Dynamic rheometry measurements

SAOS tests were performed using a Bohlin CVO controlled stress rheometer (Bohlin Instruments, Inc. Cranbury, NJ). The measurements were carried out using parallel-plate geometry (20 mm diameter and 1 mm gap). Gels were cut from Petri dishes into disk-shaped slices 20 mm diameter and 1 mm thickness with a slicing machine (570 S.T.E, Germany). Samples were allowed to rest for 15 min before analyses to ensure both thermal and mechanical equilibrium at the time of measurement. Samples were prepared in the same way as for puncture tests and were covered with a thin film of Vaseline oil (Codex purissimum) to avoid evaporation. The temperature was controlled within 0.1 °C by a Peltier element in the lower plate that was kept at 25.0 °C. No evidence of specimen slippage at the bottom plate was detected in any case.

2.2.4.1.Stress sweep tests. To determine the linear viscoelastic (LVE) region, stress sweeps were run at 6.28 rad/s and 25 °C with the shear stress (σ) of the input signal varying from 0.24 to 1000 Pa. 300 points on the continuous mode were used in all instances. Changes in storage modulus (G'), loss modulus (G''), and complex modulus (G^*) were recorded. The limit values of shear strain (γ_{max}), shear stress (σ_{max}) and the G^* values were evaluated.

2.2.4.2. Mechanical spectra. Samples were subjected to stress that varied harmonically with time at variable frequencies. The strain amplitude was set at γ= 0.5 % within the LVE range

and frequency sweeps were run from 10 to 0.1 Hz. Viscoelastic moduli were obtained as a function of frequency at 25 °C.

Data from the above analyses of lots A3 and B3 at 25 °C were taken from a previous study (Herranz et al., 2012).

2.2.5. Scanning electron microscopy (SEM)

2-3 mm cubes were cut from the gels for microscopic examination. Samples were fixed (1:1 v/v) in formaldehyde (4%) and gluteraldehyde (0.2%) in 0.1 M phosphate buffer (pH 7.3) and post-fixed with OsO_4, ashed and dried in increasing concentrations of acetone, and critical-point dried as described by Moreno, Cardoso, Solas, and Borderias (2009).They were then sputter-coated (Balzer, SCD004) with gold/palladium and examined by a Jeol Scanning Microscope (Jeol, JSC 6400, Akishima, Tokyo, Japan), at 20 kV.

2.2.6. Statistical analyses

Statistical analysis was carried out using Microsoft Excel software. Data are presented as mean values of at least five independent batches and were tested for each experiment with expanded uncertainty limit (EUL) data as the maximum and minimum deviation from the respective mean value. Trends were considered significant when means of compared sets differed at $p<0.05$ (Student's t-test).

3. RESULTS AND DISCUSSION

3.1. Water Binding Capacity (WBC)

The effect of T_{ht} on WBC was different for gels made with 3% glucomannan (A3 and B3) according to the alkali used (Fig. 1). Although non-significant differences ($p<0.05$) were found among the different heating temperatures, if the alkali agent was 0.6 N KOH (A3), WBC tended to increase as T_{ht} increased from 25 to 50 °C, whereas if the alkali was 1N NaOH (B3), WBC decreased a little more sharply between 25 and 50°C. The reason for this difference could be that gel B3 had a more rigid physical network than A3 (Herranz et al., 2012), probably because the gelation rate was higher due to its higher ionic strength (Gao & Nishinari, 2004). This means that at higher ionic strength, as in the case of B3, the resulting junction zones may join more water molecules (more hydrogen bonds). In

addition, the higher alkali concentration (1N NaOH) makes for a greater electrostatic charge density, which would be depleted by when T_{ht} increased. This could result in some loss of KGM functionality, which could explain the decrease of WBC with T_{ht} in B3 gel.

Higher glucomannan concentrations (A5 and B5) do not seem to have reinforced effective WBC (76.7-78%) in the case of lower concentrations (A3 and B3) (64.8%-78.5%) at 25 °C. At higher T_{ht} (50 and 90 °C), it seems that an increase in glucomannan concentration in gels deacetylated with 1N NaOH (B3 and B5) improved the WBC of gels ($p<0.05$). This higher WBC in B5 gels could be due to lower moisture content, so that the breakage of hydrogen bonds (polymer-solvent) diminished when T_{ht} increased. However, no significant differences were found at any temperature when the alkali was 0.6N KOH (A3 and A5), indicating that thermo-stabilization capacity was greater at high T_{ht} irrespective of the glucomannan concentration. Therefore, it seems that the use of KOH could minimize the effect of temperature in a food product with added glucomannan.

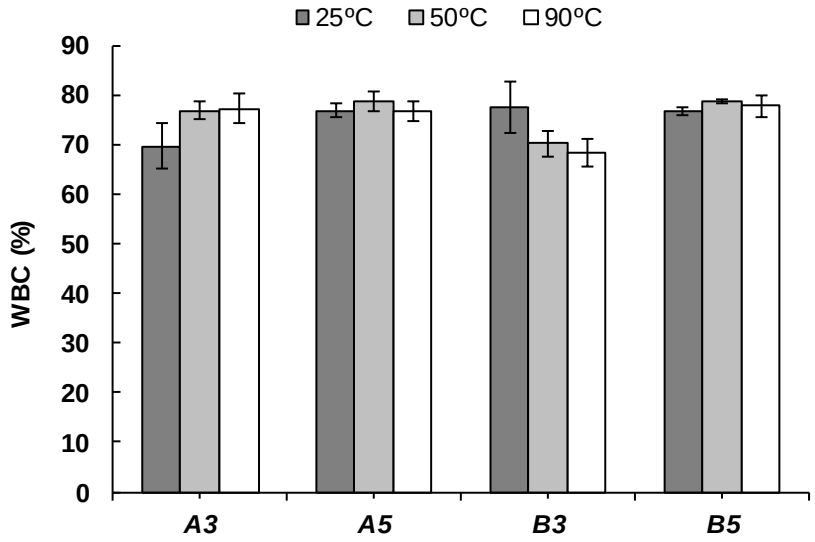

Fig. 1. WBC (%) at heat treatment temperature (T_{ht}) = 25, 50 and 90 °C of glucomannan gels deacetylated with 0.6N KOH at 3-5% glucomannan (*A3-A5*) and 1N NaOH at 3-5% glucomannan (*B3-B5*).

3. 2. Colour

As Table 1 shows, all gels were translucent, with intermediate lightness (L^*) values at any temperature; these values were between 41 and 51.3, the range needed to make a restructured fish product. This L^* was higher in gels made with NaOH (B3 and B5) than with

KOH (A3 and A5) for all T_{ht}, except at 25 °C with NaOH. This could be because of a non uniform distribution of polymer and water causing intense light-scattering (Sperling, 2001), as reported in section 3.6.

There were significant differences ($p<0.05$) at 50 and 90 °C. L^* values were higher in the gels with the higher glucomannan concentration (5%) than in the ones with the lower concentration (3%) for both alkaline agents. This could be explained because the gels at higher concentration have less water content and more density of cross-links between polymer chains. Therefore, their gel networks, have more numerous and extended junction zones that may form traces of cristallinity which may scatter the light becoming more translucent gels.

Table 1. Heat treatment temperature (T_{ht}) dependence on lightness values (L^*) of gels deacetylated with 0.6N KOH and 3-5% glucomannan (A3-A5) and 1N NaOH and 3-5% glucomannan (B3-B5).

Values are given as mean ± expanded uncertainty limit (EUL).

	L*		
	25 °C	**50 °C**	**90 °C**
A3	41.0 ± 1.5e	33.9 ± 1.2g	35.28 ± 0.62g
A5	49.7 ± 1.2ab	39.09 ± 0.34f	40.15 ± 0.54e
B3	45.67 ± 0.48d	46.9 ± 1.5cd	47.19 ± 0.98c
B5	51.3 ± 1.6a	48.76 ± 0.15b	50.06 ± 0.18a

a-g Different letters indicate significant differences ($p < 0.05$).

Park (1996) reported an increase of lightness with the addition of konjac flour in whiting and pollock surimi, suggesting that konjac gels were responsible for the reduced opacity of the surimi gels. The authors have found no more information in the literature regarding colour of glucomanan gels.

Therefore, it seems that, at either glucomannan concentration, L^* tended to improve when the alkali used was NaOH, although L^* values were high in all gels, even at high temperatures.

3.3. Puncture test

The values of BF measured at 25 °C in *A3-B3* and *A5-B5* gels at increasing T_{ht} (25, 50 and 90 °C) are shown in Fig.2a and c, respectively. There was a steep increase of BF as T_{ht} increased at both glucomannan concentrations (3 and 5%). The samples differed

significantly ($p<0.05$) at 25 and 90 °C for both types of alkali at each glucomannan concentration. In addition, BF of *B3* was significantly higher at 50 and 90 °C than in samples *A3* gels. As was to be expected, a higher glucomannan concentration (5%) produced a considerable increase in BF, as the increasing glucomannan concentration favoured interlinking of molecular chains by promoting the formation of a larger number of junction zones, thus producing tighter gel networks. Fig 2b and d show a decrease in BD values in gels made with 0.6N KOH (*A3-A5*), particularly in *A3*, although they were not significantly different ($p<0.05$) from the ones made with glucomannan (*A3-B3*) than with 5% (*A5-B5*), where values remained practically 1N NaOH (*B3-B5*). Furthermore, the T_{ht}-dependence of BD was greater with 3% constant with increasing T_{ht} (Fig. 2d). These BF and BD data can be summarized by the parameter K_f, which gives a measure of gel rigidity respect to alkali and glucomannan concentration at each T_{ht} (Fig. 2e). For instance, if the alkaline agent was 1N NaOH, K_f values were higher at both glucomannan concentrations (higher in *B3-B5* than in *A3-A5*, Fig. 2e), indicating that they were firmer at any temperature. Also, the fact that the relationship between K_f and T_{ht} was proportionate in all gels (irrespective of alkali and glucomannan concentration) suggests a permanently bonded network. Although not covalent in nature, this seems to consist of chemically cross-linked networks (Lapasin and Pricl, 1999), confirming the thermo-irreversibility of these KGM gels as reported elsewhere (Nishinari & Zhang, 2004).

3.4. Effect of temperature (T_{ht}) and glucomannan concentration on the region of linear viscoelasticity (LVE) at 25°C

The principal viscoelastic magnitudes (σ_{max}, γ_{max}, *G**) which define the linear viscoelastic (LVE) range of *A3-B3* gels were examined for T_{ht} dependence and for the effect of KGM concentration from 3% to 5%.

Stress (σ_{max}) and strain (γ_{max}) amplitudes were determined from an automatic analysis (Campo-Deaño & Tovar, 2009). Both $\sigma_{max}/\gamma_{max}$ may be taken as a measure of flexibility conformational and stability structural of gels (Mezger, 2006). The reinforcement of the physical structure in particular is associated with a substantial narrowing of the LVE region (Lapasin & Pricl, 1999). In general, A3-B3 gels behaved as LVE materials when stresses were below σ_{max} =90 Pa, which is comparable to other polysaccharide gels (Pai & Khan, 2002).

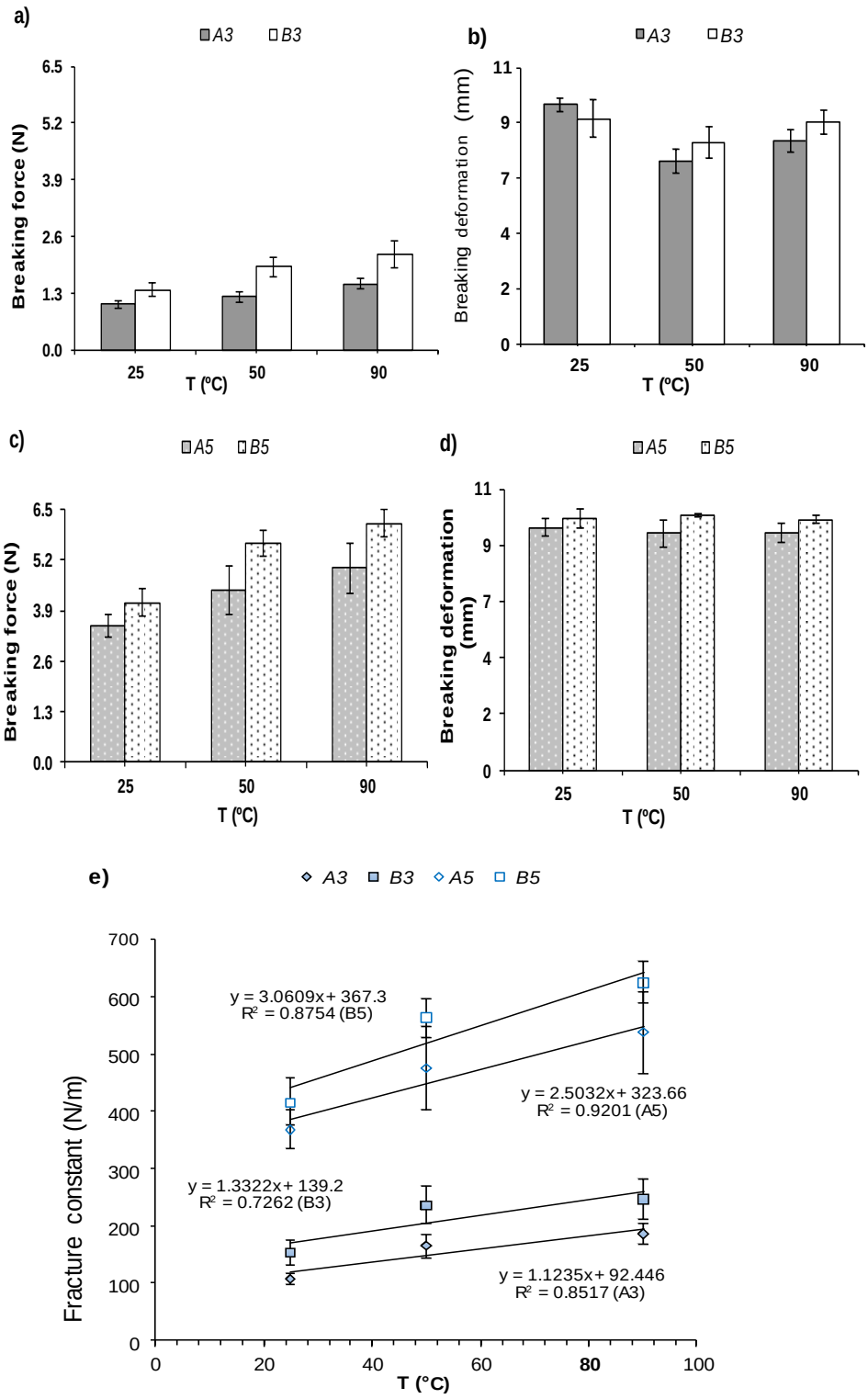

Fig. 2. Influence of heat treatment temperature (T_{ht}) =25, 50 and 90 °C on breaking force [a) - c)], breaking deformation [b) - d)] and fracture constant (e) of glucomannan gels deacetylated with 0.6N KOH at 3-5% glucomannan (*A3-A5*) and 1N NaOH at 3-5% glucomannan (*B3-B5*). T=25 °C.

As regards the effect of T_{ht} on σ_{max} and γ_{max}, the principal differences between *A3* and *B3* were found in the 25-50 °C range. Then for *A3* gel from 25 to 50 °C, γ_{max} slightly increased while for *B3* decreased but not significantly (Table 2). The same thermal effect has also been found for σ_{max} (data not shown). These SAOS results are consistent with the observed decrease of WBC in the same T_{ht} range (25 °C and 50 °C) (Fig. 1). *B3* gel was made with the alkali with the smaller ion size (Na^+) at maximum concentration; as a result, at 25 °C there was a larger number of Na^+ cations, possibly producing a larger quantity of permanently-oriented water dipoles around them. The increased level of electrostatic order, which is highly sensitive to heat (Moore, 1978, chap. 15), may have been more severely disrupted at T_{ht}= 50 °C in *B3*. This is consistent with the fact that the K_f value of B3 due to large deformations increased more between 25 °C and 50 °C (Fig. 2e), since the loss of water entrapped in the network enhanced polymer-polymer interactions, thus contributing to increased hardness.

In *A3* gels structural reinforcement occurred at $T_{ht} \geq 70$ °C, particularly at $T_{ht} = 90$ °C the γ_{max} value was the lowest in all samples (Table 2). Such an irregular trend in the thermal effect on the LVE limit is a normal consequence of the heterogeneity of the number, position and strength of physical junction zones, which fluctuate with time and temperature resulting in a very complex reticular architecture (Lapasin & Pricl, 1999).

There were no statistically significant effects of the alkali on *G** of *A3-B3* gels at fixed T_{ht} (Table 2). *G** is a measure of the overall rigidity of samples which involves both elastic and viscous deformations (Ferry, 1980). In *A3* samples, for instance, there was an unexpected increase of *G** at T_{ht}=90 °C, consistent with narrowing of the LVE limits, as mentioned above. All this indicates that the physical network of *B3* was less temperature-stable than that of *A3*, basically within the low temperature (25-50 °C) range.

Increasing the KGM concentration from 3% to 5% produced a considerable increase in σ_{max}, which was similar in *A5-B5* gels. At 5% of KGM, the LVE region was within $\sigma_{max} \leq 250$Pa, and *G** was naturally greater than at 3% KGM, but without significant differences between alkalis (*A5* and *B5*) at fixed T_{ht} (Table 2).

As regards the T_{ht} effect at 5% KGM, between 25 °C and 50 °C *G** decreased, so that there was some structural weakening of the gel network. In this way the rupture of some elastically active chains into dangling chains may contribute to the net decrease of *G** and

consequently to the softening of this physical network. This is compatible with the increase of firmness (BF) since dangling chains do not contribute to reticular order (Tanaka & Edwards, 1992), but do contribute to the overall mechanical resistance. At $T_{ht} \geq 50$ °C, *G** data were practically indistinguishable irrespective of alkali.

3.5. Influence of temperature (T_{ht}) and glucomannan concentration on mechanical spectra at 25 °C

Mechanical spectra of *A3-B3* gels showed a highly elastic behaviour, with *G'* significantly larger than *G''* (Fig. 3a and b). *G'* moduli showed little variation over the frequency range studied (irrespective of T_{ht} and alkali) and fitted the power law (equation 1).

$$G' = G_0^{'} \cdot \omega^{n'} \qquad (1)$$

In both *A3-B3* gels, *n'* parameters were low, varying slightly with T_{ht}, (Table 3). These low *n'* values show the time-stability of the physical networks and confirm that these are true gels (Ross-Murphy, 1995), which are solid-like in spite of their high water content (95-97%).

Table 2. Heat treatment temperature (T_{ht}) dependence on strain amplitudes (γ) and critical complex moduli (G^*) from stress sweeps at 1Hz in 25 ˚C of glucomannan gels made with 0.6 N KOH and 3-5% glucomannan (*A3-A5*) and 1 N NaOH and 3-5% glucomannan (*B3-B5*).

	γ (%)				G* (kPa)			
	25 °C	**50 °C**	**70 °C**	**90 °C**	**25 °C**	**50 °C**	**70 °C**	**90 °C**
A3	1.24 ± 0.22abc	1.79 ± 0.40c	1.46 ± 0.39abc	1.01 ± 0.22a	5.74 ± 0.96a	5.07 ± 1.2a	4.5 ± 1.4a	7.1 ± 1.5a
B3	1.47 ± 0.26abc	1.29 ± 0.15ac	1.21 ± 0.27abc	1.86 ± 0.68abc	5.7 ± 1.2a	4.94 ± 0.55a	5.6 ± 1.2a	5.7 ± 1.9a
A5	1.18 ± 0.08a	1.49 ± 0.36abc	1.45 ± 0.41abc	1.43 ± 0.39 abc	20.6 ± 1.4c	14.8 ± 3.7b	16.7 ± 5.6bc	15.2 ± 4.7bc
B5	0.99 ± 0.32a	1.24 ± 0.25abc	1.39 ± 0.35abc	1.49 ± 0.25 abc	21.8 ± 6.4bc	15.6 ± 2.9b	14.9 ± 3.2b	16.6 ± 2.7bc

Values are given as mean ± expanded uncertainty limit (EUL).
a-c Different letters indicate significant differences ($p < 0.05$).

Table 3. Heat treatment temperature (T_{ht}) dependence on *Go'* and *n'* parameters from equation of mechanical spectra at 25 °C of glucomannan gels made with 0.6 N KOH and 3-5% glucomannan (*A3-A5*) and 1N NaOH and 3-5% glucomannan (*B3-B5*).

	Go' ± S.D. (kPa·s$^{n'}$)				***n' ± S.D.***				**r^2**			
	25 °C	**50 °C**	**70 °C**	**90 °C**	**25 °C**	**50 °C**	**70 °C**	**90 °C**	**25 °C**	**50 °C**	**70 °C**	**90 °C**
A3	4.81 ± 0.01	4.90 ± 0.01	4.15 ± 0.01	6.29 ± 0.02	0.063 ± 0.001	0.052 ± 0.002	0.044 ± 0.001	0.053 ± 0.002	0.956	0.905	0.906	0.885
B3	6.05 ± 0.01	4.44 ± 0.01	5.05 ± 0.01	5.09 ± 0.01	0.063 ± 0.001	0.055 ± 0.002	0.045 ± 0.001	0.049 ± 0.001	0.950	0.898	0.906	0.921
A5	17.44 ± 0.03	12.94 ± 0.04	10.51 ± 0.03	13.97 ± 0.04	0.056 ± 0.001	0.053 ± 0.002	0.059 ± 0.002	0.055 ± 0.002	0.963	0.890	0.887	0.892
B5	17.15 ± 0.03	14.73 ± 0.03	12.76 ± 0.03	14.29 ± 0.04	0.049 ± 0.001	0.048 ± 0.001	0.050 ± 0.001	0.052 ± 0.002	0.957	0.922	0.921	0.904

Values of *Go'* and *n'* are given as mean ± standard deviation.

In *A3-B3* gels, T_{ht}-dependence on *G'* may be quantified by means of the parameter G_0' (eq. (1)), that is the storage modulus at ω=1 rad/s. Thus, whereas in *A3* gels G_0' values at T_{ht} =90 °C were 23.5% higher than at 25 °C, in *B3* gels they were 16% lower (Table 3). This shows that at high temperature, a gel made with 0.6N KOH (*A3*) entraps water molecules better, which enhances its macroscopic connectivity and hence the storage modulus. This thermal response of *G'* in *A3-B3* gels is consistent with the fact that WBC increased and decreased in *A3* and *B3* respectively with T_{ht}, as mentioned above (Fig. 1). Moreover this result is also consistent with the significant decrease and increase of *L** observed in *A3* and *B3* samples respectively at high T_{ht} (Table 1). When the elasticity of network intensifies as in *A3* samples at high T_{ht}, the network is more homogeneous. The number of non-uniformities diminishes and so the light-scattering is less intense and consequently L* is significantly reduced (Sperling, 2001).

In the case of loss moduli, between 25 and 50 °C *G''* generally decreased at intermediate frequencies. At T_{ht} ≥50 °C in B3, *G''* moduli overlapped (Fig. 3b), whereas in *A3* there were small (non-significant) differences, with an irregular trend in temperatures (Fig. 3a).

On the other hand, in both *A3-B3* gels the *G''* moduli increased only a little in the low frequency range. Table 4 compares experimental *G''* values at the highest (10Hz) and the lowest (0.1Hz) frequencies of the mechanical spectra. At a fixed T_{ht}, when the oscillation frequency diminishes the shear time scale increases, which favours relaxation processes (Lefebvre & Doublier, 2005), so that a larger number of molecular segments connected to junction zones or elastically active chains (EC) are released from them. This increases the number of dangling chains (DC), which can dissipate more energy, and hence *G''* increases. Note in this respect that in *B3* at 25 °C the relative increase of *G''* value from low (0.1 Hz) to high (10 Hz) frequencies was 40%, and this relative difference increased slightly (50%) at T_{ht} ≥50°C (Table 4). Conversely, in *A3* gel, at 25 °C the relative difference between *G''* at 0.1 and 10 Hz was small and non-significant ($p<0.05$), and at T_{ht} ≥50 °C it was about 45% but only significant between 70 °C and 90 °C (Table 4). All this indicates that *A3* gels formed physical networks whose junction zones were more stable in number, size and position with time and temperature.

In general, the irregular trend of G' and G'' data with T_{ht} for each alkali is a characteristic of the complex microstructure of physical networks, since the combined effects of hydrogen bonds weakened with increasing temperature and hydrophobic interactions strengthened with increasing temperature generate a complex thermal response (Case & Hamann, 1994).

At a fixed T_{ht}, increasing the KGM concentration again produced a strong and significant ($p<0.05$) increase in both G' and G'' moduli, as explained above in connection with stress sweeps and puncture data.

The principal difference is that here the T_{ht} increase produced a similar trend in *A5* and *B5* gels, where in both samples G' and G'' were highest at the lowest T_{ht}, (Fig. 3c, d) and almost frequency-independent (Table 3). At high KGM concentrations, the physical networks lost their conformational flexibility, producing a practically unchanging interconnected morphology, irrespective of the alkali. Thus, if T_{ht} increases, the thermal energy will exceed the critical bonding energy, producing excessive molecular rigidity, with minor changes in viscoelastic moduli with time and temperature (Fig. 3c, d).

Table 4. Heat treatment temperature (T_{ht}) dependence on experimental values of loss modulus (G'') at 10 and 0.1Hz from mechanical spectra of glucomannan gels made with 0.6 N KOH and 3-5% glucomannan (*A3-A5*) and 1N NaOH and 3-5% glucomannan (*B3-B5*).

	G"(kPa) at 10 Hz				***G" (kPa) at 0.1 Hz***			
	25 °C	**50 °C**	**70 °C**	**90 °C**	**25 °C**	**50 °C**	**70 °C**	**90 °C**
A3	0.54 ± 0.06cd	0.38 ± 0.07ab	0.28 ± 0.05a	0.41 ± 0.07abc	0.60 ± 0.12cde	0.69 ± 0.24bdefh	0.52 ± 0.10bcd	0.75 ± 0.05ef
B3	0.68 ± 0.18defi	0.34 ± 0.06a	0.34 ± 0.03a	0.36 ± 0.08ab	1.15 ± 0.13gk	0.77 ± 0.20defh	0.66 ± 0.09de	0.76 ± 0.18defh
A5	1.57 ± 0.22kj	0.94 ± 0.16fg	0.71 ± 0.23cdefh	1.20 ± 0.49egj	1.78 ± 0.28j	1.34 ± 0.43hj	1.29 ± 0.36ghj	1.82 ± 0.62jk
B5	1.53 ± 0.33kj	1.20 ± 0.44fgj	1.16 ± 0.55degj	1.36 ± 0.61eghj	1.98 ± 0.45j	1.80 ± 0.45j	1.71 ± 0.87ghij	1.96 ± 0.85jk

Values are given as mean ± expanded uncertainty limit (EUL).
a-k Different letters indicate significant differences ($p < 0.05$).

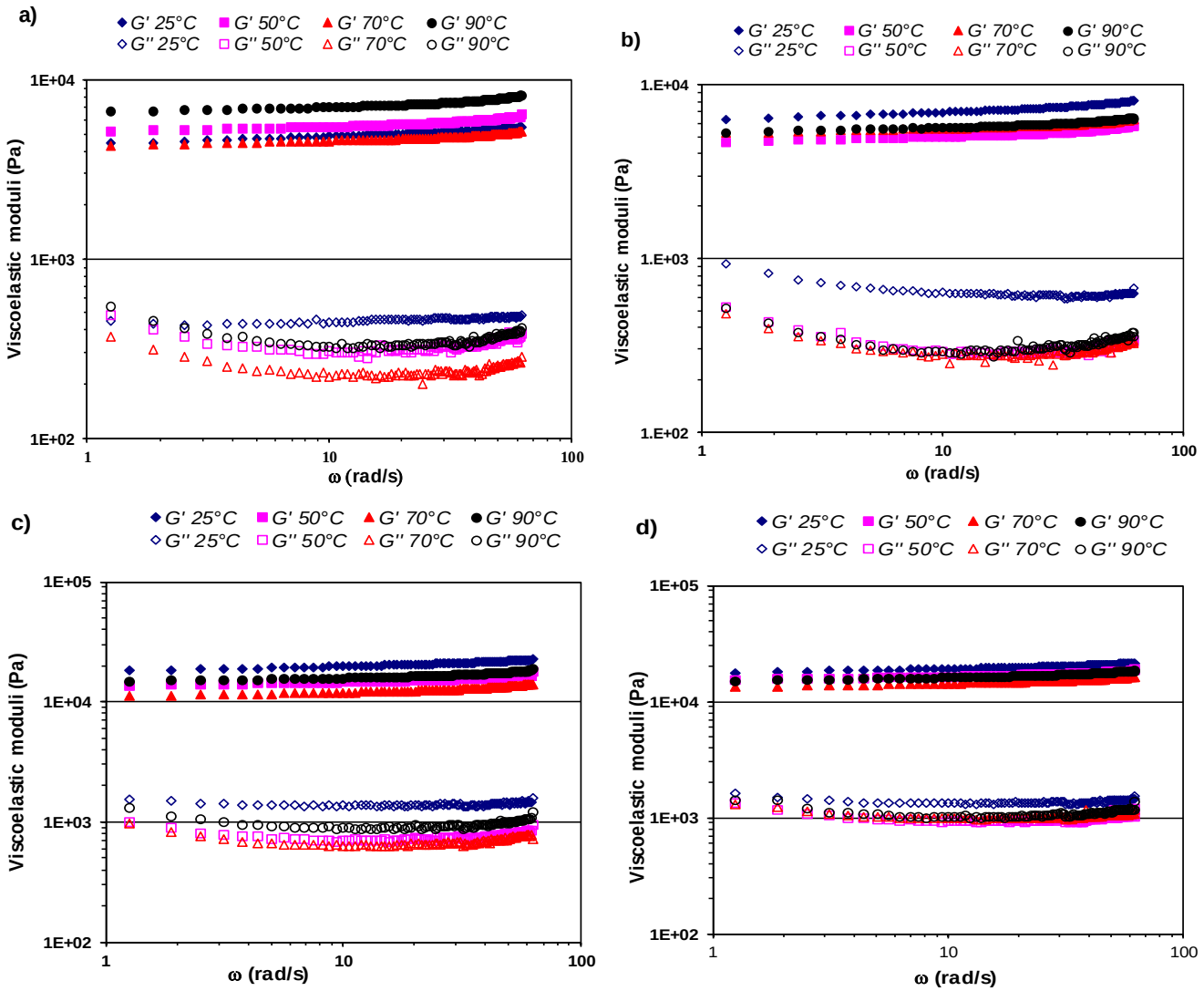

Fig. 3. Influence of heat treatment temperature on Mechanical Spectra at 25 °C, of glucomannan gels deacetylated with 0.6 N KOH at 3-5% glucomannan (*A3-A5*) (a-c) and 1N NaOH at 3-5% glucomannan (*B3-B5*) (b-d). Closed symbols storage modulus (*G'*), open symbols loss modulus (*G"*). T= 25 °C.

3.6. Scanning Electron Microscopy

Fig. 4 shows scanning electron microscopy (SEM) images at 3500x magnification of the 3% glucomannan gels deacetylated with 0.6N KOH and 1N NaOH at T_{ht} = 25 °C (a, b) and T_{ht} = 90 °C (c, d) respectively. In general, the photographs corroborate the behaviour of the gels reported up to now in all the foregoing analysis. Note that at 25 °C both samples *A3* and *B3*, (Fig. 4a, b) showed spongy networks with well-defined holes embedded homogeneously in the matrix, allowing the water molecules to settle there, as indicated by the high WBC values (section 3.1). On the other hand, *A3* (Fig. 4a) had a more homogeneous network than *B3* (Fig. 4b), with much smaller and more compact particles. When the heat treatment temperature was increased to 90 °C (Fig. 4c, d), both networks became more compact and denser, as reflected in the increase in K_f from large deformations. However, the network of *A3* (Fig. 4c) was still homogeneous with few holes, which were much smaller than at 25 °C (Fig. 4a). This is consistent with the simultaneous increase in WBC values, which were significantly higher ($p<0.05$) than at 25 °C, the decrease in L*, which was significantly lower than at 25 °C, and the increase in net elasticity (increase of *G'* and decrease of *G''*), as reported in previous sections.

There were also some large fibre-like fragments (Fig. 4c), as Chin, Gob, and Xiong (2009) found in myofibrillar protein gels made with konjac flour, although in general the structure was very homogeneous. However, when the alkali added was 1N NaOH, although the structure was also spongy at 25 °C (Fig. 4b) as noted above, the particles became larger and more compact and the network denser. This was reflected first in a more rigid network with higher K_f values, and second in a more time-unstable network due to the greater difference between *G''* moduli measured at 10 and 0.1Hz, as reported in previous sections. This increased structural rigidity was enhanced at 90 °C (Fig. 4d), at which point the sponginess disappeared completely and WBC decreased. The network became much more compact and heterogeneous and less orderly because of its higher ionic strength (see Section 3.1). As a result, in this sample with added 1N NaOH, numerous thicker fibre-like filaments appeared entangled among the other coarse particles, producing an appearance like "*joined pieces*" indicating less connectivity, as borne out by the fact that it was less elastic than *A3*.

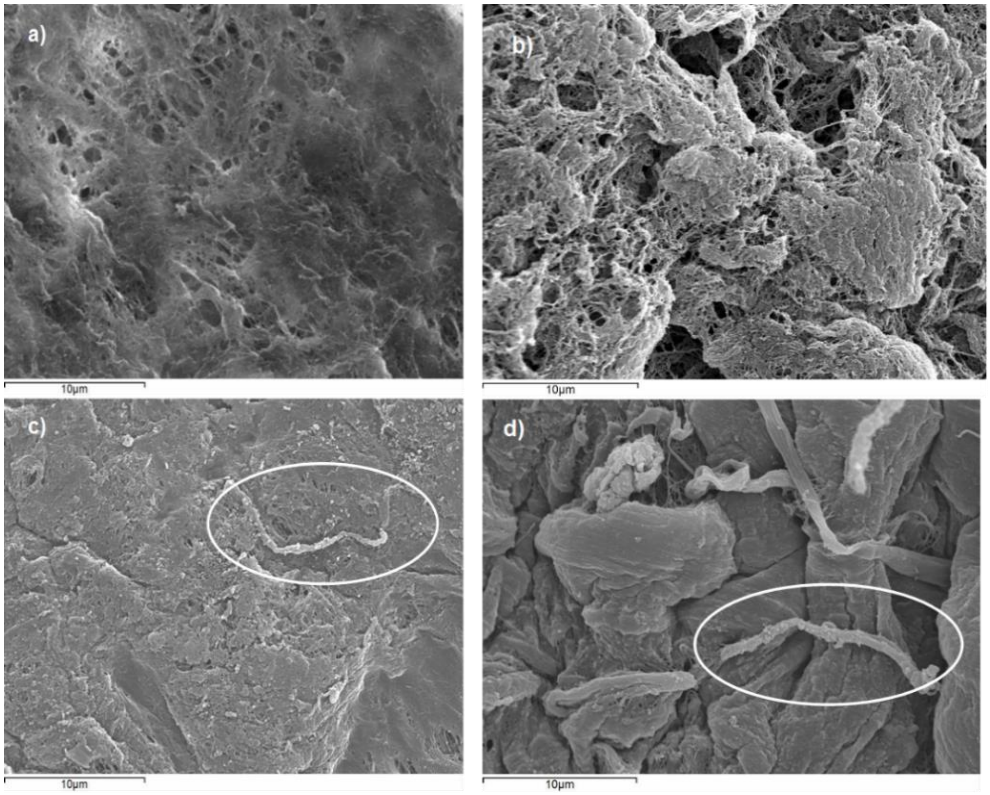

Fig. 4. Scanning electron micrograph (magnification: x3500) of 3% glucomannan gels deacetylated with 0.6N KOH (*A3*) at heat treatment temperature (T_{ht})=25 °C (a), *A3* at T_{ht} = 90 °C (b), with 1N NaOH (B3) at T_{ht} = 25 °C (c), and *B3* at T_{ht} = 90 °C (d).

The images (not shown) of the 5% glucomannan gels (A5 and B5) indicate much more aggregated and denser networks with a "rough" appearance, especially at 90 °C, and hardly any differences between the two alkalis. These networks were characterized by larger particles and many thicker filaments, even at 25 °C, which spread through the network as temperature increased.

4. CONCLUSIONS

Mechanical, rheological and structural data concur in that 0.6N KOH provides better gels with less rigid and more elastic physical networks, which are more stable with time and temperature. This thermo-physical stability was reflected in a slight increase of WBC, and also in increased elasticity and less influence of frequency on viscoelastic parameters due to greater bond flexibility in the network. As a result, the gel matrix was more ordered and homogeneous and had a high level of connectivity.

Testing of linear T_{ht}–dependence of the fracture constant also showed that both kinds of gels were thermo-irreversible and their microstructure was reinforced with increasing T_{ht}. When the glucomannan concentration was increased to 5%, gels were considerably more rigid, retaining their high WBC, colour and strong gel networks but with less temperature-induced strength.

In order to determine the thermal resistance of these gels when restructured fish products are consumed, a follow-up thermo-rheological study is now in progress in which this type of gel is measured at different temperatures to evaluate the thermo-mechanical response when these restructured products are being eaten.

ACKNOWLEDGEMENTS

This work has been supported by the Consejo Superior de Investigaciones Científicas for Postdoctoral Scholarship JAE-DOC-2008 and Predoctoral Scholarship BES-2009-018321 under proyect AGL2008-04892-CO3-C3. The authors wish to thank the Xunta de Galicia for its financial support under the Consolidation and restructuring program of competitive research units: Strategic Research Partnerships (2009/060).

REFERENCES

Bowland, E. L., & Foegedin, E. A. (1999). Small strain oscillatory shear and microstructural analyses of a model processed cheese. Journal of Dairy Science, 84, 2372–2380.

Campo-Deaño, L., & Tovar, C. (2009). The effect of egg albumen on the viscoelasticity of crab sticks made from Alaska pollock and Pacific whiting surimi. Food Hydrocolloids, 23, 1641–1646.

Case, S. E., & Hamann, D. D. (1994). Fracture properties of konjac mannan gel: effect of gel temperature. Food Hydrocolloids, 8, 147–154.

Chen, J., Li, J., & Li, B. (2011). Identification of molecular driving forces involved in the gelation of konjac glucomanann: Effect of degree of deacetylation on hydrophobic association. Carbohydrate Polymers, 86, 956-871.

Chin, K. B. Gob, M. Y., & Xiong, Y. L. (2009). Konjac flour improved textural and water retention properties of transglutaminase-mediated, heat-induced porcine myofibrillar protein gel: Effect of salt level and transglutaminase incubation. Meat Sciences, 81, 565–572.

Ferry, J. D. (1980). Viscoelastic properties of polymers. (3rd ed.). New York: John Wiley and Sons, Inc.

Gao, S., & Nishinari, K. (2004). Effect of Degree of Acetylation on Gelation of Konjac Glucomannan. Biomacromolecules, 5, 175–185.

Herranz, B., Tovar, C.A., Solo-de-Zaldívar, B., & Borderías, A. J. (2012). Effect of alkalis on konjac glucomannan gels for use as potential gelling agents in restructured seafood products. Food Hydrocolloids, 27, 145-153.

Huang, L., Takahshi, R., Kobayshi, S., Kawase, T., & Nishinari, K. (2002). Gelation behaviour of native and acetylated konjac glucomannan. Biomacromolecules, 3, 1296-1303.

Lapasin, R., & Pricl S. (1999). Rheology of Industrial Polysaccharides: Theory and applications. (1st ed.). Gaithersburg: Aspen Publishers,.

Lefebvre, J., & Doublier, J.L. (2005). Rheological behaviour of polysaccharides aqueous systems. In S. Dumitriu (Ed.), Polysaccharides Structural Diversity and Functional Versatility. (pp. 357-394). New York: Marcel Dekker.

Mezger, T. (2006). The Rheology Handbook. Vincentz Network, Hannover, Germany.

Moore W. J. (1978) Química Física, Tomo 2. Urmo, S.A. De Ediciones, Bilbao (Chapter 15).

Moreno, H. M., Cardoso, C., Solas, M. T., & Borderias, A. J. (2009). Improvement of Cold and Thermally Induced Gelation of Giant Squid (Dosidicus gigas) Surimi. Journal of Aquatic Food Product Technology, 18, 312–330.

Nishinari, K., & Zhang, H. (2004). Recent advances in the understanding of heat set gelling polysaccharides. Trends in Food Science and Technology, 15, 305–312.

Pai, V. B., & Khan, S. A. (2002). Gelation and rheology of xanthan/enzyme-modified guar blends. Carbohydrate Polymers, 49, 207–216.

Park, J. W. (1996). Temperature-tolerant fish protein gels using konjak flour. Journal of Muscle Food, 7, 165– 174.

Ross-Murphy, S.B. (1995). Structure-property relationships in food biopolymer gels and solutions. Journal of Rheology, 39, 1451–1463.

Sperling, L.H. (2001). Introduction to physical polymer science. Third Edition. Wiley-Interscience. John Wiley and Sons, Inc., New York.

Tanaka, F., & Edwards, S. F. (1992). Viscoelastic properties of physically cross-linked networks. Transient network theory. Biomacromolecules, 25,15-16- 1523.

INFLUENCE OF ALKALI AND TEMPERATURE ON GLUCOMANNAN GELS AT HIGH CONCENTRATION

Beatriz Herranz[a*], Clara A. Tovar[b,] Beatriz Solo-de-Zaldívar[a], and. A. Javier Borderias[a]

[a]Department of Products, Instituto de Ciencia y Tecnología de los Alimentos y Nutrición (ICTAN-CSIC), José Antonio Nováis 10, 28040 Madrid, Spain

[b]Department of Applied Physics, Faculty of Sciences, University of Vigo, As Lagoas, 32004 Ourense, Spain

LWT-Food Science and Technology, 51, 500-506.
(DOI: 10.1016/j.lwt.2012.11.023)

ABSTRACT

The effect of type and concentration of alkali on 5 g/100mL of glucomannan gels was examined in depth by mechanical and thermo-rheological analysis. Four KGM gels were made from aqueous glucomannan solutions deacetylated with 0.6 mol/L and 1 mol/L of KOH (A_0 and A_1) and NaOH (B_0 and B_1), respectively. At 25 °C all physical networks were too tight and dense due to the large number of extended junction zones, as indicated by the high breaking force (BF), complex modulus (G^*) and low strain amplitude (γ_{max}). The influence of alkali concentration was noticeable only in KOH gels, of which A_0 was significantly more elastic and time stable than A_1 over both short and longer time scales. 0.6 mol/L KOH was the most suitable alkali, producing the most stable and homogeneous gel, with the highest level of connectivity, the lowest relaxation exponent (n) and the highest gel strength (S).

Increasing temperature resulted in loss of elasticity in A_0, where γ_{max}, G^* and S decreased, and the loss factor ($tan\delta$), creep and recovery compliances (J) and n increased. Thus, the networks become less flexible and more unstable at high temperatures, especially between 25 and 50 °C.

KEYWORDS: Glucomannan; Rheological analysis, Gelling agent, Deacetylation

1. INTRODUCTION

Konjac glucomannan (KGM), a neutral hydrocolloid extracted from *Amorphohallus konjac* C. Koch, is a gelling agent proposed in previous work for making restructured fish products (Herranz, Tovar, Solo-de-Zaldívar, & Borderias, 2012) so that it forms thermally stable gels with alkaline coagulant. Williams, Foster, Martin & Norton (2000), among others, had studied deeply the role of the alkali in deacetylation mechanism at low KGM concentrations. Based on this property, it was thought that KGM could act as a gelling agent by forming a network in which the fish particles could be incorporated as a filler.

Some alkalis at various concentrations were assessed for 3 g/100mL aqueous glucomannan dispersions. 0.6 mol/L KOH and 1 mol/L NaOH were chosen as the best alkaline agents for making well-structured gels. 0.6 mol/L KOH in particular produced good gels with better elasticity and structural stability with time (Herranz, Tovar, et al., 2012). In addition, these glucomannan gels, deacetylated with 0.6 mol/L KOH and 1 mol/L NaOH at

two different concentrations (3 and 5 g/100mL), were assessed for thermostability; however, this study focused only on a pre-heating treatment (T_{ht}) from 50 to 90 °C, before low-temperature storage (5 °C) (Herranz, Borderias, Solo-de-Zaldívar, Solas, & Tovar, 2012).

In this study (Herranz, Borderias, et al., 2012), small amplitude oscillatory shear (SAOS) data indicated that interactions among KGM chains involved sizable regions, which formed multi-extended junctions of different strengths, distributed heterogeneously within the gel network (Lapasin & Pricl, 1999). These physical cross-links were transient in nature so their number and position fluctuated with time and temperature (Rao, 2007, chap. 6). However, 5 g/100mL glucomannan gel networks made with 0.6 KOH and 1mol/L NaOH were more stable to frequency and much less temperature-dependent than those with 3 g/100mL glucomannan made with the same alkalis. However, this paper did not include a thorough rheological study of the use of these alkalis (KOH and NaOH) at both concentrations (0.6 and 1 mol/L) in order to choose the most suitable at glucomannan concentration of 5 g/100mL. Glucomannan gels at these high concentations (5 g/100mL) are of paramount importance for making restructured seafood products from certain cephalopod and other seafood muscles, so that a more rigid and elastic texture is required to imitate the “peculiar” texture of these seafood muscles.

Therefore, the aim of this paper was to examine the influence of concentration and type of alkali on the structural conformation of networks containing 5 g/100mL glucomannan, and to assess the thermal and time stability of the gel made with the most suitable alkali by means of mechanical and SAOS analyses.

2. MATERIALS AND METHODS

2.1. Preparation of glucomannan gels

Aqueous solutions (5 g/100mL (w/v) of glucomannan from konjac (glucomannan purity 100 g/100g, Guinama, Valencia, Spain) were prepared by continuous stirring for 30 min at low speed in a homogenizer (Stephan UM5, Stephan u. Söhne GmbH & Co., Hameln, Germany) at 60 °C. Then, 5 mL/100mL (v/v) of alkali coagulant (NaOH or KOH) at a

concentration of 0.6 or 1 mol/L of (Panreac Química, S. A., Barcelona, Spain) was added, mixing for 1 min at 50 rpm to induce gel formation. After that, the methodology described in Herranz, Tovar, et al. (2012) was followed. Petri dishes and cylindrical containers (diameter 3 cm x 3.5 cm height) were filled with these mixtures; then, the samples were set first by heating at 30 °C for 1 h and then at 5 °C for 4 h to obtain heat-stable gels. Finally, the high pH of the samples (12.26-12.19) was brought down to 6.68-6.52 by placing them in pH 5 citrate-phosphate buffer (gel:buffer proportion 1:10) for 20 h at 5 °C. These neutral gels, with a high moisture content (95.29-94.90 g/100g), were then kept refrigerated (5 ±1 °C). Analyses were performed at day 1 after gel preparation.

The different lots were named A_0 and A_1 for gels made with 0.6 and 1mol/L of KOH respectively, and B_0 and B_1 for the gels made with 0.6 and 1g/100mL of NaOH respectively.

2.2. Analyses

2.2.1. Proximate analysis

The pH was measured using a model 9165BNWP pH probe (Analítica Instrumental, S.A., Barcelona) inserted in the gel. The pHmeter was an Orion model 720A (Analítica Instrumental, S.A., Barcelona).

Water content was determined by drying the sample to constant weight at 110 °C and the results are expressed as a percentage (AOAC, 2000). The measurements were carried out in triplicate.

2.2.2. Water binding capacity (WBC)

Gels were cut into small pieces (2 g) and placed in a centrifuge tube (diameter 10 mm) with enough filter paper (2 filters Whatman no. 1, diameter 90 mm). Then the samples were centrifuged in a Jouan MR1812 centrifuge (Saint Nazaire, France) for 10 min at 3000g at room temperature. WBC was expressed as per cent water retained per 100 g water present in the sample prior to centrifuging.

2.2.3. Colour measurements

Lightness (L^*), was analysed using a CIELab scale. Measurements were made using a colorimeter (Minolta Chroma Meter Cr-200, Japan). The colour coordinates were measured five times on the surface of the gel at three different analysis times (on days 0, 1 and 10 of chilled storage). Before use, the colorimeter was standardized using a white calibration plate.

2.2.4. Puncture tests

Cylindrical samples (diameter 3 cm x height 3.5 cm) were filled after gel preparation. After neutralization in the buffer probes they were removed from the cylindrical cells and placed in thermostable plastic bags. They were heated in a water bath at 25 °C (for gels A_0, A_1, B_0, B_1), 50 °C and 70 °C (for A_0) for 20 min. Gels were then immediately pierced to breaking point using a TA-XTplus Texture Analyser (Stable Micro System Ltd., Surrey, UK) with a 5 mm–diameter round–ended metal probe. Crosshead speed was 1 mm/s, and a 5 kg load cell was used. The load as breaking force (BF) and the depth of depression as deformation (BD) when the gel sample lost its strength and ruptured were recorded. These data were used to determine the ratio (BF/BD), i.e. the fracture constant (K_f), which is a measure of the rigidity of gels at the failure point (Herranz, Borderias et al., 2012). The measurements were carried out at least in sextuplicate for each T_m value.

2.2.5. Dynamic rheometry measurements

Small amplitude oscillatory shear (SAOS) testing was performed using a Bohlin CVO controlled stress rheometer (Bohlin Instruments, Inc. Cranbury, NJ). The measurements were carried out using parallel-plate geometry (20 mm in diameter and 1 mm gap). Definitive gels were cut into disk-shaped slices 20 mm in diameter and 1 mm thick on a 570 S.T.E slicer (Germany). Any excess sample protruding beyond the upper plate was carefully removed. Samples were allowed to rest for 15 min before analysis to ensure both thermal and mechanical equilibrium at the time of measurement. Samples were covered with a thin film of Vaseline oil (Codex purissimum) to limit evaporation. The temperature was controlled to within 0.1°C by a Peltier element in the lower plate and was kept at 25.0°C (for gels A_0, A_1, B_0, B_1),, and 50.0, and 70.0 °C for A_0.

2.2.5.1. Stress sweep tests. To determine the linear viscoelastic (LVE) region, stress amplitude sweeps were run at 6.28 rad/s, and 25, 50 and 70 °C. The amplitude sweeps were conducted by varying the shear stress (σ) of the input signal from 0.24 to 1000 Pa. 300 points in the continuous mode were used in all instances. Changes in storage modulus (G'), loss modulus (G'') and complex modulus (G^*) were recorded. The critical (maximum) values of the amplitude sweeps—shear stress (σ_{max}) and shear strain (γ_{max}) at which the G^* values are just beginning to show a noticeable deviation from the previously constant values—were determined from these data. The range of tolerable deviation (± 10 %) was proposed and verified in previous works (Herranz, Borderias et al., 2012).

2.2.5.2. Mechanical spectra. Samples were subjected to stress that varied harmonically with time at a variable frequency. The shear strain amplitude was fixed at γ = 0.5 %; within the linear viscoelastic range. Frequency sweeps were run from 10 to 0.1 Hz, and measurements were made at 25, 50 and 70 °C. The complex modulus (G^*), storage modulus (G'), loss modulus (G''), and loss factor ($tan\delta$), were measured for each frequency value.

Data on stress and frequency sweeps of lots A_0 and B_0 at 25 °C were taken from a previous paper (Herranz, Borderias et al., 2012).

2.2.5.3. Creep and recovery tests. An instantaneous stress σ_0 (95 Pa) was applied for 600 s to each sample in the creep tests and the resulting change in strain over time was monitored. When the stress was released, some recovery was also observed for 600s. Creep measurements were made over the linear viscoelastic range on each sample (σ_0 corresponding to 0.5%shear strain). The creep and recovery results are described in terms of the shear compliance function, $J(t) = \gamma(t)/\sigma_0$.

For gels A_0, A_1, B_0, B_1 the measurements were made at 25.0 °C, and for A_0 also at 50.0 °C, and 70.0 °C.

2.2.6. Statistical analyses

At least five independent batches were tested for each experiment and data are presented as averages. Statistical analysis was carried out using Microsoft Excel software. Trends were considered significant when means of compared sets differed at $p < 0.05$ (Student's t-test).

3. RESULTS AND DISCUSSION

3.1. Selection of the most suitable alkali agent for 5g/100mL glucomannan gels

3.1.1 Water Binding Capacity (WBC) and colour measurements (L)*

All samples (A_0, A_1, B_0 and B_1) showed high WBC (g/100g), around 70g/100g without any significant differences among them (Table 1). This high capacity of water absorbency has been reported previously (Kök, Abdelhameed, Ang, Morris, & Harding, 2009; Yassen, Herald, Aramouni, & Alavi, 2005). Although there were no significant differences among them, the values for gels made with NaOH at 0.6 and 1mol/L (B_0 and B_1) were slightly higher than those with KOH (A_0 and A_1,). This trend was also observed in 3g/100mL glucomannan gels and may be related to the fact that Na^+ has a higher hydratation number than K^+ (Moore, 1978, chap. 10).

Table 1. Water binding capacity WBC (%) and lightness values (L*) of 5 g/100mL glucomannan gels.

	WBC (%)	L*
A0	70.2 ± 2.7a	49.7 ± 1.1d
A1	66.4 ± 4.9a	51.57 ± 0.70e
B0	72.1 ± 6.6a	52.99 ± 0.61f
B1	73.5 ± 7.4a	51.25 ± 1.5def

Values are given as mean ± expanded uncertainty limit (EUL).
a-f Different letters in the same column indicate significant differences ($p < 0.05$) among 5 g/100mL of glucomannan gels at 25°C.

As regards colour (*L**), all gels were translucent with statistically indistinguishable values ($p<0.05$) (Table 1) and therefore may be suitable for the further use as functional ingredients for restructured fish products.

3.1.2 Puncture test

With a 5 g/100mL concentration of glucomannan, gels made with NaOH (B_0 and B_1) were more rigid, with higher breaking force (BF), although not significantly so, than those deacetylated with KOH irrespective of the alkali concentration (Table 2). In addition, BF was not affected by increasing the concentration of each kind of alkali from 0.6 to 1 mol/L (Table 2).

At 5 g/100mL glucomannan, physical gels have larger number of extended junctions, heterogeneously distributed in a tighter network. Na^+ cations have a denser electrostatic charge (the same positive charge in the smaller radius) than K^+ cations, and so Na^+ cations can produce stronger ion-dipole interactions with water molecules and with OH groups of KGM chains, producing a higher level of dipolar order within the network (Herranz et al., 2012a), which is entirely consistent with the higher WBC of gels B_0 and B_1, (3.1.1 section).

The breaking deformation (BD) of A_0, B_0 and B_1 were practically indistinguishable; however, it was significantly lower in A_1 ($p<0.05$), so that gel A_1 was brittle and less stable than the other gels. This result was reflected in the fact that the fracture constant (K_f), which provides an average measurement of gel rigidity, was significantly ($p<0.05$) higher than in gel A_0 (Table 2) (Herranz, Borderias, et al., 2012).

Table 2. Breaking force (BF), breaking deformation (BD) and fracture constant (K_f) of 5 g/100mL KGM gels deacetylated with different alkalis and concentration at 25 °C.

	BF (N)	BD ($x10^{-3}$ m)	K_f (N/m)
A_0	3.50 ± 0.28a	9.49 ± 0.36c	369 ± 40d
A_1	3.71 ± 0.61a	7.19± 0.88b	516 ± 95e
B_0	4.14 ± 0.47a	9.40 ± 0.57c	440 ± 57de
B_1	4.11 ± 0.36a	9.85 ± 0.39c	417 ± 40de

Values are given as mean ± expanded uncertainty limit (EUL).
a-e Different letters in the same column indicate significant differences ($p<0.05$) among 5 g/100mL glucomannan gels at 25 °C.

3.1.3. Influence of the alkaline agent in 5 g/100mL glucomannan gels on SAOS measurements at 25 °C.

3.1.3.1. Stress sweeps at 25 °C. The linear viscoelastic (LVE) range was determined to test the influence of the alkaline agent and its concentration on the conformational stability of the four 5 g/100mL KGM gels.

Stress amplitudes were all similar in A_0, A_1, B_0 and B_1 gels (σ_{max}~210 Pa) irrespective of the alkali concentration, and strain amplitudes were likewise similar for all gels (γ_{max} ~ 1%). The same applies to the rest of the viscoelastic parameters at the limit of the LVE region, such as complex modulus (G^*~19 kPa) and loss factor (tanδ~0.11). All these data indicated that with a 5 g/100mL glucomannan (GM) concentration physical networks were too rigid and tight (high G^* values and low γ_{max}), so that the effect of a particular alkaline agent and

its concentration on conformational flexibility could not be distinguished at the limit of the LVE region.

However, when the same alkaline agents at the same concentration were used at a GM concentration of 3 g/100mL, there were significant differences in σ_{max} and γ_{max} gels at 25 °C (Herranz, Tovar, et al., 2012).

3.1.3.2. Frequency sweeps at 25 °C. Fig. 1 shows the mechanical spectra of the four gels deacetylated with the different alkalis. All gels showed solid-like behaviour and elastic character under sinusoidal stress at a small strain and variable frequency. Values of *G'* were considerably larger than those of *G''* over the entire frequency range. *G'* showed little frequency dependence, i.e. when frequency decreased *G'* diminished slightly (top-Fig. 1). Frequency had the opposite effect on *G''* moduli: at low frequencies, *G''* increased a little and was highest at the lowest frequency (bottom-Fig. 1). This particular behaviour of *G''* can be clearly seen if we compare the experimental values of *G''* at the highest frequency (10 Hz) and the lowest (0.1 Hz) (Table 3). There was a relative increase of *G''* from 10 Hz to 0.1 Hz, which was lowest in A_0 (13 %) and highest and significant in A_1 (51%). As regards of gels deacetylated with NaOH, the relative increase of *G''* in this frequency range was similar at both alkali concentrations: i.e. 35% and 29% in B_0 and B_1 respectively (Table 3). Thus, in the case of 5 g/100mL glucomannan gels A_0 was the more stable under small strain conditions and short test times. Conversely, A_1 was more time-dependent. This behaviour of *G''* in the A_1 gel is consistent with its brittle character, as reflected in the highest K_f value under high deformation (discussed above). This suggests that when an alkali agent with a greater number of larger (K^+) cations is used for deacetylation, these cations may progressively break down the three-dimensional network into small clusters (Lapasin & Pricl, 1999) which are released into the network, raising the molecular mobility, and consequently causing an increase of *G''* moduli at low frequencies.

3.1.3.3. Creep and recovery at 25 °C. This transient test produces creep and recovery compliance data, *J(t)*, over longer time scales than SAOS measurements (Mezger, 2006). So, these experiments can cause irreversible breakdown of short-range interactions; thus providing information about the relative long-range properties of these physical networks (Steffe, 1996, chap. 5).

Table 3. Influence of type of alkali and concentration on experimental values of loss moduli at 10 and 0.1 Hz from mechanical spectra for 5 g/100mL KGM gels at 25 °C.

	G'' (kPa) 10Hz	G'' (kPa) 0.1Hz
A_0	1.57 ± 0.23a	1.78 ± 0.28ab
A_1	1.40 ± 0.15ac	2.11 ± 0.28b
B_0	1.54 ± 0.18a	2.08 ± 0.61ab
B_1	1.53 ± 0.33ab	1.98 ± 0.45bc

Values are given as mean ± expanded uncertainty limit (EUL).
a-c Different letters indicate significant differences ($p < 0.05$).

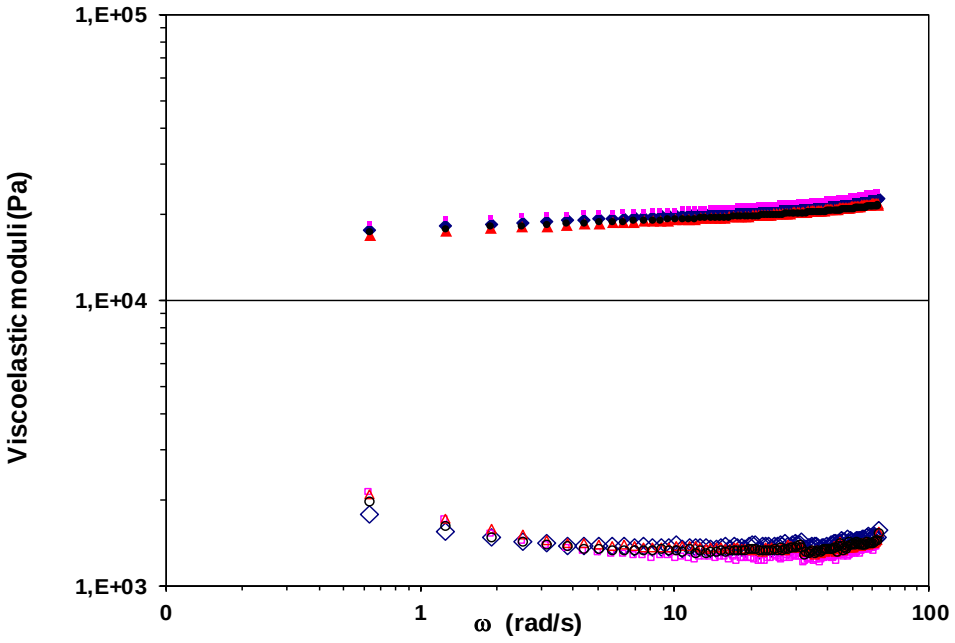

Fig. 1. Mechanical spectra of 5 g/100mLKGM gels deacetylated with different alkalis (KOH and NaOH) and concentrations (0.6 and 1 mol/L) at 25 °C. ◆A_0, ■A_1, ▲B_0, ●B_1. Closed symbols G', open symbols G''.

From J_{max} and J_{min} values at the end of the creep and recovery curves respectively, the percentage of elasticity (equation (1)) can be calculated for the different structures (Herranz, Tovar, et al., 2012):

$$Elasticity\ (\%) = \left(\frac{J_{max} - J_{min}}{J_{max}} \right) \cdot 100 \qquad (1)$$

Fig. 2 shows creep-recovery compliances for the four glucomannan gels. There are clearly two trends depending on the type of alkali. Gels made with KOH at both alkali concentrations (A_0 and A_1) showed lower compliances (J) than those with NaOH over the entire time interval. Also, during recovery time, $J(t)$ data were slightly higher in A_1 than in A_0. These results indicate that the network was less elastic in A_1 than in A_0 (Table 4), given that the network in gel A_1 was rather less ordered (more time-dependent).

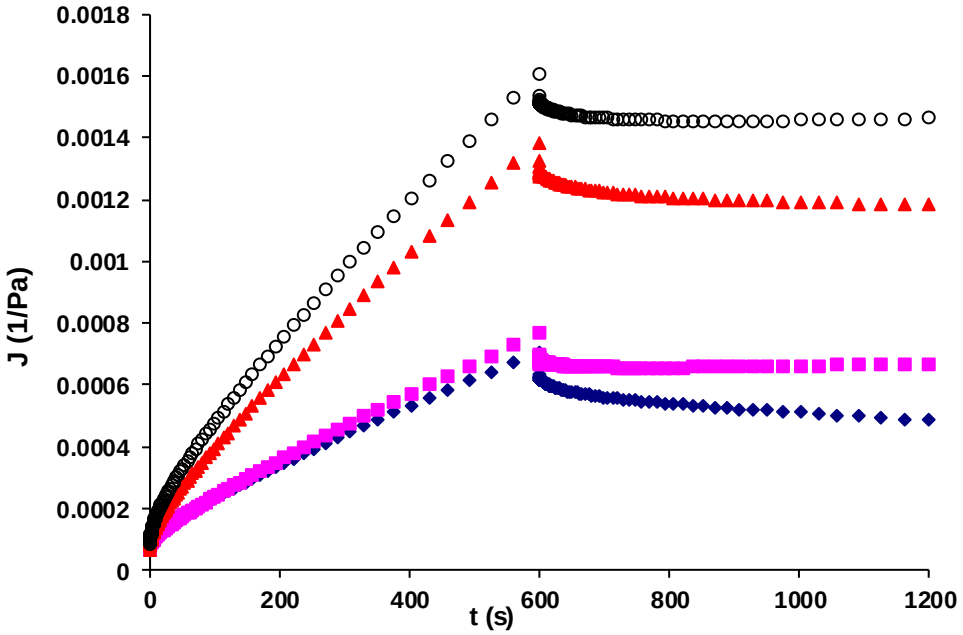

Fig. 2. Creep and recovery compliance $J(t)$ data of 5 g/100mL KGM gels deacetylated with different alkalis (KOH and NaOH) and concentrations (0.6 and 1mol/L) at 25 °C.
◆A_0, ■A_1, ▲B_0, ○B_1.

The greater influence of frequency on G'' moduli from mechanical spectra in gels A_1, B_0 and B_1, discussed earlier, is consistent with their low elasticity (Table 4), since if the network is less cohesive, the time-stability of the networks diminishes over short (time intervals oscillatory tests) and longer ones (transient tests).

Moreover, the $J(t)$ values from the creep curves can be used to calculate the relaxation modulus $G(t)$, (Ferry, 1980), which can be fitted with time to the power-law using the equation 2.

$$G(t) = S \cdot t^{-n} \qquad (2)$$

where S (Pa·s^n) is the gel strength and n is the relaxation exponent, both characteristic parameters of each kind of structure (te Nijenhuis, 1997, chap. 1).

Table 4. Effect of type of alkali and concentration on power-law parameters of equation 2 for 5 g/100mL KGM gels at 25 °C.

	S (kPa)	*n*	r^2	% Elasticity
A_0	14.34 ± 0.26a	0.279 ± 0.011e	0.863	31
A_1	13.55 ± 0.27b	0.276 ± 0.012e	0.844	13
B_0	12.25 ± 0.25c	0.359 ± 0.013f	0.897	15
B_1	10.24 ± 0.19d	0.360 ± 0.011f	0.914	9

a-f Different letters in the same column indicate significant differences ($p < 0.05$).

Table 4 shows that gels made with KOH at both alkali concentrations (A_0 and A_1) had higher values of S and lower values of n (high degree of connectivity in the networks) than gels B_0 and B_1. These data indicate that, during creep time, gels made with KOH, over longer times preserve the initial level of connectivity in the networks better than gels made with NaOH, since if S increases, that means that the network has more numerous and more widespread junctions, especially at 0.6 mol/L concentration (A_0). Also, the fact that there are smaller number of larger K^+ cations, which have less polarization ability (a lower hydratation number) (Moore, 1978, chap. 10), makes for a more homogeneous distribution of the junctions and hence more flexible and elastic networks, as in the case of A_0. This was also observed in 3 g/100mL glucomannan gels, where 0.6 mol/L KOH was the most suitable alkali although the differences were greater (Herranz, Tovar, et al., 2012). At higher glucomannan concentrations, then, the type and concentration of alkali seem also to influence the spread of the junction zones, producing networks that are elastic but less so, as these networks are denser.

In short, while in the case of NaOH the effect of raising the concentration from 0.6 to 1 mol/L had little impact on physicochemical and rheological properties, when the alkali was KOH the influence of the same increase was crucial, so that at low concentration (0.6 mol/L) the networks were less rigid (higher elasticity), more time stable and had greater WBC. This essential distinction between both alkalis could have to do with the difference in effective radius between hydrated K^+ (0.17 nm) and Na^+ (0.24nm) (Moore, 1978, chap. 10), so that if the hydrated radius decreases there are more free water molecules in the medium.

The increase in the alkali concentration (KOH) may thus have considerably more impact, causing a reduction of the total water, particularly the free water, and hence an increase of the overall rigidity of the network.

When KGM concentration was 3 g/100mL, a similar increase of the alkali produced an opposite effect. With the same increase of concentration from 0.6 to 1 mol/L, the gel networks produced with the alkali NaOH were more rigid (from puncture tests) and less elastic (from transient experiments) (Herranz, Tovar et al., 2012). This opposition of trends is consistent with the analysis set out in this paper regarding the greater ability of Na^+ cations to polarize and retain water dipoles, which is enhanced at lower polymer concentrations.

3.2. Evaluation of the thermostability of 5 g/100g glucomannan gels deacetylated with 0.6N KOH

Following the conclusion that gels made with 0.6 mol/L KOH (A_0) had more elastic networks with a high degree of internal cohesion and time stability, these gels were subjected to a thermo-rheological study to determine whether they could be used to make restructured seafood products that can be cooked or thermally treated in an industrial process.

3.2.1 Water Binding Capacity (WBC) and colour measurements (L)*

Increasing temperature did not modify the values of WBC (data not shown), which were around 70g/100g, or L* (around 50). There were no significant differences at the three temperatures (25 °C, 50 °C and 70 °C).

3.2.2 Puncture test

Fig. 3 shows the values of BF, BD and K_f of A_0 measured at 25 °C, 50 °C and 70 °C. BF and K_f increased with the temperature (Fig. 3a and c respectively) while BD remained practically constant at all temperatures (Fig. 3b).

The rigidity of A_0 increased linearly with T (Fig. 3c), indicating that heating produces a firmer structure. Given that fracture involves both the force required to break the network and the ability to transfer breaking energy among molecular fragments (Foegeding,

González, Hammann, & Case, 1994), if the number of loose fragments increases with T, more energy is needed to transfer the micro-cracks inside the network, thus increasing the rigidity.

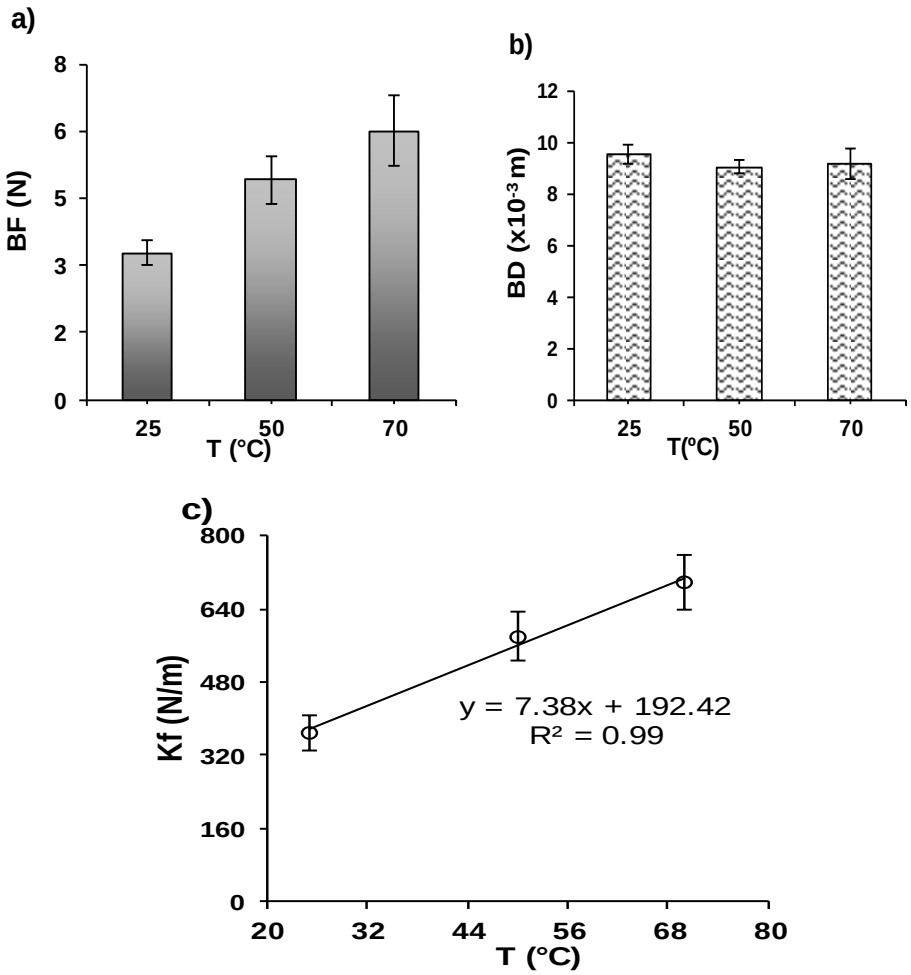

Fig 3. Influence of temperature (25 °C, 50 °C and 70 °C) on breaking force (a), breaking deformation (b) and fracture constant (c) of 5 g/100mL KGM gel deacetylated with 0.6mol/L KOH. T=25 °C.

3.2.3. Dynamic rheometry measurements at 25 °C, 50 °C and 70 °C

3.2.3.1. Stress sweeps. As can be seen in Table 5, increasing temperature produced a decrease in characteristic stress (σ_{max}) and strain (γ_{max}) amplitudes in the linear viscoelastic (LVE) range, making networks less flexible and more unstable. This viscoelastic effect may also be reflected in increasing loss factor ($tan\delta$), indicating a higher level of internal friction among the molecular fragments with increasing T. As a result, frictional heat is produced increasing the G'' component (Mezger, 2006), and hence tanδ.

*G**, a measure of the overall viscoelastic rigidity, involves both elastic and viscous deformations (Ferry, 1980). Between 25 °C and 50 °C, *G** decreased significantly (39%) and remained constant from T>50 °C (Table 5). When T increases, some physical interactions break down, particularly after the cleavage of hydrogen bonds (inter-chains and among water molecules), which may be more pronounced between 25 °C and 50°C, as also indicated by the significant increase of both $tan\delta$ and BF (Fig. 3a). This may be a consequence of some "*disaggregation*" into the network, contributing to the loss of elasticity. The decrease of *G'* was greater than the increase of *G''*, which is consistent with the breakdown of polar interactions in junctions such as ion-dipole (among K^+–water molecules and K^+–OH groups of KGM) and dipole-dipole (polymer-polymer and polymer-water). In addition, the proximity of values of *G** between 50 °C and 70 °C may indicate a "compensatory effect" of hydrophobic interactions, becoming stronger at high T (Case & Hamann, 1994).

Table 5. Influence of temperature on the viscoelastic parameters of the LVE range of 5 g/100mL KGM deacetylated with 0.6N KOH.

T(°C)	σ_{max} (Pa)	γ_{max}(%)	G* (kPa)	tanδ
25	242 ± 24a	1.18 ± 0.08d	20.6 ± 1.4f	0.101 ± 0.073h
50	125 ± 13b	1.01 ± 0.15d	12.6 ± 1.6g	0.185 ± 0.041h
70	66 ± 7c	0.56 ± 0.19e	12.8 ± 3.5g	0.198 ± 0.097h

Values are given as mean ± expanded uncertainty limit (EUL).
a-h Different letters indicate significant differences ($p < 0.05$).

3.2.3.2. Frequency sweeps. Fig. 4a shows the influence of temperature on mechanical spectra of the gel A_0. As happened at 25 °C (section 3.1.3), *G'* moduli were also greater than *G''* over the entire range of frequencies at 50 °C and 70 °C. When the temperature rose, *G'* and *G''* showed opposite trends, with frequency (low frequencies) *G'* (decreasing) and *G''* (increasing), the same as was observed at 25 °C.

As regards experimental values, *G'* moduli at 50 °C and 70 °C were lower than at 25 °C, meaning that there was a loss of elasticity in the network again as a consequence of some breakage of junctions as mentioned above. This loss of elasticity shows the damage caused by heat in the network, particularly at low frequencies. Fig. 4b shows a significant decrease of *G'* (at 0.1Hz) with temperature from 25 °C to 50 °C (38%), whereas between 50 °C and 70 °C the decrease was smaller (14%) and not significant. The overall result can be seen in the high and negative slope (-0.138 ± 0.054 kPa/ °C) indicating severe damage to the network

due to the breakdown of polar interactions in junctions as detailed above.

This structural effect can also be seen in Fig. 4c, which represents *G"* (also at 0.1Hz) versus T. In this case the positive slope (0.038±0.006 kPa/ °C) indicates a continuous increase of the loose energy which is spent by the irreversible deformation. Both slopes show the overall damage caused in the network by heating, which was more intense between 25 °C and 50 °C as in the stress sweeps. These results are consistent with the difference in the intensity of the increase of K_f and BF, which was also greater between 25 °C and 50 °C and somewhat weaker between 50 °C and 70 °C (from large deformation tests).

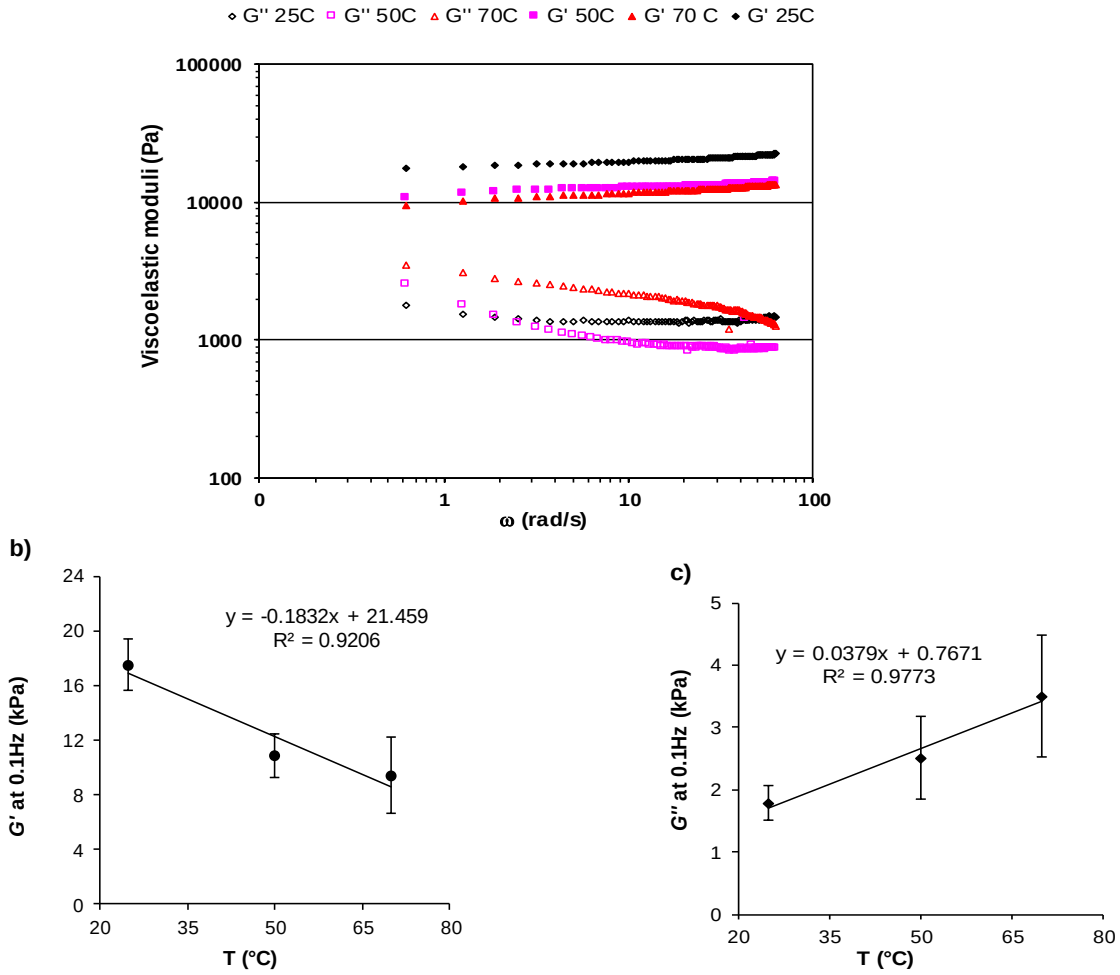

Fig. 4. Mechanical spectra of 0.6 mol/L KOH glucomannan gel at several temperatures: 25, 50 and 70 °C (a). Closed symbols *G*', open symbols *G*''. Influence of temperature on *G'* (b) and *G"* (c) of 0.6 N KOH gel at 0.1 Hz.

3.2.3.3. Creep and recovery. Transient experiments on 5 g/100mL KGM gels with increasing temperature can be used to visualize the progressive contribution of heat to the breakage

of polar interactions (hydrogen bonds, and electrostatic interactions) and consequently to gel strength. These tests only leave intact the physical interactions which act as permanent bonds supporting the stress (Chronakis, 1996).

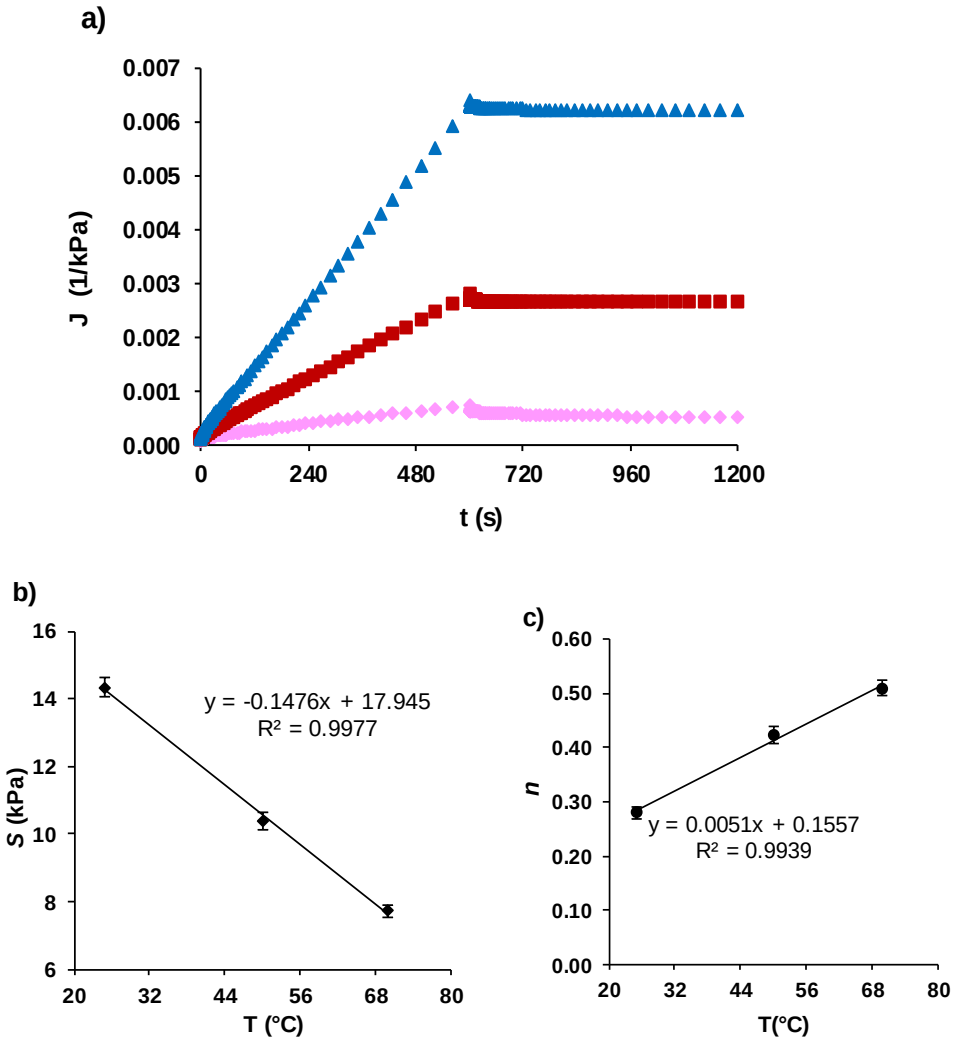

Fig. 5. Creep and recovery compliance *J(t)* values of 0.6 mol/L KOH glucomannan gel at 25, 50 and 70 °C (a) ◆ 25 °C, ■ 50 °C, ▲ 70 °C .Influence of temperature on gel strength (*S*) (b) and relaxation exponent (*n*) (c) of 0.6 mol/L KOH gel from equation (2).

Using a load stress within the LVE range (Fig. 5a), there was a continuous increase of creep and recovery compliance *J(t)* as the temperature rose, indicating softening and loss of viscoelastic rigidity in KGM gels. The gels were not in true equilibrium and junctions changed slowly with time through a disaggregation process (mentioned previously), suggesting some flow from dangling chains and other free segments from breakage of

chains. This rheological response may be quantified through the behaviour of *S* and *n* parameters (equation 2) with increasing T. Note in particular that the negative slope of linear fit between *S* and T (Fig. 5b) is similar (slightly lower) to the slope of fit *G'* –T (Fig. 4b). Moreover, the constant rise of *n* with T (Fig. 5c) is similar to the increase of *G''* with T (Fig. 4c) in that if the number and density of junctions diminishes, *n* increases (te Nijenhuis, 1997, chap. 1), and hence the loss of energy by heat increases (*G''* increases).

Combining both trends (*G'*-*S* and *G''*-*n*) the pattern of the structural rigidity observed from SAOS testing at short experiment times seems to be in agreement with creep parameters from longer-time tests, showing that transient tests provide structural magnitudes that reflect the permanent junction zones in the network.

In short, 0.6 mol/L KOH was the best alkaline agent at both 3 and 5 g/100 mL glucomannan concentrations for making restructured seafood products so that it produces more elastic and time-stable networks. The temperature increase caused similar relative increase of rigidity, and loss of elasticity and cohesiveness in the network, at both GM concentrations. This trend was much more noticeable between 25 °C and 50 °C than 50 °C and 70 °C.

4. CONCLUSIONS

Gels made with a high concentraction (5 g/100mL) of glucomannan showed dense and tight networks with a large number of extended junction zones. The effect of the alkali concentration was significant only in the case of gels made with KOH. Networks with 0.6 mol/L KOH were more time-stable, less rigid and had a high level of connectivity, Hence, 0.6 mol/L KOH was the most suitable alkaline agent for the deacetylation of 5 g/100mL glucomannan gels.

The thermo-rheological study of gels deacetylated with 0.6 mol/L KOH indicated that the heat produced a dissagregation effect, making networks less flexible and more unstable. This process was more marked between 25 °C and 50 °C.

ACKNOWLEDGEMENTS

This work has been supported by the Consejo Superior de Investigaciones Científicas

for Postdoctoral Scholarship JAE-DOC-2008. The authors wish to thank the Xunta de Galicia for its financial support under the Consolidation and restructuring program of competitive research units: Strategic Research Partnerships (2009/060).

REFERENCES

AOAC (2000). Official method of analysis (17th Ed.). Maryland, USA: Association of Official Analytical Chemists

Case, S. E., & Hamann, D. D. (1994): Fracture properties of konjac mannan gel: effect of gel temperature. Food Hydrocolloids, 8, 147–154.

Chronakis, I. S. (1996). Network formation and viscoelastic properties of commercial soy protein dispersions: effect of heat treatment, pH and calcium ions. Food Research International, 29, 123–134.

Ferry, J. D. (1980). Viscoelastic properties of polymers. (3rd Ed.)(pp. 1–32). New York. John Wiley and Sons, Inc.,

Foegeding, E. A., González, C., Hammann, D. D. & Case, S. (1994). Polyacrylamide gels as elastic models for food gels. Food Hydrocolloids, 8, 125–134.

Herranz, B., Tovar, C. A., Solo-de-Zaldívar, B. & Borderias, A. J. (2012). Effect of alkalis on konjac glucomannan gels for use as potential gelling agents in restructured seafood products. Food Hydrocolloids, 27, 145–153.

Herranz, B., Borderias, J. A., Solo-de-Zaldívar, B, Solas, M. T., & Tovar, C. A. (2012). Thermostability analices of glucomannan gels. Food Hydrocolloids, 29, 85–92.

Kök, M. S., Abdelhameed, A. S., Ang, S., Morris, G. S., & Harding, S. E. (2009). A novel global hydrodynamic analysis of the molecular flexibility of the dietary fibre polysaccharide konjac glucomannan. Food Hydrocolloids, 23, 1910–1917.

Lapasin, R., & Pricl S. (1999). Rheology of Industrial Polysaccharides: Theory and Applications. (1st Ed.) (pp., 250–494).Gaithersburg: Aspen Publishers.

Mezger, T. (2006). The Rheology Handbook. Hannover, Germany: Vincentz Network.

Moore W. J. (1978). Química Física, Tomo 1. Bilbao. Urmo, S.A. De Ediciones.

te Nijenhuis, K. (1997). Thermoreversible networks. Viscoelastic properties and structure of gelsIn Advances in polymer science 130. Berlin: Springer-Verlag.

Rao, M. A. (2007). Rheology of Fluid and Semisolid Foods. Principles and Applications (2nd Ed.). New York: Springer.

Steffe, J. F. (1996). Rheological Methods in Food Process Engineering. (2nd Ed.). East Lansing: Freeman Press.

Williams, M. A. K., Foster, T. J., Martin, D. R., Yoshimura, M., Nishinari, K. & Norton, I. T. (2000). A molecular description of the gelation mechanism of konjac mannan, Biomacromolecules, 1, 440–450.

Yassen, E. I., Herald, T. J., Aramouni, F. M., & Alavi, S. (2005). Rheological properties of selected gum solutions. Food Research International, 38, 111–119.

CAPÍTULO 2

OPTIMIZACIÓN DE LA METODOLOGÍA PARA LA OBTENCIÓN DE REESTRUCTURADOS DE PESCADO-GM. CARACTERIZACIÓN REOLÓGICA Y ESTRUCTURAL

RESEARCH NOTE

OBTAINING A RESTRUCTURED SEAFOOD PRODUCT FROM NON- FUNCTIONAL FISH MUSCLE BY GLUCOMANNAN ADDITION: FIRST STEPS.

Beatriz Herranz, Beatriz Solo-De-Zaldivar, and A. Javier Borderias.

Institute of Food Science, Technology and Nutrition (ICTAN) and Spanish National Research Council (CSIC), Madrid, Spain

Food Product Technology, 22(2), 201-208.
(DOI:10.1080/10498850.2011.632114)

ABSTRACT

A procedure of fish minced muscle gelation has been deviced in order to reestructure mince muscle which lacks functionality due to previous processing. This consists in adding glucomannan, dispersed in water in concentrations of 3-6 %, to the mince. Then, 0.6N KOH is added to bring the pH up to 11.8-12 and the sample is kept to set at 30°C for 1 h and then at 5°C for 4 h, so that the glucomannan gels with the mince incorporated as a "filler". This gel is subsequently neutralized by placing it in a buffer. The result is a thermostable gel with the same aspect than raw fish muscle and oral sensory properties similar to those of muscle when both are cooked. Therefore, this gel could be used to mimic muscle fibres or myotomes.

KEYWORDS: Glucomannan, Restructured fish product, Alkaline coagulant, Deacetylation

INTRODUCTION

Restructured fishery products made from mince and/or chopped muscle and which are used, with or without ingredients, to make other products with a new appearance and texture. Various types of gelate processes can be used in the restructuring of minced fish muscle to produce gels with different characteristics. These include cold gelation using ingredients such as microbial transglutaminase (Moreno et al., 2008), Fibrimex® (Flores et al., 2007), calcium alginate (Shand et al., 1993), or thermally induced gelation of minced chopped muscle or surimi, after solubilization of myofibrillar proteins using salt and mechanical methods. When fish mince presents lack of functionality due to previous processing, or it has too much fat, the ingredients and methods mentioned above are not efficient to gelate and form fibre- or myotome–like structures. The technological properties of Konjac glucomannan (KGM) offer an interesting new and unexplored possibility for restructuring products with these characteristics. KGM is a neutral polysaccharide derived from the tuber of *Amorphophallus konjac* C. (Nishinari et al., 1992) and consists of *β*-1,4-linked D-mannose and D-glucose in the ratio 1.6:1 (Kato and Matsuda, 1969), with about 1 in 19 units (Maekaji, 1978a) being acetylated. When KGM lose their acetyl groups due to the addition of alkaline coagulant, a thermally stable gel is formed (Maekaji, 1978a, 1978b; Yoshimura and Nishinari, 1999). This gelation occurs through the formation of a network

structure of junction zones which are stabilized mainly by hydrogen bonding, electrostatic and hydrophobic interactions (Rao, 2007) although the gelation mechanism has not been fully understood yet (Yoshimura & Nishinari, 1999).

Since 1994, KGM has been generally recognized as safe (GRAS; Khanna and Tester, 2006; Takigami, 2000). In Europe, KGM has been given in E425 agreement number by the European Safety Authority (EFSA; FSA, 2007). It has long been used as a thickener and gelation agent in traditional Asian foods and is consumed as a functional food in the form of noodles, tofu and snacks (Brown, 2000; Chua et al., 2010; Douglas et al., 2005; Long, 1998; Wootton et al., 1993). In Western countries there has been an increase in the use of KGM due to its potential for use as a dietary fibre. It is one of the most viscous dietary fibres known because of its effective water-absorbing ability (Chua et al., 2010). Thus, it has been used as a binder in meat (Chua et al., 2010) and as a fat replacer in low-fat and fat-free meat products (Chin et al., 1999; Jiménez-Colmenero et al., 2010; Lin and Huang, 2003, 2008; Osburn and Keeton, 2004; Takigami, 2000). However it is not a common ingredient in the seafood industry; there are only a few known studies of its use in the scientific literature on fish products (Iglesias-Otero et al., 2010 Park, 1996; Xiong et al., 2009) and one patent (Kawano, 2000). In some previous studies at our laboratory (data not shown) we assayed the best conditions for dispersing glucomannan in water, the most suitable alkaline method for deacetylation and consequently for getting thermostable gels, and the appropiate time and temperature setting. A neutralization methodology to reduce the high pH of these thermostable gels has also been defined. Against this background, we think it could be very interesting to use this property of KGM by adding it to the non-functional fish mince to make thermostable gels with the mince incorporated like a filler, for use in restructured seafood products.

The aim of this research note is to explore the possibility of making fish muscle gels by adding KGM to non-functional mince. This gel will be the base for different structures to be used in restructured fish products.

MATERIALS AND METHODS

Materials

Purified glucomannan from konjac (KGM ≥ 99%; MW: 200,000-2,000,000 Da) was

purchased from Guinama, Valencia, Spain. Fish muscle "sawdust" obtained from sawing frozen hake (*Merluccius capensis)* and frozen in blocks was supplied by Frinova, Pontevedra, Spain. All chemical reagents were supplied by Panreac, Química S.A., Barcelona and were of reagent grade.

Proximate analysis, pH and Functional Quality of Raw Materials

The moisture of the KGM gels and fish muscle sawdust (mince) were determined (AOAC Official method no 950.46.2000 5.43, AOAC, 2000) in triplicate. The ash content and lipid content was determined in triplicate (AOAC Official method no 9030.30.2000 and 996.06.20022.5.57, respectively) according to AOAC (2000). The raw protein content of the fish muscle sawdust was measured in triplicate with a LECO FP-2000 Nitrogen Analyser (Leco Corporation, St. Joseph, MI, USA), using a conversion factor nitrogen- protein of 6.25.

The pH was determined in quadruplicate, using a pH meter (Thermo-Orion 720, Spain) with a specific electrode for direct measuring on the mixture (Thermo-Orion Sure-flow 9165BNWP, Spain).

To determine the functional quality of the fish muscle sawdust, apparent viscosity was measured in a muscle dispersion on NaCl following the method described by Borderias et al. (1985). Measurements were carried out in triplicate with results expressed in centipoises (cP).

Sample preparations

Preparation of KGM dispersions

Aqueous glucomannan dispersions (AGD) were prepared at 3, 6 and 10 % (w/v) total polysaccharide concentration using distilled warm water at 80 °C in a Stephan vacuum homogenizer machine (Stephan Machinery GmbH and Co., Hameln, Germany) for 30 min and then left to cool at room temperature.

To make fish mince gels, different proportions of AGD at 3, 6 and 10% (w/v) and mince (50:50, 25:75 and 15:85 (w/w), respectively) were mixed in a Stephan vacuum homogenizer at below 30 °C during 5 min to obtain a final glucomannan concentration of 1.5%. Then, 0.6N KOH was added to bring the pH up to 11.8- 12. At this pH, a deacetylation of

95- 100 % is obtained (Solo-de-Zaldivar et al., 2011). The samples were placed in petri dishes (1.5 cm thick and 9.0 cm diameter). Then they were set by heating for 1 h at 30 °C and then for 4 h at 5 °C. The last step was to neutralize the pH of the gels; this was done by keeping them for 20 h in a 0.2M citrate-phosphate buffer at pH 5 (gel:buffer proportion 1:10) at chilled temperature. The end products were heat-stable gels with an approximate pH of 6.5-7.

The samples were denominated 15R (proportion of AGD:mince, 15:85), 25R (25:75) and 50R (50:50). Samples were compaired with whole fresh hake (*Merluccius merluccius)* muscle to discover the degree of similarity of the restructured prototype with a real muscle.

Gel quality analyses

Water Binding Capacity (WBC)

WBC was determined as described by Moreno et al. (2010). Samples were cut into small pieces, weighed (approx. 2g) and then placed in a centrifuge tube (10 mm diameter) with pipette filter as absorber. Samples were then centrifuged in a Heraeus Multifuge 3 Plus centrifuge (Asheville, North Carolina, USA) for 10 min at 3,000 × *g* at room temperature. WBC was expressed as percentage of water retained per 100 g water present in the sample prior to centrifuging. Measurements were carried out in triplicate.

Color

The surface colour of the samples was evaluated on a colorimeter (Minolta. CR-400 Konica-Minolta, Japan) (D65/2°), using the CIELAB scale (Lightness, L^*; redness, a^* and yellowness, b^*). Whiteness was determined using the following formula: $100 - [(100-L^*)^2 + a^{*2} + b^{*2}]^{1/2}$ (Park, 1995). Colour determinations were carried out after neutralizing. Determinations were performed on five points per formulation.

Puncture test

The cilindrical blocks taken out from Petri dishes, approximately the same thickness as hake fillets, were penetrated to breaking-point using a TA-XT plus Texture Analyzer (Texture Technologies Corp., Scarsdale, NY) equipped with a rod with a spherical tip (5 mm diameter (P/5S)). The same was done in the loin part of hake fillet. Cross-head speed was

1mm/s and a 5 kg load-cell connected to the crosshead of the Texture Analyzer was used. Breaking force (N) and breaking deformation (mm) were determined in the force-deformation curves. Puncture tests were carried out with the gels and fillets kept at room temperature. All determinations were carried out at least in quadruplicate.

Sensory test

A sensory test with non-structured scales was performed with six members of the authors' departament who are previously semi-trained in the sensorial assessment of fish products. Sensory analysis was performed according to the criteria of the UNE-ISO 6658 (ISO, 2008). For that purpose, a 9 mm non-structured scale were used to assess flavour and and texture with anchor points: *poor flavour* (0) to *good flavour* (9) and *poor texture* (0) to *good texture* (9), respectively. Restructured samples were steam-cooked for 10 min and compaired with hake fillets cooked in the same way. Then, they were presented to the panelists. The evaluation was performed in a standard panel room designed according UNE-ISO 8589 (ISO, 2010) and was done between meals (after breakfast and before the midday day). The tasters were given unsalted crackers and room temperature water to clean the palate between samples. The samples were left for 20 min to reach room temperature. The evaluations were performed in a room with white fluorescent light.

Statistical analysis

Analysis of variance (One–way ANOVA) was carried out to evaluate the statistical significance ($p < 0.05$). Statistical analysis was performed using Statgraphics Plus version 5.0 (Statpoint Technologies, Inc., Warrenton, VA, USA).

RESULTS AND DISCUSSION

Proximate Analysis and Technological Functionality of Fish Mince Used as Raw Material (Fish Muscle Sawdust)

The proximate analysis (protein content = 18.41 ± 0.32 %, lipid content = 1.50 ± 0.30 %, ash content = 1.19 ± 0.01 %, and moisture content = 79.00 ± 0.01 %) was within the normal range for the fish species studied (Moreno et al., 2011). Due to the heat produced by sawing the original block, the protein in the sawdust was denatured and therefore did not

interact with the aqueous solution, so that the apparent viscosity measured was 0 cP. This data indicates that the fish sawdust was not suitable for gelation by itself in a medium of adequate ionic strength .

Gelation of Mince Adding AGD

Sample 15R was a very weak gel with insufficient consistency to hold the fish particles, so this sample was rejected. The other AGD:mince proportions (50:50 and 25:75, denominated 50R and 25 R respectively) formed homogeneous gelated prototypes with good appearance and a firm enough texture for restructuring to mimic fibres or myotomes .Table 1 shows the breaking force and breaking deformation of samples 25R and 50R compared with whole fresh hake fillet (*Merluccius merluccius)*. Samples 25R and 50R showed the same breaking force that was different significantly ($p<0.05$) from that of the hake fillet. There was a significant ($p < 0.05$) difference in breaking deformation between 25R and hake fillet but not between 50R and hake fillet. It is worth noting that despite these differences between the samples with added AGD and hake fillet, all values were of the same order of magnitude and depend on the reference fish muscle chosen.

Table 1.- Breaking force (N) and breaking deformation (mm) in restructured fish products and in hake fillet.

Sample	*Breaking force (N)*	*Breaking deformation (mm)*
25R	1.3 ± 0.09^{b}	6.14 ± 0.44^{a}
50R	1.35 ± 0.15^{b}	5.40 ± 1.25^{ab}
Hake fillet	2.33 ± 0.43^{a}	4.96 ± 0.34^{b}

Samples: 25R = AGD: mince in proportion 25:75; 50R = AGD: mince in proportion 50:50; and Hake fillet = fish fillet.
a–b Different superscripts in the same column indicate significant differences ($p < 0.05$).

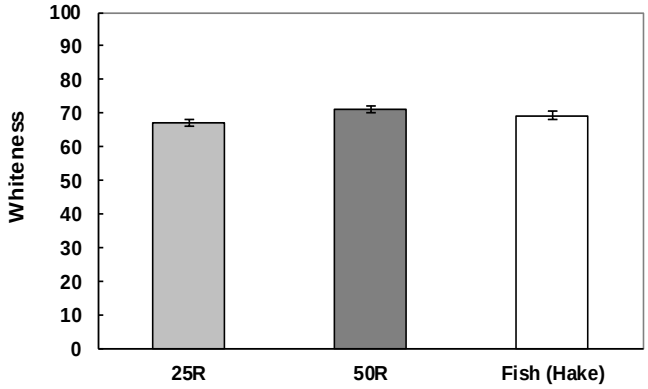

Figure 1.-Whiteness index of the fish fillet and the restructured fish products elaborating with different proportions of AGD:mince using 0.6N KOH as gelling agent (Samples: 25R = AGD:mince in proportion 25:75, 50R = AGD:mince in proportion 50:50 and hake fillet).

WBC was around 80% in all samples, with no significant differences ($p > 0.05$) among them (Fig. 2). Xiong et al. (2009) found that addition of KGM improved the water-holding properties of surimi gels from grass carp; but, as noted above, glucomannan was added in powdered form, not in an aqueous dispersion (at 3 or 6%) as was done in this paper, so the final mixture was much higher (around 90%). No more data were found in the literature

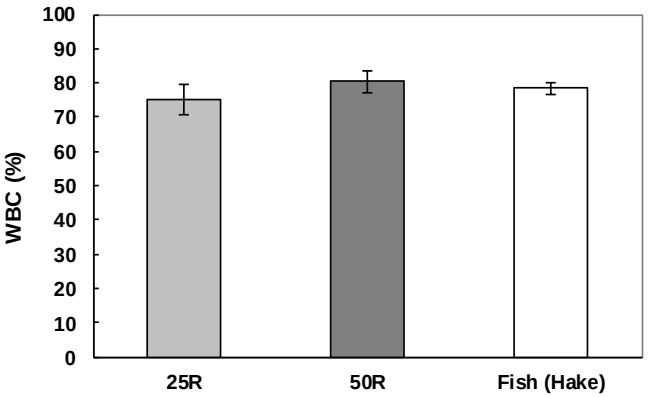

Figure 2. - Water binding capacity (WBC) of the fish fillet and the restructured fish products elaborating with different proportions of AGD:mince using 0.6N KOH as gelling agent (Samples: 25R = AGD:mince in proportion 25:75; 50R = AGD:mince in proportion 50:50 and hake fillet).

The sensory test showed (Table 2) that the 25R sample, with a higher proportion of fish mince, had a more pronounced fish flavour than the 50R sample, which is logical given the higher mince content, and both presented an acceptable texture for restructured seafoods, similar to fish muscle. Sample 25R did not differ significantly ($p > 0.05$) in flavour from hake muscle. In the case of texture, although it differed significantly from hake muscle

($p < 0.05$), that difference was small and depends on the type of muscle with which it is to be compared. For its part, sample 50R had the weakest flavour but was acceptable, and its texture did not differ from that of 25R. As noted earlier, the only sample that was rejected was 15R because of its poor consistency, which was unlike that of any fish muscle.

Table 2.-Sensory test in restructured fish products and in hake fillet.

Sensorial attributes	*25R*	*50R*	*Hake fillet*
Flavor	7.37 ± 0.21^{ab}	5.15 ± 0.82^{b}	8.79 ± 0.51^{a}
Texture	6.56 ± 0.40^{b}	5.94 ± 0.62^{b}	7.33 ± 0.76^{a}

Samples: 25R = AGD:mince in proportion 25:75; 50R = AGD:mince in proportion 50:50; and Hake fillet = fish fillet.

a–b Different superscripts in the same raw indicate significant differences ($p < 0.05$).

CONCLUSION

Addition of aqueous glucomannan dispersions (at 3 and 6% w/v) to minced fish muscle in proportions of 50:50 and 25:75 combined with 0.6N KOH is a feasible procedure for including a non-functional mince on a glucomannan gel as a filler, so as to mimic the texture of whole muscle. More rheological studies are necessary to complete the examination of the structure and thermostability of these gels and restructured fish products based on them.

ACKNOWLEDGEMENTS

This work has been supported by the Consejo Superior de Investigaciones Científicas in Postdoctoral Scholarship JAE-DOC-2008 and by Ministerio de Ciencia e Innovación in the project AGL2008-04892-CO3-C3.

REFERENCES

AOAC. 2000. Official method of analysis of the Association of Official Analytical Chemists. 17th Ed. Gaithersburg, MD: Author.

Borderias, A. J., Jiménez-Colmenero, F. and Tejada, M. 1995. Viscosity and emulsifying ability of fish and chicken muscle protein. J. Food Sci. 20: 31-42.

Brown, D. 2000. Aroids, Plants of the Arum Family. Portland, OR: Timber Press.

Chin, K. B., Keeton, J. T., Longnecker, M. T. and Lamkey, J. W. 1999. Utilization of soy protein isolate and konjac blends in a low-fat bologna (model system). Meat Sci. 53: 45-57.

Chua, M., Baldwin, T. C., Hocking, T. J. and Chan, K. 2010. Traditional uses and potential health benefits of Amorphophallus konjac K. Koch ex N.E.Br. J. Ethnoph. 128: 268-278.

Douglas, J.A., Follett, J.M. and Waller, J.E. 2005. Research on konjac (Amorphophallus konjac) production in New Zealand. Acta Hortoc. 670: 173–180.

Flores, N. C., Boyle, E. A. E., and Kastner, C. L. (2007). Instrumental and consumer evaluation of pork restructured with active (TM) or with fibrimex (TM) formulated with and without phosphate. Lwt - Food Sci. Tech.40: 179-185.

FSA. 2007. Current EU approved additives and their E numbers. Retrieved from http://www.food.gov.uk/policy-advice/additivesbranch/enumberlist

Iglesias-Otero, M. A., Borderias, J. and Tovar, C. A. 2010. Use of konjac glucomannan as additive to reinforce the gels from low quality squid surimi. J. Food Engineering 101: 281- 288.

ISO. 2008. Sensory analysis. Methodology. General guidance. (UNE-ISO 6658) Geneva, Switzerland: International Organization for Standarization.

ISO. 2010. Sensory analysis - General guidance for the design of test rooms (UNE-ISO 8589). Geneva, Switzerland: International Organization for Standarization.

Jiménez-Colmenero, F., Cofrades, S., Lopez-Lopez, I., Ruiz-Capillas, C., Pintado, T. and Solas, M. T. 2010. Technological and sensory characteristics of reduced/low-fat, low-salt frankfurters as affected by the addition of konjac and seaweed. Meat Sci. 84: 356-363.

Kato, K., and Matsuda, K. (1969). Studies on the chemical structure of konjac mannan. Agric. Biol. Chem.33: 1446-1453.

Kawano, N. 2000. U.S. Patent No 6,146,684. Washington, DC: U.S. Patent and Trademark Office.

Khanna, S. and Tester, R. F. 2006. Influence of purified konjac glucomannan on the gelatinisation and retrogradation properties of maize and potato starch. Food Hydrocol. 20: 567–576.

Lin, K. W. and Huang, H. Y. 2003. Konjac/gellan gum mixed gels improve the quality of reduced-fat frankfurters. Meat Sci. 65: 749-755.

Lin, K. W. and Huang, C. Y. 2008. Physicochemical and textural properties of ultrasound-degraded konjac flour and their influences on the quality of low-fat Chinese-style sausage. Meat Sci. 79: 615-622.

Long, C. L. 1998. Ethnobotany of Amorphophallus of China. Acta Bot Yunnanica 20(10):1-3.

Maekaji, K. 1978a. Determination of acidic components of konjac mannan. Agric. Biol. Chem.42: 177-178.

Maekaji, K. 1978b. A method for measurement and kinetic analysis of the gelation process of konjac mannan (kinetic study on the gelation of konjac mannan). Nippon Nogeikag. 52: 251-257

Moreno, H. M., Carballo, J., and Borderias, J. 2008. Influence of alginate and microbial transglutaminase as binding ingredients on restructured fish muscle processed at low temperature. J Sci. Food Agric. 88: 1529–1536.

Moreno, H. M., Carballo, J., and Borderias, J. 2010. Gelation of fish muscle using microbial transglutaminase and the effect of sodium chloride and pH levels. J Muscle Foods 21: 433-450.

Moreno, H. M., Carballo, J., and Borderias, J. 2011. Application of response surface methodology to study the effect of different calcium sources in fish muscle-alginate restructured products. Ciéncia e Tecnlogia de Alimento, 31: 209-216.

Nishinari, K., Williams, P. A. and Phillips, G. O. 1992. Review of the physico-chemical characteristics and properties of konjac mannan. Food Hydrocol. 6: 199-222.

Osburn, W. N. and Keeton, J. T. 2004. Evaluation of low-fat sausage containing desinewed lamb and konjac gel. Meat Sci. 68: 221-223.

Park, J. W. 1995. Surimi gel colors as affected by moisture-content and physical conditions. J. Food Sci. 60: 15-18.

Park, J. W. 1996. Temperature-tolerant fish protein gels using konjak flour. J. Muscle Food 7: 165-174.

Rao, M. A. 2007. Rheology of Fluid and Semisolid Foods: Principles and Applications (2nd Ed). New York: Springer.

Shand, P. J., Sofos, J. N., and Schmidt, G. R. 1993. Properties of algin calcium and salt phosphate structured beef rolls with added gums. J Food Sci. 58: 224–1230.

Solo-de-Zaldivar, B., Herranz, B. and Borderias, J. 2011. First steps in using glucomannan to make thermostable gels for potential use in mince fish restructuration. Int J Food Eng. 8(1), 1-10

Takigami, S. 2000. Konjac mannan. In: Handbook of hydrocolloids. Phillips, G. O. and P. A. Williams (Eds.). New York, NY: Woodhead. pp. 413–424.

Wootton, A. J., Luker-Brown, M., Westcott, R. J. and Cheetham, P. S .J. 1993. The extraction of a glucomannan polysaccharide from konjac corms (Elephant Yam, Amorphophallus rivierii). J. Sci. Food Agric. 61: 429–433.

Xiong, G., Cheng, W., Ye, L., Du, X., Zhou, M., Lin, R., Geng, S., Geng, S., Chen, M., Corke, H. and Cai, Y. Z. 2009. Effect of konjac glucomannan on physicochemical properties of myofibrillar protein and surimi gels from grass carp (Ctenopharyngodon idella). Food Chem. 166: 413- 418.

Yoshimura, M., and Nishinari, K. 1999. Dynamic viscoelastic study on the gelation of konjac glucomannan with different molecular weights. Food hydrocol., 13, 227-233.

EFFECT OF FREEZING AND FROZEN STORAGE ON RESTRUCTURED FISH PROTOTYPES MADE WITH GLUCOMANNAN AND FISH MINCE

Solo-de-Zaldívar, B.[a]*. Herranz, B.[a], Borderías, A. J.[a] and Tovar, C. A.[b]

[a]Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN-CSIC), José Antonio Novais 10, 28040 Madrid, Spain.

[b]Department of Applied Physics, Faculty of Sciences, University of Vigo, As Lagoas, 32004 Ourense, Spain.

Food Hydrocolloids, 41, 233-240.
(DOI: 10.1016/j.foodhyd.2014.04.019)

ABSTRACT:

This paper examines the physicochemical and rheological properties to determine the freezing and frozen stability of restructured fish prototypes made with glucomannan (GM) and fish mince lacking any protein functionality. The effect of adding salt and fish oil to the fish prototype formulae is also examined. Three prototype fish gels at 1.25% final GM concentration were made: control lot (C); oil lot (O): control gels with 5% added fish oil, and salt lot (S): control gels were made with 0.8% NaCl. The analyses were performed after 24h refrigerated storage (5 °C) and 30, 90 and 150 days frozen storage at -20 °C.

Freezing produced hardening of gels due to partial dehydration caused by ice crystal formation, particularly in Lot S where the gels were more rigid with less water binding capacity (WBC) and more cooking loss (CL) after 30 days storage. Conversely, lot C exhibited some synergy between GM and protein molecules, giving time-stable networks with higher WBC after freezing. Lot O showed less CL and a more regular trend in mechanical and viscoelastic parameters between 90 and 150 days frozen storage, demonstrating the stabilizing effect of the "oil-protein-water" complex in a GM matrix.

KEYWORDS: Glucomannan gels; Restructured seafood products; Frozen storage.

INTRODUCTION:

Konjac glucomannan (KGM) is a neutral hydrocolloid from *Amorphophallus konjac* C. (Nishinari, Williams, & Phillips, 1992) which has been used in traditional Asian cooking for more than 2000 years because of its gelling and high water absorbing ability (Chua, Baldwin, Hocking, & Chan, 2010). It also has physiological properties and is effective in the treatment of obesity (Kraemer, et al., 2007), diabetes (Vuksan, et al., 2008) and in the reduction of cholesterol levels (Levrat-Verny, Behr, Mustad, Remesy, &and Demigne, 2000). Thanks to all these characteristics, demand has greatly increased in Western countries, where it is used in industrial and medical applications.

In the fish industry in particular it is not widely used, and serves mainly as a filler in powder form (Cardoso, Ribeiro, & Mendes, 2012; Iglesias-Otero, Borderías, & Tovar, 2010; Park, 1996) or as a cryoprotectant (Xiong et al., 2009).

Herranz, Solo-De-Zaldivar, and Borderias (2013) studied the possibility of making restructured seafood products using glucomannan's ability to form a thermostable gel when deacetylated. In that way the glucomannan acts as a gelling agent, forming a gel network containing fish particles as filler. This new alternative offers the possibility of upgrading any seafood material, even non-functional minces, such as muscle that has been previously heated during processing, or to use mince from high-fat fish muscle. A number of studies have been carried out to try and improve the characteristics and verify the possibilities of using glucomannan as a gelling agent in the manufacture of this type of fish restructurates. For instance, different alkalis (NaOH and KOH) have been tested at different concentrations (0.6 and 1N) for glucomannan deacetylation (Herranz, Tovar, Solo-de-Zaldívar, & Borderias, 2012); the conclusion has been that the most suitable alkali input to produce an elastic gel with greater time- and thermal-stability for the manufacture of restructured products is 0.6N KOH (Herranz, Borderias, Solas, & Tovar, 2012; Herranz, Borderias, Solo-de-Zaldívar, Solas, & Tovar, 2012).

As regards the influence of freezing in konjac gels, Jimenez-Colmenero, Cofrades, Herrero, Solas, and Ruiz-Capillas (2013) reported that the freezing/thawing process strongly affected the texture and water binding capacity of glucomannan gels with a glucomannan concentration of around 5%, but the changes were not attributable to frozen storage.

This paper is an assessment of physicochemical and rheological properties during freezing and frozen storage of restructured fish prototypes made with glucomannan and low-functionality fish mince (sawdust from the frozen fish sawing process). It also reports on the influence of addition of salt and fish oil on gel formulation.

2. MATERIALS AND METHODS

2.1. Raw material, additives and reagents

The raw material was fish muscle "sawdust" from the sawing of frozen blocks of hake (*Merluccius capensis*), supplied by the company Frinova (Pescanova).

The fish oil used was Omevital© (18/12 TG Gold, Cognis GMBH, Illertissen, Germany) and the salt used was NaCl (Panreac Química, S.A. Barcelona, Spain).

All the chemicals used were analytical grade supplied by Panreac Química S.A. (Barcelona, Spain)

2.2. Preparation of fish prototypes

First, aqueous glucomannan dispersions (AGD) at 5% were prepared as described in Herranz, Solo-De-Zaldivar et al., (2013). Then, fish mince gels were made as follows: the fish was homogenized in a Stephan UM5 Universal Machine (Stephan u. Söhne GmbH & Co., Hameln, Germany) at 2 °C for 5 min under vacuum. The 5% (w/v) AGD was added in a proportion of 25:75 (w/w) to the homogenized fish muscle and the mixture homogenized again for 10 min more along with the appropriate ingredients (salt, oil) depending on the lot. Then, 0.6 N KOH was added to bring the pH up to 11.0-11.1 and gelate the mixture. Cylindrical containers (diameter 3 cm x height 3.5 cm) and Petri dishes were filled with this mixture. All samples were then set, first at 30°C for 1 h and then at 5 °C for 4 h, to obtain thermostable gels. High pH values were neutralized by soaking the samples in 0.2 M citrate-phosphate buffer at pH 5.1 (gel: buffer ratio 1:10) for 16 h at 5 °C. In this way three neutral pH gels were prepared with a final glucomannan concentration of 1.25%: control lot (C); oil lot (O): control samples with 5% added fish oil, and salt lot (S). A control lot was prepared with 0.8% NaCl.

For each lot, some of the gels were kept chilled (5 °C) and the rest were frozen (-40 °C) in a Sabroe horizontal plate freezer (Hanst-Moller, Germany) which cooled the thermal core to -20 °C. Frozen samples were then vacuum packed in plastic bags and stored (1 day) at -18 °C. The chilled samples at 5 °C were analysed after 24h of chilling storage, and frozen samples were thawed for analysis after 30, 90 and 150 days of frozen storage. The different samples were coded as shown in Table 1.

Table 1. Nomenclature of control samples (C), samples containing fish oil (O) and samples containing salt (S) during storage.

Storage time (days)	Storage T (°C)	Lot C	Lot O	Lot S
1	5	C1	O1	S1
30	-18	C30	O30	S30
90	-18	C90	O90	S90
150	-18	C150	O150	S150

2.3. Analyses

2.3.1 Proximate analyses and apparent viscosity of raw material

Moisture, fat and ash content of raw fish were determined (AOAC, 2000) in quintuplicate. Crude protein content was measured in quadruplicate with a LECO FP-2000 Nitrogen Determinator (Leco Corporation, St Joseph, MI, USA).

To determine the protein functional quality of the fish muscle sawdust, apparent viscosity was measured in a muscle dispersion on NaCl following the method described by Borderias, Jimenez-Colmenero, and Tejada (1985). Measurements were carried out in triplicate and results expressed in centipoises (cP).

2.3.2. Water binding capacity (WBC)

Water binding capacity (WBC) was measured according to Eide, Borresen, and Strom (1982). Samples were cut into small pieces and then placed in centrifuge tubes and centrifuged (3000g) at room temperature for 10 min in a Jouan MR1812 centrifuge (Saint Nazaire, France). WBC was expressed as percent water retained relative to the amount of water present in the sample prior to centrifuging. All determinations were carried out in triplicate.

2.3.3. Oil binding capacity (OBC)

Restructured prototypes formulated with 5% fish oil (Lot O) were tested for fat release according to Gómez-Guillén, Montero, Hurtado, and Borderías (2000) and the result was expressed as oil binding capacity (OBC) per 100 g initial weight of product. All determinations were carried out in triplicate.

2.3.4. Cooking loss determination

A sample (40 g) was cut into small pieces and placed in a plastic bag where small holes had been made to drain the drip. Then, this bag with the sample inside was put inside another bag, hung with the holes at the bottom and cooked in that position in an oven (Rational Combi-Master CM6) for 20 min at 100 °C. The sample was then cooled and

weighed. Cooking loss was expressed as g/100 g by weight difference between uncooked and cooked samples.

2.3.5. Colour measurement

The lightness parameter (L*) was measured five times on the surface of the fish mince gels using a colorimeter (Minolta Chroma Meter Cr-200, Japan) which was standardized using a white calibration plate.

2.3.6. Puncture tests

Cylindrical samples (diameter 3 cm x height 3.5 cm) were pierced to breaking point using a TA-XTplus Texture Analyser (Stable Micro System Ltd., Surrey, UK) with a 5 mm-diameter round-ended metal probe at room temperature. Crosshead speed was 1 mm/s, and a 5 kg load cell was used. The load was recorded as breaking force (BF) and the depth of depression as breaking deformation (BD) when the fish mince gels sample lost its strength and ruptured. The measurements were carried out at least in sextuplicate.

2.3.7. Dynamic rheometry measurements

SAOS tests were performed using a Bohlin CVO controlled-stress rheometer (Bohlin Instruments, Inc. Cranbury, NJ). The measurements were obtained using parallel-plate geometry (20 mm diameter and 1 mm gap). Before measurements, the fish mince gels were tempered at ambient temperature and cut from Petri dishes into disk-shaped slices 20 mm in diameter and 1 mm thick with a 570 S.T.E slicing machine (Germany). They were then put on the lower plate of the rheometer for measuring. Any excess sample protruding beyond the upper plate was carefully removed. Samples were allowed to rest for 15 min before analyses to ensure both thermal and mechanical equilibrium at the time of measurement. Samples were covered with a thin film of Vaseline oil (Codex purissimum) to avoid evaporation. The temperature was controlled to within 0.1°C by a Peltier element in the lower plate that was kept at 25.0 °C.

2.3.7.1. Stress sweep tests. To determine the linear viscoelastic (LVE) region, stress sweeps were run at 6.28 rad/s at 25 °C with the shear stress (σ) of the input signal varying from 10 to 1000 Pa. 300 points on the continuous mode were used in all instances. Changes in

storage modulus (G'), loss modulus (G''), complex modulus (G^*) and loss tangent ($tan\delta$) were recorded. The critical (maximum) values of shear strain (γ_{max}), and shear stress (σ_{max}) on the limit of LVE range were obtained according to the method previously described in Campo-Deaño and Tovar, (2009).

2.3.7.2. Frequency sweep tests. Samples were subjected to stress that varied harmonically with time at variable frequencies from 10 to 0.1 Hz. The strain amplitude was fixed at γ= 2 % within the LVE range.

2.3.7.3. Creep and recovery tests. An instantaneous stress σ_0, corresponding to 0.5% shear strain within the LVE range, was applied for 600s in the creep tests and the resulting change in strain over time $\gamma(t)$ was monitored. When the stress was released, some recovery was also observed for 600s. The creep and recovery results were described in terms of the shear compliance function $J(t) = \gamma(t)/\sigma_0$. Compliance curves generated at different linear stress levels overlap, making it possible to examine and compare the structural properties of the different food gels on larger time scales (Steffe, 1996 chap. 5).

All viscoelastic measurements were carried out at least in quintuplicate.

2.3.8. Sensory evaluation

Three lots were tested for sensory evaluation in terms of taste (TAA) and texture acceptability (TEA). These acceptability tests were performed according to standard UNE-ISO 6658 (ISO, 2008). Twenty-five semi-trained panellists from the staff of the Institute of Science, Technology and Nutrition (ICTAN) were asked to evaluate the three lots (C, O and S) at 1 and 21 days of frozen storage using a non-structured hedonic scale in which samples were given scores of 1 (for dislike extremely) to 9 (for like extremely). The evaluation was performed in a standard panel room designed according to UNE-ISO 8589 (ISO, 2010) and was done between meals (after breakfast and before the midday meal). The tasters were given unsalted crackers and room-temperature water to clean the palate between samples. The samples were cut into pieces of approximately 20 g and grilled for 3 minutes with a small amount of sunflower oil. The results are the means of the scores from the twenty-five tasters.

2.3.9. Statistical analyses

Statistical analysis was carried out using Microsoft Excel software. Data are presented as mean values of at least five independent batches and were tested for each experiment with expanded uncertainty limit (EUL) data as the maximum and minimum deviation from the respective mean value. Trends were considered significant when means of compared sets differed at $p<0.05$ (Student's t-test).

Statistical correlations between parameters were determined by multiple regression with confidence intervals of 95% ($p<0.05$) using the SPSS Statistics 17.0 software.

3. RESULTS AND DISCUSSION

3.1. Proximate analysis and viscosity analysis

The proximate analysis of the mince (sawdust) used in the preparation of fish mince gels was as follows: moisture content (79.00 ± 0.01) %, crude protein (18.41 ± 0.32) %, fat (1.50 ± 0.30) % and ashes (1.19 ± 0.03) %. Apparent viscosity of a homogenate of sawdust in a sodium chloride solution was tested following the method of Borderias et al., (1985) to ascertain the protein functionality of the sawdust. The value obtained was 0 cP which means that the fish muscle protein showed nule or very low functionality, probably due to the heat produced by the sawing of the original fish blocks. In spite of this apparent viscosity data, the dynamic-mechanical thermo-analysis of the lots C, O and S, showed thermal-gelation profiles which indicate some gelling capacity (data not shown).

Samples C and S contained 88 % moisture and sample O 84 %. The increase of moisture with respect to the mince was due to the high moisture content of the aqueous glucomannan dispersions, as reported elsewhere (Herranz, Borderias et al., 2012; Herranz, Tovar, Solo-de-Zaldívar, & Borderias, 2013; Solo-de-Zaldivar, Herranz, & Borderias, 2012).

3.2. Water binding capacity (WBC) and oil binding capacity (OBC)

The effects of freezing/thawing and frozen storage on WBC data are shown in Table 2. In all lots there was a significant decrease of WBC ($p<0.05$) due to freezing, as also reported by Jiménez-Colmenero et al. (2013) in konjac gels. The WBC loss was higher in lot S (around 9%) than in lot O (6.4%) or lot C (4.6%). During freezing, the changes in the physical state

of the water (ice crystal formation) caused partial dehydration of the network (Hui et al., 2006, chap 16), which was slightly more pronounced in lot S than in the rest. So, when gel is being frozen, polymer-rich regions are formed in the unfrozen matrix, enhancing the inter-chain associations. These form superjunctions based on thick strings, which produce larger pore structures (Mao, Tang & Swanso, 2001). The increased pore size reduces the capillary pressure, causing the release of the water, so that WBC is reduced after freezing/thawing (Sahin & Sumnu, 2006, chap. 1).

This partial dehydration continued through frozen storage, so that WBC was gradually reduced in lot C until 150 days; in lots O and S, WBC remained stable until 90 days, but over the final period from 90 to 150 days, as during freezing, the loss of WBC was again greater in lot S (~28%) than in O (~20%) or in C (~9%).This higher dehydration in lot S could be related to the greater polymeric freezing-concentration which increase the ionic strength damaging the proteins (Walstra, 2003, chap. 16) and reducing consequently its ability to retain water (Table 2).

Table 2. WBC (%), lightness value (L*) and cooking loss (%) of samples C, O and S after 1, 30, 90 and 150 days of storage.

		WBC (%)	Cooking loss (%)	L*
LOT C	C1	85.5 ±1.7dA	9.0 ±1.3aA	67.94 ±0.38bA
	C30	81.54 ±0.57cB	17.3±2.4bA	65.36 ±0.45aA
	C90	76.9 ±3.8bA	23.0 ± 2.1cA	65.0 ±0.31aA
	C150	69.66 ±0.97aB	22.0 ±1.3cA	65.49 ±0.65aA
LOT O	O1	82.8 ±1.6 cA	6.3 ±1.7aB	82.6 ±1.2bB
	O30	77.5 ±1.9bA	12.69 ±0.87bB	78.2 ±1.0aB
	O90	76.8 ±1.2bA	12.6 ±1.7bB	78.95 ±0.28aB
	O150	61.2 ±1.1aA	12.0 ±1.3bB	79.44 ±1.1aB
LOT S	S1	87.49 ±0.48aB	7.7 ±1.4aAB	71.01 ±0.63aC
	S30	79.7 ±2.2bAB	18.4 ± 2.1bA	68.03 ±0.73bC
	S90	76.9 ±3.3bA	23.3 ± 2.2cA	66.23 ±0.19cC
	S150	55.6 ±1.0cC	24.7 ± 1.6cA	66.81 ±0.28cC

Values are given as mean ± expanded uncertainty limit (EUL).
a-c Different small letters in the same column indicate significant differences ($p < 0.05$) in a particular lot (C, O or S) at different storage period.
A-C Different capital letters in the same column indicate significant differences ($p<0.05$) among lots C, O and S over a fixed storage period ($p < 0.05$).

Significant ($p<0.05$) differences in oil binding capacity (OBC) were found during freezing (86.6±2.6%). In frozen storage, OBC values were stable from 30 (81.3±3.2%) to 90 days (75.2±3.8%) but had decreased significantly by 150 days (64.0±2.7%). The mechanism of OBC is thought to be the combination of physical entrapment of oil molecules in the network and the hydrophobicity of the material when the hydrophobic groups in the protein chain are exposed (He, Franco, & Zhang, 2013). The ability to trap oil molecules in the gel is likely related to the hydrophilic nature and smallness of pores in the gel network. During frozen storage the formation of larger ice crystals promote the formation of large pore structures, increasing the diffusivity of both water and oil droplest (Sahin & Sumnu, 2006, chap.1) , thus reducing OBC.

3.3. Determination of cooking loss

Partial dehydration during freezing may also be reflected in an increase of initial cooking loss (CL) values, reducing the stability of the KGM network and affecting polysaccharide-water interaction (Jimenez-Colmenero et al., 2013). Moreover, as the mince had poor water binding capacity (45.2±1.2 %) because of the denatured proteins, the formation of ice crystals aggravated its negative effect on both protein-water and KGM-water ratios, thus increasing CL. The principal differences in CL among lots were found during frozen storage: in lots C and S, CL increased significantly from 30 to 90 days frozen storage. In lot O on the other hand, water loss remained stable (around 12%) throughout storage. Moreover, CL was significantly lower ($p<0.05$) in lot O than in lots C or S at any storage time. This suggests that in lot O, the KGM network was the principal system (continuous phase), containing the oil-protein-water emulsion as a filler which produce a compact structure which entraps water in a more efficient way after cooking. As a result lot O exuded less water after heating than lots C or S (Damodaran & Paraf,1997). This structural improvement produced by heating in lot O does not affect WBC data obtained at 25 °C. The loss of WBC was similar in the three lots, while CL was lower in lot O. The centrifugal force produced by WBC measurements was thus a mechanical effect which, along with the pressurizing caused by ice crystals during frozen storage, produced physical damage in the network (similar in all three lots). Thus, the loss of water binding capacity was similar in all three lots while CL was lower in lot O than in lots C or S.

3.4. Colour measurement

Lightness values (*L**) ranged between 65.00±0.31 and 82.6±1.2 (Table 2). At initial time L* was significantly ($p<0.05$) higher in sample O1 than in S1 or C1. This shows that the oil-protein-water emulsion in the KGM network (lot O) had better light scattering ability (high L*) than lots S or C, which indicates a more aggregated and compacted network (Solo-de-Zaldivar, Tovar, Borderías, & Herranz, 2014) in lot O.

Freezing significantly ($p<0.05$) reduced L* to a similar extent (around 4-5%) in all three lots as a natural consequence of the partial breakage of hydrogen bonds by the formation of ice crystals and subsequent re-aggregation among biopolymer chains after the removal of water molecules from the initial network. Thus quasi-crystalline structures were formed among protein-polysaccharide chains, thus this regular organization reduces the light scattering ability (low L*) (Damodaran & Paraf, 1997; Sperling, 2001, chap. 3 and 11). Throughout frozen storage L* values remained practically constant in all three lots, maintaining the trend seen at initial time with L* higher in lot O than in lots C or S at any point during frozen storage. This trend shows the stabilizing role of the oil-in-water emulsion in the KGM matrix during frozen storage, as also reflected in CL measurements.

3.5. Puncture test

Fig. 1a and b respectively show the evolution of breaking force (BF) and breaking deformation (BD) in the three lots with increasing storage time. Freezing produced considerable hardening in all samples, especially in lot S, as reflected in the noticeable increase in BF between 1 and 30 days (Fig. 1a).

This is consistent with the fact that the decrease in WBC and the increase in CL were both greater during the same time period. Thus, the partial dehydration and the pressurizing effect of ice crystals produced large pore structures by freezing causing polymer-polymer associations and resulting in new aggregation (Shenouda, 1980) that promote stronger superjunctions, increasing BF (Fig.1a). In the case of lot S, when ice formed, the electrostatic interactions in the unfrozen matrix would increase, as a result ions in the concentrated matrix would compete with the existing electrostatic bonds, breaking some in the proteins (Shenouda, 1980) and forming others, such as ion-dipole bonds, with

KGM chains. In this way the increased ionic strength would cause the formation of a less ordered and more rigid network (Herranz, Tovar et al., 2012).

With respect to the effect of frozen storage, note that in lot O BF increased significantly between 30 and 90 days then remained constant up to 150 days frozen storage (Fig.1a). This result could be related to the growth of ice crystals during storage that could lead to a re-aggregation of junction zones, building up a rigid gel network, and so raising the BF.

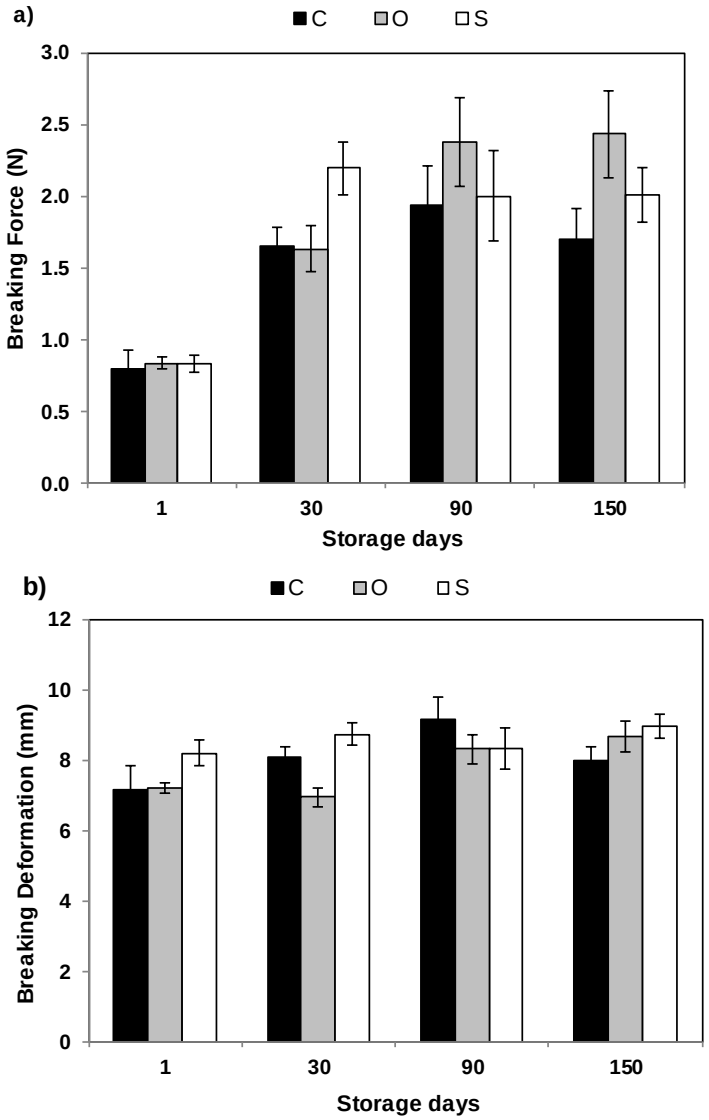

Fig. 1. Influence of storage time (1 day of refrigeration and 30, 90 and 150 days of frozen storage) on breaking force (a) and breaking deformation (b) of three lots of fish mince-glucomannan gels: control (C), 5% fish oil (O), 0.8% salt (S). Error bars represent the expanded uncertainty limits (EUL).

Changes in breaking deformation (BD) were generally not significant in any of the three lots during freezing, but there was a slight increase in BD in lots C and S and a slight decrease in lot O between 1 and 30 days. Given that BD is controlled by the shape of the junctions and their ability to change shape (spread out) when force is applied (Leksrisompong, Lanier, & Foegeding, 2012), the lack of significant changes in BD suggests that the basic interactions that support the junctions (hydrogen bonds, van der Waals forces and hydrophobic interactions) remained practically unchanged during freezing. However, during frozen storage there was a significant increase of BD in lot O between 30 and 90 days (Fig.1b). This may be a consequence of the dehydration effect (ice-crystal growth) increasing the migration of water molecules in network that could cause a local rearrangement that favours the formation of an oil-rich side in KGM matrix which may act as a lubricant between junction zones so that more distance is needed to break the junctions and to propagate the crack through the network (Sperling, 2001,). BD in lot C also increased within the same time interval (between 30 and 90 days), although not significantly (Fig.1b). However, in lot S BD remained practically constant throughout frozen storage, indicating greater mechanical stability in the network.

3.6. Oscillatory tests

3.6.1. Linear viscoelastic (LVE) range

Stress sweeps were carried out to determine the influence of freezing and frozen storage on structural stability within the limits of the LVE range. For this purpose, stress (σ_{max}) and strain (% γ_{max}) amplitudes were calculated following the same methodology as in previous work (Solo-de-Zaldivar et al., 2014). At initial time (1 day chilled), σ_{max} was similar in C1 and O1 (354±35 Pa and 338±34 Pa respectively); however γ_{max} was significantly lower in O1 (3.73±0.37%) than in C1 (5.88±0.98%). This indicates that lot O1 possessed a less flexible network than C1, which could be related to the fact that the moisture content was lower in O1 gel (84%) than in C1 (88%) and to strengthening caused by the formation of oil-protein-water emulsions within the KGM network. Thus, the system formed in O1 was strongly structured and more heterogeneous, giving a more shear-strain sensitive network (Mezger, 2006. Chap.8) than in C1.

The addition of salt to the control sample (S1) formula caused a significant increase of σ_{max} (487±49 Pa) and consequently a slight (but non-significant) increase in γ_{max}=6.0±2.1% compared to C1. These results suggest that at initial time, salt enhanced structural stability, making for a more homogeneous KGM matrix, which is consistent with the higher BD in S1 mentioned above.

These amplitude values generally increased during freezing and up to 90 days' frozen storage, reaching a maximum of σ_{max} ~ 800 Pa and γ_{max}~7% in all three lots. However, by the end of frozen storage (150 days), it was the control sample (C150) that had sustained the most damage. Its critical parameters decreased considerably (σ_{max}=103±10 Pa and γ_{max}=0.93 ±0.30), so that at the end of storage the network was much more brittle than in C1. This could be related to the considerable decrease of BF and BD observed between 90 and 150 days (Fig. 1) in C gel. Nevertheless, stress and strain amplitudes in O150 (σ_{max} =926±93 Pa and γ_{max}=7.3 ±1.4%) and S150 (σ_{max} =679±68 Pa, and γ_{max}=6.3 ±3.3%) remained practically the same as in O90 and S90 and were noticeably higher than in C150. This would seem to indicate that oil (lot O) and salt (lot S) rendered samples more stable than the control during frozen storage, particularly up to 150 days.

3.6.2. Frequency sweeps

3.6.2.1. Mechanical spectra. At initial time, mechanical spectra of the three lots showed that storage moduli (G') (Fig. 2a-c) were larger than viscous moduli (G'') (Fig. 2d-f); and specifically G_0' (storage modulus at 1 rad/s) was greater than G_0'' (loss modulus at 1 rad/s) (Table 3). Both parameters are derived from power-law fit (equations (1) and (2)):

$$G' = G_0' \cdot \omega^{n'} \quad \textbf{(1)}$$

$$G'' = G_0'' \cdot \omega^{n''} \quad \textbf{(2)}$$

Exponents n' and n'' may be related to the time-stability of the network given the frequency-dependence (ω) of viscoelastic moduli (Mezger, 2006, chap.8). In general, frequency dependence was slight in all three lots and in certain samples there was no fit (Table 3), indicating that both moduli were practically independent of ω. These data show that all samples were strong (true) gels (Clark & Ross-Murphy, 1987), with S1 similar to C1.

In the case of lot O1, both viscoelastic moduli, G' (Fig. 2b) and G'' (Fig. 2e), were greater than in the other lots; in particular, G_0'' was 16% larger than in C1 or S1, and G_0' was 10% greater than in C1 or S1 (Table 3). This trend suggests that "oil-protein-water" emulsion caused a firmer network in O1 than in C1 or S1. This suggests that the "oil-protein-water" emulsion acts as an effective filler, which is consistent with the fact that L* was significantly higher in lot O1 than in C1 or S1 (section 3.4). Moreover, n' and n'' values (O1) were both slightly higher than in C1 and the n' value higher than in S1. This indicates a less time-stable matrix (O1) resulting in a more shear-strain sensitive network as reported in stress sweeps.

All three lots underwent considerable hardening during freezing, with G' and G'' increasing greatly throughout the frequency range (Fig. 2). Lot S in particular was the most sensitive to freezing, with G_0' increasing 90% and G_0'' 67% between S1 and S30. In S30 freezing produced a strong packing effect so that the elasticity of the network increased more than the viscosity. The dense packing of the network in S30 from SAOS test is consistent with the fact that BF was highest in the case of large deformation (puncture test). At the same time, the time-stability of the network increased, as reflected in the fact that frequency-dependence was less (lower n' and n'' exponents) in S30 than in S1 (Table 3).

In the case of the control gel (C30), G_0' and G_0'' increased by 58% and 39% respectively relative to C1, and at the same time n' decreased (40%), indicating a greater degree of connectivity. Thus, there was some synergy between proteins and polysaccharides in lot C after freezing, which produced a more stable conformation in the C30 gel.

In the case of the control gel (C30), G_0' and G_0'' increased by 58% and 39% respectively relative to C1, and at the same time n' decreased (40%), indicating a greater degree of connectivity. Thus, there was some synergy between proteins and polysaccharides in lot C after freezing, which produced a more stable conformation in the C30 gel.

Conversely, the effect of freezing was significantly lower in lot O than in S or C, where viscoelastic parameters increased, but in a smaller proportion. In particular, G_0' increased (33%) in O30 relative to O1 and G_0'' in 13% more than in O1 (Table 3). These results suggest that the "oil-protein-water" system as a filler played a stabilizing role in the KGM matrix, partially reducing the formation of ice crystals as compared to C30 and S30 gels. Therefore, the filler may stabilize the continuous phase (solid matrix) in the gel, thus improving its ability to immobilize fluid in the network by lowering the degree of molecular association

due to freezing comparing with lots C30 and S30. This behaviour was also reflected in the rheological parameters *n'* and *n''*, which registered a similar decrease up to 30 days in C30, S30 and O30.

As regards the effect of frozen storage, in general from 30 to 150 days both viscoelastic moduli increased with time, and parameters *n'* and *n''* changed only slightly during storage (Table 3). This is a normal response to the detrimental effect on networks associated with frozen storage as a consequence of the growth of ice crystals over storage that produces stronger junctions that increase the viscoelastic parameters (rigidity) of gels (Mao et al., 2001). In lot C the increase of viscoelastic moduli was more progressive, reaching the highest values of G_0' and G_0'' at 150 days (C150) (Table 3). The negative effect of frozen storage was particularly apparent in the fact that *G''* values were highest (practically constant throughout the frequency range) in C150 (Fig. 2d). Thus, when *G''* increases, the number of dangling chains (free ends) also increases and the ability of the network to store energy in the continuous phase is diminished (te Nijenhuis, 1997, chap.1). This is consistent with the brittleness of C150 gel deduced from puncture tests and corroborated by the very low amplitude parameters from stress sweeps (discussed above). The mechanical spectrum in lot O was similar, except that *G'* and *G''* values versus initial time were highest at 90 days (O90) (Fig. 2b and e). In this case *G''* modulus increased with decreasing frequency, i.e. n''=-0.072 (Table 3). This result suggests that after 90 days ice recrystallization could partially attack the continuous gel phase (KGM matrix) as reported by Jimenez-Colmenero et al. (2013), causing the formation of new dangling substructures which, together with the molecular fragments in the dispersed phase could increase the frictional forces at low frequencies (high oscillatory time), thus causing *G''* to increase. An analogous effect was noted in the case of large deformations: there was a sharp increase of BF between O30 and O90, and particularly a significant increase in BD (O30-O90), which is entirely consistent with the high *G''* modulus in O90 (Fig. 2e), given that when dissipated energy increases, more distance is needed to propagate cracking (Sperling, 2001,chap.3 and 11). In addition, the two kinds of measurements (large and small oscillatory deformations) provide different rheological data, and these showed the same trend between 90 and 150 days. Therefore, the viscoelastic parameters remained the same between O90 and O150 (Table 3), as in the case of BF and BD over the same time interval (discussed earlier).

Table 3. Power law parameters: *Go'* and *n'* from equation (1) and, *Go''* and *n''* from equation (2) of samples C, O and S after 1, 30, 90 and 150 days of storage. T=25°C.

		Go' (kPa)	n'	r^2 Eq. (1)	Go'' (kPa)	n''	r^2 Eq. (2)
LOT C	C1	7.93±0.01	0.071±0.001	0,986	1.149±0.002	0.036±0.001	0,877
	C30	12.57±0.02	0.042±0.001	0,999	1.59±0.01	n.f*	n.f*
	C90	15.65±0.05	n.f*	n.f*	1.77±0.01	0.034±0.002	0,706
	C150	17.84±0.02	0.0518±0.001	0,970	2.28±0.01	n.f*	n.f*
LOT O	O1	8.79±0.01	0.081±0.001	0,991	1.384±0.004	0.053±0.002	0,9
	O30	11.71±0.01	0.0547±0.0004	0,995	1.583±0.001	0.035±0.001	0,978
	O90	16.79±0.01	0.0273±0.0004	0,974	2.438±0.008	0.075±0.002	0,915
	O150	16.32±0.01	0.042±0.001	0,980	2.36±0.01	n.f*	n.f*
LOT S	S1	7.56±0.01	0.064±0.001	0,975	1.015±0.001	0.051±0.001	0,97
	S30	14.41±0.01	0.048±0.001	0,986	1.698±0.003	0.034±0.001	0,875
	S90	13.08±0.04	n.f*	n.f*	1.242±0.006	n.f*	n.f*
	S150	15.05±0.02	0.043±0.001	0,911	2.11±0.01	n.f*	n.f*

Values are given as power law parameters ± standard deviation of fitted parameters
*n.f: not fitted to power law equations (1) or (2).

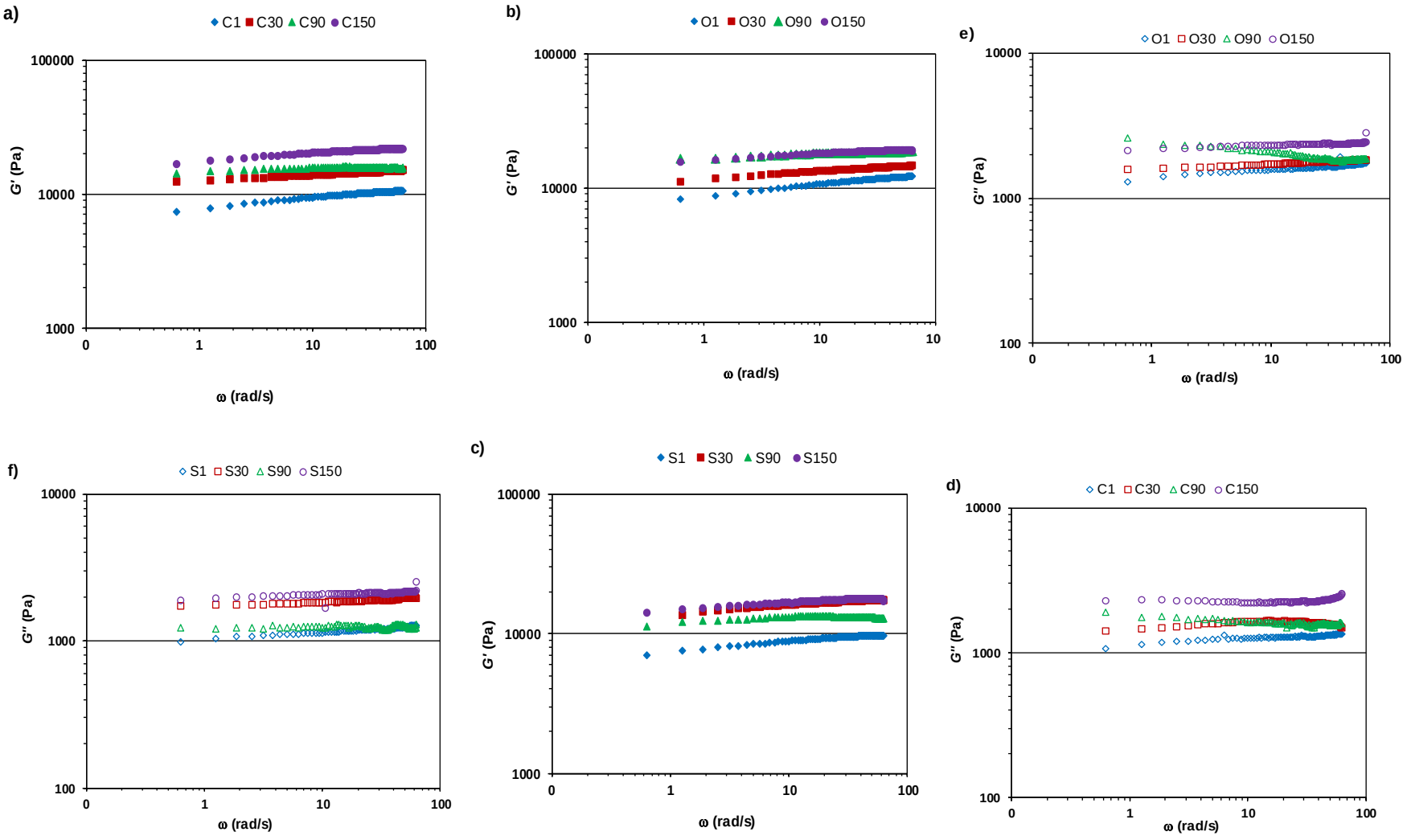

Fig. 2. Influence of storage time on mechanical spectra at 25°C of three lots of fish mince -glucomannan gels: control (C), 5% fish oil (O), 0.8% salt (S) storage modulus (G') (a–c) and loss modulus (G'') (d-f).

In lot S the influence of frozen storage time was weaker and less uniform than in lots C or O. Both G_0' and G_0'' decreased slightly between S30 and S90, and conversely both parameters increased between S90 and S150, considerably more in the case of G_0'' (70%) than of G_0' (16%). This higher increase of viscous parameters at the end of storage indicates an absence of synergy (protein-polysaccharide). This suggests some "microphase separation" of protein substructures and KGM fragments from the principal matrix caused by the growth of ice crystals in lots S90-S150, possibly related to the high loss of water binding in the S150 network (analysed in Section 3.2).

3.6.3. Creep and recovery tests

In creep tests a constant stress (σ_0) within the linear viscoelastic (LVE) range was applied to the three gels and the dimensions were recorded with time in terms of the time-dependent creep-recovery compliance *J(t)* (data not shown). The relaxation modulus *G(t)* was obtained from *J(t)*, *G(t)* =1/*J(t)*, which can be fitted with time by applying equation 3 (Ferry, 1980).

$$G(t) = S \cdot t^{-n} \qquad \textbf{(3)}$$

where *S* is the gel strength factor and *n* the relaxation exponent, which are both characteristic network parameters (Lapasin & Pricl, 1999, chap.4). At initial time, lot O1 had a higher *S* value than S1 or C1, indicating a greater degree of packing in the network, but with less crosslinking as evidenced by the fact that *n* was greater in O1 than in C1 (Table 4). This may be a consequence of the natural heterogeneity present in gel O1, caused by the ("oil-protein-water") emulsion in the KGM network; this would strengthen the network as it is maintained over longer time scales. The three lots underwent some strengthening during freezing, the lot with salt (S) more than the others (O and C). Thus the *S* value increased considerably (96%) between S1 and S30, while in lots C and O the increase in the *S* parameter was noticeably smaller, around 16% (Table 4). This could be related to the fact that the decrease in WBC and the increase in CL were greatest in S1-S30 (section 3.2), presumably because salt reinforces the partial dehydration associated with freezing (Kitazawa, Kawai, Inoue, & Shinano, 1995). The presence of Na^+ cations in lot S may have facilitated the generation of ice crystals, resulting in an increase of the amount of frozen water. In this way the degree of association among the polymers would increase, resulting

in a firmer matrix (high *S*), but with some internal discontinuity (Jimenez-Colmenero et al., 2013) as reflected in a high *n* value (Table 4). Thus, S30 was more sensitive to freezing than the control (C30). The onset of freezing damage occurred later (90 days) in the control gel. Therefore, C90 had the highest gel strength and relaxation exponent (Table 4), indicating that at least for the first 30 days there was some KGM–protein-water synergy in the system that improved the degree of connectivity in the network (lower *n* values in C30) by increasing the amount of water bound to the network. Thus, the network connectivity in the control was maintained until 30 days of frozen storage, as reflected by the fact that *S* -parameter and *n* values were lower in C30 than in O30 or S30 (Table 4).

Table 4. Gel strength (*S*) and relaxation exponent (*n*) from equation (3) for gels C, O and S after 1 and 150 days of storage. T=25°C.

		S (kPa)	n	r^2
LOT C	C1	6.78±0.02	0.117±0.002	0.976
	C30	7.88±0.03	0.118±0.003	0.957
	C90	17.30±0.15	0.183±0.01	0.930
	C150	13.02±0.03	0.134±0.001	0.990
LOT O	O1	8.42±0.03	0.125±0.002	0.972
	O30	9.75±0.04	0.156±0.003	0.971
	O90	12.51±0.08	0.147±0.004	0.932
	O150	17.92±0.08	0.135±0.003	0.963
LOT S	S1	5.82±0.02	0.123±0.002	0.969
	S30	11.43±0.04	0.158±0.002	0.987
	S90	15.91±0.09	0.154±0.004	0.954
	S150	11.70±0.02	0.128±0.001	0.991

Values are given as power law parameters ± standard deviation of fitted parameters.

As regards frozen storage, there was a considerable loss of gel strength (around 25%) at 150 days in both S150 and C150 (Table 4). The decrease of gel strength (*S*) between C90-C150 and S90-S150 may be compatible with the increase of connectivity (low *n* parameters) in these networks after 150 days. For instance, in a KGM matrix the crosslinks are sizeable regions that can break and recombine (Herranz, Tovar et al., 2012). Conversely, in O150 by

the end of storage, *S* had increased and *n* decreased with respect to O90 (Table 4). In this case (O150) it seems that the filler ("oil-protein-water") effectively stabilized the network, especially at the end of the frozen storage period.

3.7. Sensory analysis

The taste acceptance test of unfrozen samples (C1, O1 and S1) scored lots C1 and S1 in the middle of the scale (1-9). S1 scored better (6.05±0.52) than C1 (5.29±0.70), although not significantly ($p<0.05$). However, sample O1 was scored much lower (3.93±0.62) by the panellists, probably because of the oily flavour (Table 5). In the case of texture acceptability, samples C1 and S1 also received a higher score (5.30±0.75 and 6.02±0.68 respectively) than O1 (2.59±0.31). The differences in average values of samples C1 and S1 were not significant ($p<0.05$). The fact that taste and texture acceptability values were lowest in O1 could be related to the slippery mouthfeel of the sample (O1), probably due to the addition of oil, causing the panellists to reject the sample. This score could further be related to the greater heterogeneity of this network, which was more sensitive to shear-strain (lower γ_{max}).

Table 5. Taste acceptability (TAA) and texture acceptability (TEA) of samples C, O and S after 1 and 150 days of storage.

		TAA	TEA
LOT C	C1	5.29 ± 0.70 aA	5.30 ± 0.75 aA
	C150	4.18 ± 0.87 aA	5.04 ± 0.30 aA
LOT O	O1	3.93 ± 0.62 aB	2.59 ± 0.31 aB
	O150	4.73 ± 0.96 aA	3.85 ± 0.90 bA
LOT S	S1	6.05 ± 0.52 aA	6.02 ± 0.68 aA
	S150	6.52 ± 0.58 aB	5.81 ± 0.22 aB

Values are given as mean ± expanded uncertainty limit (EUL).
a-b Different small letters in the same column indicate significant differences ($p<0.05$) between 1 and 150 days of storage for each lot.
A-B Different capital letters in the same column indicate significant differences among lots C, O and S over the same storage period ($p<0.05$).

After 150 days of frozen storage, the panellists did not find significant differences in the taste and texture acceptability of each lot as compared to the scores awarded at 1 day of storage. As before freezing, lot S scored best for both attributes (6.52±0.58 for taste and

5.81±0.22 for texture), but in this case they were significantly different ($p<0.05$). Conversely, O150 scored the lowest. This means that oil addition is not well accepted from a sensory point of view, as the oil added in these samples lent it slippery feel which is not "usual" in restructured fish products. Moreover, the panellists seemed to prefer a better-formed texture with a higher degree of cohesion among the particles for this kind of products.

4. CONCLUSIONS

The three prototypes made with non functional fish muscle and glucomannan behaved as true gels during freezing and frozen storage. At initial time (before freezing), lot O had a more compacted and heterogeneous network than lot C or S due to the formation of an "oil-protein-water" system in the KGM matrix. Freezing in particular, but also frozen storage, produced irregular hardening of the three lots depending on storage time and sample because of the partial dehydration associated with the formation of ice crystals, which made for more polymer-polymer interactions. The "oil-protein-water system" in lot O, as a filler inside the KGM matrix (continuous phase), had a stabilizing effect during freezing and frozen storage, reducing the number of polymer-polymer associations and improving post-cooking water-binding capacity. Samples C and S scored better in the sensory evaluations because they had a more homogeneous network and lacked the slippery mouthfeel conferred by the oil.

ACKNOWLEDGEMENTS

The authors would like to thank the Spanish Ministry of Economy and Competitiveness for its support under Project AGL-24693, and the Consejo Superior de Investigaciones Científicas for Predoctoral Scholarship BES-2009-018321. The authors also wish to thank the Xunta de Galicia for its financial support under the Consolidation and restructuring program of competitive research units: Strategic Research Partnerships (2009/060).

REFERENCES

AOAC. (2000). Official method of analysis (17th ed.). Maryland, USA: Association of Official Analytical Chemists.

Borderias, A. J., Jimenez-Colmenero, F., & Tejada, M. (1985). Viscosity and emulsifying ability of fish

and chicken muscle protein. Journal of FoodTechnology, 20(1), 31–42.

Campo-Deaño & Tovar (2009). The effect of egg albumen on the viscoelasticity of crab sticks made from Alaska pollock and Pacific whiting surimi. Food Hydrocolloids, 23, 1641–1646.

Cardoso, C., Ribeiro, B., & Mendes, R. (2012). Effects of dietary fibre and microbial transglutaminase addition on the rheological and textural properties of protein gels from different fish species. Journal of Food Engineering, 113(4), 520–526.

Chua, M., Baldwin, T. C., Hocking, T. J., & Chan, K. (2010). Traditional uses and potential health benefits of Amorphophallus konjac K. Koch ex N.E.Br. Journal of Ethnopharmacology, 128(2), 268–278.

Clark, A. H., & Ross-Murphy, S. B. (1987). Structural and mechanical properties of biopolymer gels. Advances in Polymer Science, 83, 57–192.

Damodaran, S. &. Paraf, A. (1997). Food Proteins and their Applications (Vol. Chapter 4 and 16).New York: Inc.

Eide, O. L. A., Borresen, T., & Strom, T. (1982). Minced Fish Production From Capelin (Mallotus villosus). A New Method for Gutting, Skinning and Removal of Fat from Small Fatty Fish Species. Journal of Food Science, 47(2), 347–349.

Ferry, J. D. (1980). Viscoelastic properties of polymers (3rd ed.). New York: John Wiley and Sons, Inc.

Gómez-Guillén, M. C., Montero, P., Hurtado, O., & Borderías, A. J. (2000). Biological characteristics affect the quality of farmed atlantic salmon and smoked muscle. Journal of Food Science, 65(1), 53–60.

He, S., Franco, C., & Zhang, W. (2013). Functions, applications and production of protein hydrolysates from fish processing co-products (FPCP). Food Research International, 50(1), 289–297.

Herranz, B., Borderias, A. J., Solas, M. T., & Tovar, C. A. (2012). Influence of measurement temperature on the rheological and microstructural properties of glucomannan gels with different thermal histories. Food Research International, 48(2), 885–892.

Herranz, B., Borderias, A. J., Solo-de-Zaldívar, B., Solas, M. T., & Tovar, C. A. (2012). Thermostability analyses of glucomannan gels. Concentration influence. Food Hydrocolloids, 29(1), 85–92.

Herranz, B., Solo-De-Zaldivar, B., & Borderias, A. J. (2013). Obtaining a Restructured Seafood Product from Non-Functional Fish Muscle by Glucomannan Addition: First Steps. Journal of Aquatic Food Product Technology, 22(2), 201–208.

Herranz, B., Tovar, C. A., Solo-de-Zaldívar, B., & Borderias, A. J. (2012). Effect of alkalis on konjac glucomannan gels for use as potential gelling agents in restructured seafood products. Food Hydrocolloids, 27(1), 145–153.

Herranz, B., Tovar, C. A., Solo-de-Zaldívar, B., & Borderias, A. J. (2013). Influence of alkali and temperature on glucomannan gels at high concentration. LWT - Food Science and Technology, 51(2), 500–506.

Hui, Y.H., Cross, N., Kristinsson, H.G. Lim, M.H., Nip, W.K. Siow, L.F. & Stanfield, P.S. (2006). Biochemistry of seafood processing (Chapter 16) in Food biochemistry and Food processing . Blackwell publishing Oxford.

Iglesias-Otero, M. A., Borderías, J., & Tovar, C. A. (2010). Use of Konjac glucomannan as additive to reinforce the gels from low-quality squid surimi. Journal of Food Engineering, 101(3), 281–288.

ISO (2008). Sensory analysis–Methodology–General guidance (UNE-ISO 6658). Geneva, Switzerland: International Organization for Standardization.

ISO (2010). Sensory analysis–General guidance for the design of test rooms (UNE-ISO 8589). Geneva, Switzerland:International Organization for Standardization.

Jimenez-Colmenero, F., Cofrades, S., Herrero, A. M., Solas, M. T., & Ruiz-Capillas, C. (2013). Konjac gel

for use as potential fat analogue for healthier meat product development: Effect of chilled and frozen storage. Food Hydrocolloids, 30(1), 351–357.

Kitazawa, H., Kawai, Y., Inoue, N., & Shinano, H. (1995). Influence on the decrease of Ca2+-ATPase activity and solubility of carp myofibrils during frozen storage. Fisheries Science, 61, 1037–1038.

Kraemer, W. J., Vingren, J. L., Silvestre, R., Spiering, B. A., Hatfield, D. L., Ho, J. Y., Fragala, M. S., Maresh, C. M., & Volek, J. S. (2007). Effect of adding exercise to a diet containing glucomannan. Metabolism, 56(8), 1149–1158.

Lapasin, R., & Pricl, S. (1999). Rheology of industrial polysaccharides: Theory and applications (1st ed.). Gaithersburg: Aspen Publishers. (Chapter 4).

Leksrisompong, P. N., Lanier, T. C., & Foegeding, E. A. (2012). Effects of Heating Rate and pH on Fracture and Water-Holding Properties of Globular Protein Gels as Explained by Micro-Phase Separation. Journal of Food Science, 77(2), E60–E67.

Levrat-Verny, M. A., Behr, S., Mustad, V., Remesy, C., & Demigne, C. (2000). Low levels of viscous hydrocolloids lower plasma cholesterol in rats primarily by impairing cholesterol absorption. Journal of Nutrition, 130(2), 243–248.

Mao, R., Tang, J. & Swanson, B.G. (2001). Water holding capacity and microstructure of gellan gels. Carbohydrate Polymers, 46, 365–371.

Mezger, T. (2006). The rheology handbook. Hannover, Germany: Vincentz Network GmbH & Co. KG. (Chapter 8).

Nishinari, K., Williams, P. A., & Phillips, G. O. (1992). Review of the physico-chemical characteristics and properties of konjac mannan. Food Hydrocolloids, 6(2), 199–222.

Park, J. W. (1996). Temperature-tolerant fish protein gels using Konjac flour. Journal of Muscle Foods, 7(2), 165–174.

Sahin, S. & Sumnu, S. G. (2006). Physical Properties of Foods. New York: Springer Science+Business Media, LLC. (Chapter 4).

Shenouda, S. Y. K. (1980). Theories of protein denaturation during frozen storage of fish flesh. Advances in Food Research, 26, 275–311.

Solo-de-Zaldívar, B., Herranz, B. & Borderias, J. A. (2012). First steps in using glucomannan to make thermostable gels for potencial use in mince fish restructuration. International Journal of Food Engineering, 8(1), ISSN 1556-3758, DOI: 10.1515/1556-3758.2407.

Solo-de-Zaldívar, B., Tovar, C. A., Borderías, A. J., & Herranz, B. (2014). Effect of deacetylation on the glucomannan gelation process for making restructured seafood products. Food Hydrocolloids, 35, 59–68.

Sperling, L. H. (2001). Introduction to physical polymer science (3th ed.). New York: Wiley-Interscience. John Wiley and Sons, Inc. (Chapter 11).

Steffe, J. F. (1996). Rheological methods in food process engineering (2nd ed.). East Lansing: Freeman Press. (Chapter 5).

te Nijenhuis, K. (1997). Advances in polymer science. Thermoreversible Networks. Viscoelastic Properties and Structure of Gels, 130: Berlin Springer-Verlag (Chapter 1).

Vuksan, V., Sung, M.-K., Sievenpiper, J. L., Stavro, P. M., Jenkins, A. L., Di Buono, M., Lee, K.-S., Leiter, L. A., Nam, K. Y., Arnason, J. T., Choi, M., & Naeem, A. (2008). Korean red ginseng (Panax ginseng) improves glucose and insulin regulation in well-controlled, type 2 diabetes: Results of a randomized, double-blind, placebo-controlled study of efficacy and safety. Nutrition, Metabolism and Cardiovascular Diseases, 18(1), 46-56.

Walstra, P. (2003). Physical chemistry of foods. New York: Marcel Dekker Inc.

Xiong, G., Cheng, W., Ye, L., Du, X., Zhou, M., Lin, R., Geng, S., Chen, M., Corke, H., & Cai, Y.-Z. (2009). Effects of konjac glucomannan on physicochemical properties of myofibrillar protein and surimi gels from grass carp (Ctenopharyngodon idella). Food Chemistry, 116(2), 413-418.

PASTEURIZATION AND CHILLED STORAGE OF RESTRUCTURED FISH MUSCLE PRODUCTS BASED ON GLUCAMANNAN GELATION

Solo-de-Zaldívar, B.[a], Tovar, C. A.[b], Borderías, A. J.[a] and Herranz, B.[a*]

[a]Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN-CSIC), José Antonio Novais 10, 28040 Madrid, Spain.

[b]Department of Applied Physics, Faculty of Sciences, University of Vigo, As Lagoas, 32004 Ourense, Spain.

Food Hydrocolloids, 43, 418-426.
(DOI: 10.1016/j.foodhyd.2014.06.016)

ABSTRACT:

The effect of pasteurization and stability during subsequent chilled storage of three fish restructured prototypes made with 1.25% glucomannan and fish mince (sawdust from cutting of frozen fish blocks) was examined through physicochemical (water binding ability, mechanical and rheological behaviour), microbiological and sensory analyses. Three lots were made: control lot (C); lot O (with 5% added fish oil), and lot S (with 0.8% added NaCl). The three lots were pasteurized (80 °C 20 min) and stored at 5 °C. Analyses were carried out at 1, 7, 21 and 35 days of chilled storage.

Pasteurization produced a significant decrease in water binding capacity (WBC) and cooking loss (CL), less evident in lots O and S, due to changes in structure. In the case of lot O, an emulsion was formed in gel network, making this sample less acceptable from a sensory point of view. The addition of salt (lot S) produced an ionic situation more conducive to protein-polysaccharide associations, thus reinforcing the gel network.

KEYWORDS: Glucomannan; Restructured seafood products; Pasteurization

1. INTRODUCTION

The last few decades have seen an increase in the consumption of fish due to consumer perception of the health benefits of both lean and fatty fish (Rosnes, Skara, T. & Skipnes, 2011), and particularly to increasing demand for quality, variety and new seafood products based on restructuring (Kennedy, Storrs, Devoluy & Cruveiller, 2007). Various different technologies are used for seafood restructuring. Thermally-induced gelation is the most common method, but for this technology to work, the muscle proteins must retain their protein functionality so as to ensure good gelling capacity. However, there are many muscle by-products — one of the sources of raw material for making fish restructurates — which lack or have largely lost protein functionality as a result of prior thermal or mechanical processing, so that texturizing by thermally-induced gelation is impossible. A new possibility to get round this technological problem and make seafood products from muscles lacking functionality is to add konjac glucomannan (KGM) — a neutral hydrocolloid which is able to form thermostable hydrogels in the presence of an alkali — to the mince (Herranz, Borderias, Solo-de-Zaldívar, Solas & Tovar, 2012b; Herranz, Borderias, Solas &

Tovar, 2012c; Herranz, Solo-de-Zaldívar & Borderias 2013). Thus, KGM would act as a gelling agent by forming a coupled network with protein particles in the continuous phase (solid matrix). In this way, gelation could be induced in a wide variety of fish muscle by products from food processing which have lost any functionality due to heating in order to make seafood analogues (Herranz, Solo-de-Zaldívar & Borderias, 2013). Moreover, konjac flour has high water-absorbing capacity (Chua, Baldwin, Hocking & Chan, 2010) and has numerous physiological effects and therapeutic applications (González Canga et al., 2004; Zhang, Yoshimura, Nishinari, Williams, Foster & Norton, 2001). Several papers have been published on the use of glucomannan (GM) in the making of restructured seafood products, with a view to choosing the best gelling conditions and testing them for thermostability. Experimentation with alkalinization conditions to improve gelation showed that 0.6N KOH was the most suitable alkali to produce more elastic and time-stable GM gels (Herranz, Tovar, Solo-de-Zaldívar & Borderias, 2012a) and that the most suitable pH level was 11 (Solo-de-Zaldívar, Tovar, Borderías & Herranz, 2014). As regards thermostability, a comprehensive thermo-rheological study of these GM gels conducted at different temperatures concluded that pre-heating reinforced the GM network (Herranz, Borderías, Solas, et al., 2012). Based on these studies, some fish prototypes were made using GM and non-functional fish muscle ("sawdust"). The flavour and texture closest to those of fish (hake) muscle were achieved with a prototype gel made from 75% sawdust and 25% aqueous glucomannan dispersion (AGD) with 1.25% GM (final concentration) (Herranz et al., 2013).

Pasteurization is one of the commonest methods for maintaining the safety and prolonging the shelf life of chilled fish products. It reduces the microbial load of fish products, but it is important to determine how heating affects the thermostability of a GM network with sawdust, since pasteurization may induce structural changes and a loss of textural quality. Especially in so sensitive a material as the gel, it can cause protein denaturation and a decrease in water binding capacity and colour, depending on the intensity of treatment (Fernández-Segovia, Camacho, Martínez-Navarrete, Escriche & Chiralt, 2003).

Restructured products also offer the possibility of using different kinds of ingredients and additives to confer a particular taste, texture and/or functional capacity. Important

among these ingredients are salt and fish oil owing to their organoleptic and functional characteristics.

The aim of this work was to study the effect of heating (pasteurization) on the physicochemical, rheological and sensory quality of prototype fish muscle restructurates made with sawdust and glucomannan with or without added salt and fish oil, and stored for 35 days at 5 °C.

2. MATERIALS AND METHODS

2.1. Raw material, additives and reagents

Konjac glucomannan (glucomannan purity 100%, MW: $11x10^5$ Da) was purchased from Guinama, Valencia, Spain. Fish muscle "sawdust" obtained from sawing frozen blocks of hake (*Merluccius capensis*) as raw material was supplied by the company Frinova (Pescanova S.A., Vigo, Spain).

The fish oil used was from the company Omevital© (18/12 TG Gold, Cognis GmbH, Illertissen, Germany) and salt was NaCl from Panreac Química, S.A. (Barcelona,Spain). All the chemicals used were analytical grade and were supplied by Panreac Química S.A. (Barcelona, Spain).

2.2. Preparation of fish prototypes

Aqueous glucomannan dispersions (AGD) at 5% (w/v) were prepared as described in Herranz et al., (2013). Fish mince gel preparation was as follows: the fish ("sawdust") was homogenized for 5 min in a Stephan UM5 mixer (Stephan u. Söhne GmbH & Co., Hameln, Germany) at 2 °C with vacuum. After that, 5% (w/v) AGD was added in proportion 25:75 (w/w) and homogenized for another 10 min, followed by addition of salt or oil. Then, 0.6 N KOH was added to bring the pH up to 11.0-11.1 and induce gelation. Cylindrical containers (diameter 3 cm x height 3.5 cm) and Petri dishes were filled with these three different homogenous mixtures and packed in vacuum conditions. After that, all samples were set first for 1 h at 30 °C and then for 4 h at 5 °C. These setting conditions were chosen based on prior tests to reduce the setting time as much as possible but maintaining texture similar to that of whole fish muscle (data not shown). The high pH values were reduced by immersing the samples in 0.2 M citrate-phosphate buffer at pH 5.1 (gel: buffer ratio was 1:10) for 16 h

at 5 °C. In this way three lots with a 1.25% final glucomannan concentration were obtained: control lot (C); oil lot (O): control lot with addition of 5% fish oil, and salt lot (S): control lot with 0.8% NaCl, which is the regular amount in fish products. This amount of added oil (5%) was chosen based on previous experiments in which various restructured fish prototypes (in proportion 25:75 (w/w) with 1.25% final glucomannan concentration) were made with different oil levels (0%, 1%, 5% and 10%) at various pH (data not shown). The objective of these prior assays was to mimic the texture and flavour of whole fish muscle with low (1%), medium (5%) and high fat content (10%), adding an oil enriched in long chain omega-3 fatty acids without off-flavour. Texture (puncture) and sensory analyses showed that the prototype with 5% oil most resembled a fish hake fillet in texture and flavour (data not shown).

Each lot was then divided into two parts: one part of these neutral gels was kept in chilled storage (5 °C). The other was vacuum packed in plastic bags and pasteurized in an oven (Rational Combi-Master CM6, Germany) at 80 ° C for 20 min then immediately cooled in a water/ice slurry and stored at 5 °C. The non-pasteurized samples (0 day) were analysed after 24 h of chilled storage, and the pasteurized samples were kept at 5 °C for analysis after 1, 7, 21 and 35 days of chilled storage. The different samples were coded as shown in Table 1.

All the analytical data after 24 h of chilled storage (day 0), reported below, were taken from a previous study (Solo-de-Zaldívar, Herranz, Borderías, & Tovar, 2014).

Table 1. Nomenclature of control samples (C), samples containing fish oil (O) and samples containing salt (S) during chilled storage.

Storage time (days)	**Lot C**	**Lot O**	**Lot S**
Fresh restructured	C 0	O 0	S 0
1	C 1	O 1	S 1
7	C 7	O 7	S 7
21	C 21	O 21	S 21
35	C 35	O 35	S 35

2.3. Analyses

2.3.1. Proximate analyses and apparent viscosity of raw material

Moisture, fat and ash contents of raw fish were determined (AOAC, 2000) in quintuplicate. Crude protein content was measured in quadruplicate with a LECO FP-2000 Nitrogen Determinator (Leco Corporation, St Joseph, MI, USA). The apparent viscosity was measured with a Brookfield viscometer (Model DV-III, Brookfield Viscometers Ltd, Harlow, Essex, England) using a no. 1 disc spindle at a speed = 12 rpm) following the method described by Borderias, Jimenez-Colmenero & Tejada (1985).

2.3.2. Water binding capacity (WBC) and oil binding capacity (OBC)

Water binding capacity (WBC) was measured according to Eide, Borresen & Strom, (1982). Samples were cut into small pieces and then placed in centrifuge tubes and centrifuged (3000g) at room temperature for 10 min in a Jouan MR1812 centrifuge (Saint Nazaire, France). WBC was expressed as percent water retained relative to the amount of water present in the sample prior to centrifuging. Samples formulated with 5% fish oil (Lot O) were tested for fat release according to Gómez-Guillén, Montero, Hurtado & Borderías, (2000). The aqueous plus oil fraction which was retained in the filter after centrifugation was dried to constant weight. The result was expressed as oil binding capacity (OBC) per 100 g initial weight of product. All determinations were carried out in triplicate.

2.3.3. Cooking loss determination

Samples were weighed (40 g), cut into small pieces and placed in a plastic bag with small holes. This bag was then put into another bag with the holes at the bottom to drain the sample drip and was cooked hanging in an oven (Rational Combi-Master CM6) for 20 min at 100 °C. The sample was weighed after cooling. Cooking loss was expressed as g/100 g by weight difference between uncooked and cooked samples.

2.3.4. Colour measurement

Colour measurements were performed at five surface points of each sample using a colorimeter (Minolta Chroma Meter Cr-200, Japan) standardized from a white calibration

plate. Measurements were taken using the chromatic model CIE L*a*b* (CIELAB). The lightness parameter (*L**) was used to compare and characterize the samples.

2.3.5. Microbiological analysis

Samples for total microbial analyses were prepared in a vertical laminar flow cabinet (model AV 30/70, Telstar, Madrid, Spain). For each sample, 10 ± 0.5g (in duplicate) was placed in a sterile plastic bag (Sterilin, Stone, Staffordshire, UK) with 90 mL of buffered peptone water (Cultimed, Panreac Química, Barcelona, Spain) (0.1%) (Panreac Química, S.A., Barcelona, Spain). After 1 min in a stomacher blender (Colworth 400, Seward, London, UK), appropriate decimal dilutions were pour-plated on Plate Count Agar (PCA) (Merck, Germany) for 72 h at 30 °C to determine the total viable count. The results were expressed as logarithms of colony forming units per gram (log cfu/g).

Analyses were carried out in quintuplicate on day 0 (fresh sample) and after 1, 7, 21 and 35 days of chilled storage following pasteurization.

2.3.6. Dynamic rheometry measurements: stress sweep test

Viscoelastic properties were measured using a Bohlin CVO controlled stress rheometer (Bohlin Instruments, Inc. Cranbury, NJ). Measurements were made using parallel-plate geometry (20 mm diameter and 1 mm gap). Before measurement, samples from each lot were tempered at ambient temperature and cut from Petri dishes into disk-shaped slices 20 mm in diameter and 1 mm thick with a 570 S.T.E slicer (Germany). They were then placed on the lower plate of the rheometer for measuring. Any excess sample protruding beyond the upper plate was carefully removed. Samples were allowed to rest for 15 min before analyses to ensure both thermal and mechanical equilibrium at the time of measurement. Samples were covered with a thin film of Vaseline oil (Codex purissimum, vaseline oil, Panreac Química, Barcelona, Spain) to avoid evaporation. The temperature was controlled to within 0.1°C by a Peltier element in the lower plate, which was kept at 25.0°C.

Viscoelastic properties were obtained from oscillatory tests at constant frequency (6.28 rad/s), gradually increasing (300 points on the continuous mode) the input stress (10 Pa) until material deformation was complete, to determine the linear viscoelastic (LVE) region and differentiate the viscoelastic characteristics of samples (Steffe, 1996, chap.5).

These tests were carried out at least in quintuplicate at 1 and 21 days of chilled storage at T=5 °C following pasteurization.

2.3.7. Puncture tests

Cylindrical samples (diameter 3 cm x height 3.5 cm) were pierced to breaking point with a 5 mm–diameter stainless steel spherical probe at room temperature using a TA-XTplus Texture Analyser (Stable Micro System Ltd., Surrey, UK). Crosshead speed was 1 mm/s, and a 5 kg load cell was used. The load as breaking force (BF) and the depth of depression as breaking deformation (BD) when the sample lost its strength and ruptured were recorded. Measurements were carried out at least in sextuplicate at day 0 (fresh) and 1, 7 and 21 days of chilled storage following pasteurization.

2.3.8. Sensory evaluation

Three lots were tested for taste (TAA) and texture acceptability (TEA). These tests were performed according to standard UNE-ISO 6658 (ISO, 2008). Twenty-five semi-trained panellists from the staff of the Institute of Science, Technology and Nutrition (ICTAN) were asked to rate the three lots (C, O, and S) at 1, 7, and 21 days of chilled storage following pasteurization on a non-structured hedonic scale in which samples were given scores of 1 (for dislike extremely) to 9 (for like extremely). Panellists were asked to give reasons for their scores. The evaluation was performed in a standard panel room designed according to UNE-ISO 8589 (ISO, 2010) and was done between meals (after breakfast and before the midday meal). The panellists were given unsalted crackers and room-temperature water to rinse their mouths between samples. The samples were cut into pieces of approximately 20 g and grilled for 3 minutes with a small amount of sunflower oil. The results are the means of the scores from the twenty-five tasters.

2.3.9. Statistical analyses

Statistical analysis was carried out using Microsoft Excel software. Data are presented as mean values of at least five independent batches and were tested for each experiment with expanded uncertainty limit (EUL) data as the maximum and minimum deviation from the respective mean value. Trends were considered significant when means of compared sets differed at $p<0.05$ (Student's t-test).

Statistical correlations between parameters were determined by multiple regression with confidence intervals of 95% ($p<0.05$) using the SPSS Statistics 17.0 software.

3. RESULTS AND DISCUSSION

3.1. Proximate analysis, viscosity analysis and sample moisture.

The proximate analysis of the mince (sawdust) used in the preparation of gels was as follows: moisture content (79.00 ± 0.01)%, crude protein (18.41 ± 0.32)%, fat (1.50 ± 0.30)% and ashes (1.19 ± 0.03)%. The methodology reported by Borderias et al. (1985) was applied to check the protein functionality by measuring the apparent viscosity of a dispersion of muscle in NaCl. The apparent viscosity was 1.7 cP, which means that the protein functionality is very poor, probably due to the heat produced by sawing the original blocks of fish.

The water content of the three lots was high (data not shown), above 80%, and decreased slightly when they were pasteurized (~ 1%). The decrease was more pronounced in lot O (1.6%) but the level remained constant throughout storage. On the other hand, lots C and S lost 15% and 11.5 % of moisture respectively with respect to value at day 1 following pasteurization, and after 21 days of storage moisture values had reached 74% and 75% respectively.

3.2. Water binding (WBC) and oil binding capacity (OBC)

The effects of pasteurization and chilled storage on WBC data are shown in Table 2. In all lots there was a significant decrease in WBC ($p<0.05$) due to heating during pasteurization, with a greater decrease of WBC in lot C (11.3 %) than in lot O (5.3%) or lot S (5.1%). These differences could be related directly to differences in the structural organization of the three types of prototypes. In lot C the KGM and fish particles formed a coupled network (KGM-protein-water matrix). However, in lot O a "complex emulsion system" was formed as a result of oil addition. This emulsion could be a two-phase system acting a new phase filler in the aqueous glucomannan-protein matrix (Damodaran, 1997, chap.3), reducing the decrease of WBC, so that pasteurization improved water entrapment ability relative to lot C1. Pasteurization could therefore have induced some structural rearrangements in lot O, increasing the hydrophobic interactions in the network; this would

enhance the linking of filler in the biopolymer matrix, thus improving water holding ability (bonded water) (Kinsella, 1982) as compared to the control (lot C1). In the case of lot S, the larger number of Na^+ and Cl^- ions made for greater electrostatic charge density, which increased the number of ion-dipole interactions with the permanent dipoles of water, enhancing the water holding ability of the gel network (lot S1) more effectively than in the control (lot C1).

Table 2. Water binding capacity (WBC) (%), cooking loss (CL) (%) and lightness value (L*) of samples C, O and S after 0, 1, 7, 21 and 35 days of pasteurization and chilled storage.

		WBC (%)	CL (%)	L*
LOT C	C0	85.5 ±1.7cAB	9.0 ±1.3bA	67.94 ±0.38aA
	C1	75.83 ±0.88bA	5.12 ±0.16aA	71.24 ±0.34bA
	C7	77.4 ±1.6bAB	4.45 ±0.71aA	71.31 ±0.89bA
	C21	77.9 ±2.5bA	8.85 ±0.76bA	68.3 ±1.8aA
	C35	64.8 ±2.9aA	12.3 ±1.0cB	67.7 ±1.9aA
LOT O	O0	82.8 ±1.6cA	6.3 ±1.7bA	82.60 ±1.2bB
	O1	78.4 ±2.3bA	4.51 ±0.87aA	81.81 ±0.38bB
	O7	77.83 ±0.51bA	4.4 ±1.2aA	81.4 ±1.5 abB
	O21	76.8 ±1.7bBA	6.55 ±0.86bC	80.97 ±0.27 aB
	O35	73.0 ±1.1aB	9.17 ±0.84cA	81.84 ±0.51bB
LOT S	S0	87.49 ±0.48dB	7.7 ±1.4bA	71.01 ±0.63bC
	S1	83.0 ±2.2cB	5.46 ±0.70aA	71.1 ±1.3bA
	S7	76.0 ±1.3bB	4.80 ±0.36aA	70.40 ±0.98bA
	S21	75.49 ±0.29bA	11.49 ±0.53cB	69.93 ±0.51bA
	S35	63.0 ±2.6aA	12.1 ±2.0cAB	66.78 ±0.58aA

Values are given as mean ± expanded uncertainty limit (EUL).
a-d Different small letters in the same column indicate significant differences among storage period (days) at a fixed sample (C, O and S) ($p < 0.05$).
A-C Different capital letters in the same column indicate significant differences among samples C, O and S at the same storage period ($p < 0.05$).

During days 1-21 of chilled storage there were no significant differences ($p<0.05$) in WBC values in lots C and O with respect to the value at day 1 following pasteurization, but lot S registered a significant decrease at day 7 of chilled storage, slightly higher (~8%) than at day 1 after pasteurization (~5%). This difference in the decrease of WBC in S7 could be a consequence of the higher ionic strength of this lot S, reducing the number of water molecules surrounding the GM-protein matrix and thus causing partial dehydration. This electrostatic effect would be naturally reinforced at low temperature during chilled storage and could have caused some rigidity in S7 sample, as evidenced by its lower breaking deformation (BD), which is discussed in section 3.7 (puncture data). Nevertheless, even at day 21 the WBC of all lots was still above 75%, indicating high stability during chilled storage. By day 35, there was another significant decrease in WBC in all three lots, but particularly in lots C (C35) and S (S35) (~16%), as compared to C21 and S21 respectively, indicating irreversible structural damage in both gel networks. However, this decrease of WBC was considerably smaller (~5%) in sample O (O35) than in samples C35 and S35, showing the stabilizing role of oil in network.

With regard to oil binding, the emulsion system formed in lot O did not remain stable over the course of storage. Pasteurization induced a significant reduction in oil binding capacity (OBC) from 86.6±2.6 (initial value: sample O0) to 74.6±4.7 (sample O1), particularly during chilled storage. The OBC mechanism combines physical entrapment of oil droplets in capillaries of the gel network (Solo-de-Zaldívar, Herranz, et al., 2014; Solo-de-Zaldívar, Tovar, et al., 2014) with the structural ability of the gel network to link oil by attractive forces such as van der Waals and hydrophobic interactions among oil functional-groups (non polar) and non functional fish protein (He, Franco & Zhang, 2013). Because the sawdust protein is practically denatured, from the outset it has more hydrophobic groups which can be associated with oil droplets, thus preventing droplet aggregation and consequently stabilizing the emulsion (McClements, 2004). When the temperature is raised, the oil becomes more fluid (less viscous) and at the same time the interfacial tension is reduced (Sahin & Sumnu, 2006), causing increased oil diffusivity in the network as evidenced by the lower OBC after pasteurization.

During the first 7 days of chilled storage, OBC decreased by around 17% and remained stable from days 7 (61.1±3.5%) to 21 (59.0±1.9%) of storage. However, by day 35 of chilled storage it had dropped to as low as 38.5 ±3.2%. The cause of this loss of ability to trap oil

molecules in the network during storage could be that at low temperature emulsions become thermodynamically unstable and can partially break down over time; thus, cooling could reinforce repulsive forces like electrostatic and steric forces (in opposition to the attractive interactions) (McClements, 2004), consequently reducing emulsion stability.

3.3. Determination of cooking loss (CL)

The heat treatment (pasteurization) produced a significant decrease of cooking loss (*CL*) in all three lots, with a greater decrease in lot C (~43%) than in lots O and S (~28%) (Table 2). This is consistent with the decrease of WBC in relation to structural differences (section 3.2). In general, the amounts of cooking drip exudate were small. After 7 days of chilled storage, cooking loss values were approximately the same in all samples (C7, O7 and S7), with no significant differences among them.

These values had increased in all lots by days 21 and 35, although proportionally less in lot O (Table 2); this corroborates the earlier discussion regarding enhancement of water binding due to structural stabilization produced by the emulsion in the GM network.

3.4. Colour measurement

Lightness values (*L**) are also shown in Table 2. All three lots registered from 66.78±0.58 to 82.6±1.2, although *L** was significantly ($p < 0.05$) higher in lot O than in lots S and C throughout the experimental period due to the formation of a complex emulsion system in network. In the case of lot O, more radiant energy was reflected or transmitted than in lots C and S. This means that the emulsion (as a filler) produced a less regular and more heterogeneous network with larger particles than in lots C and S, enhancing the light scattering and making gels (lot O) appear whiter and more opaque (high *L**) (Sperling, 2001, chap. 3).

Pasteurization did not seem to have any great effect on the colour of any of the lots. *L** only increased significantly in lot C when it was pasteurized (C1 sample), which is consistent with thermo-induced reinforcement of the GM network at high temperature (Herranz, Borderias, Solas, et al., 2012). *L** remained stable up to day 7 of chilled storage; it increased significantly up to day 21, then remained stable up to day 35 (C35). Also, there was a

significant decrease of L^* in lot S at the end of storage (35 days), due to the structural damage in the network (S35) as noted earlier (low WBC in S35).

3.5. Microbiological analyses in the course of chilled storage

Table 3 shows the total viable bacterial count of the three lots (C, O and S) before (day 0) and after pasteurization and at 1, 7, 21 and 35 days of chilled storage. As expected, pasteurization produced a notable decrease of the microbial total count from 10^4 cfu/g to 10^1 cfu/g in all lots. These counts remained practically stable over 7 days of chilled storage in all three lots, but at day 21 lots C (C21) and O (O21) exhibited unexpected microbial growth (around 10^6 cfu/g respectively), unlike lot S (S21) where the count increased only slightly (almost 10^2 cfu/g). This reflects the limiting role of salt in microbial growth. At the end of chilled storage (35 days), microbial growth was even higher in all three lots — ~10^6 in lot S (S35) and ~10^7 in lots C (C35) and O (O35) — meaning that the total microbial count in the latter two samples (C35 and O35) slightly exceeded the limit for microbial acceptability, set by the Official Spanish Recommendations (R.D 135/2010 B.O.E. 25/02/2010) at 10^6 cfu/g.

In view of the results of all the analyses reported above, which indicated considerable spoilage in all samples after 35 days of chilled storage (C35, O35 and S35), and given that all were unacceptable because of a strong off-odour (data not shown), it was decided to do the viscoelastic, puncture test and sensory analyses in all three lots only up to day 21 of chilled storage.

Table 3. Total viable count (log CFU/g) of restructured fish prototypes after 0, 1, 7, 21 and 35 days of pasteurization and chilled storage.

	Total viable count (log CFU/g)		
Storage (days)	**C**	**O**	**S**
0	4.672	4.672	4.672
1	1.301	1.602	1.602
7	1.977	1.301	1.389
21	6.810	6.759	1.866
35	7.301	6.801	5.977

3.6. Viscoelastic properties in the linear viscoelastic (LVE) range

The stability of gels (viscoelastic materials) to pasteurization and further chilled storage can be determined by analysing the stress (σ) and strain (γ) dependence of storage (G') and viscous (G'') moduli in the linear viscoelastic (LVE) range. At low deformation, in the LVE range, both G' and G'' curves show constant plateau values. The term 'LVE range' is derived from the linear proportionality between σ and γ resulting in the complex modulus (G^*), which is constant within the LVE range (Mezger, 2006, chap. 8). The linear relationship was considered separately for each of the two components of G^*: G', which was depicted using the linear plot of elastic stress ($\sigma_{elastic}$), and G'', which was depicted by the viscous stress ($\sigma_{viscous}$). Both were plotted versus γ, where $\sigma_{elastic}$ and $\sigma_{viscous}$ are the products of $G' \cdot \gamma$ and $G'' \cdot \gamma$ respectively (Pai & Khan , 2002). The two plots (Fig. 1A, B and C) help to visualize the LVE range through the linear fit (equation (1) and equation (2) respectively).

$$\sigma_{viscous} = c \cdot \gamma + d \quad \textbf{(1)}$$

$$\sigma_{elastic} = a \cdot \gamma + b \quad \textbf{(2)}$$

These plots further make it possible to graphically compare the strain amplitude (γ_{max}) of the different gels, which may be taken as a measure of their structural stability in terms of conformational flexibility (Mezger, 2006, chap. 8). The slope of each line serves to quantify the overall values of the deformation energy stored — *elastic slope* (a) (eq. (1)) — and the deformation energy lost as heat — *viscous slope* (c) (eq. (2)) — within the LVE interval (Pai & Khan, 2002). The independent terms b and d of equations (1) and (2) respectively have no physical meaning. Thus, the stress sweeps contain abundant information about the viscoelastic components and consequently the structural stability of the three lots (Steffe, 1996, chap. 5). This is therefore a useful tool to discriminate between the effects of pasteurization and the effects of further chilled storage on viscoelastic properties.

Regarding the effect of pasteurization, Fig. 1A (0 day) and 1b (1 day) show that in general pasteurization reduced the γ_{max} in all three lots (C1, O1 and S1), and at the same time slope a increased considerably for lots C1 (104%) and S1 (77%) with respect to C0 and S0 respectively (Table 4). This result indicates that pasteurization greatly reinforced the biopolymer matrix, increasing the crosslink density in the *GM-protein-water* complex and

hence reducing the conformational flexibility (γ_{max}), especially in lots C (C1) and S (S1) (Fig. 1A and 1B). This result is consistent on the one hand with the significant increase of L* in C1 with respect to C0 as noted above, and on the other hand with the thermal strengthening undergone by the GM gels after heating (Herranz, Borderias, Solas, et al., 2012).

The benefit of salt addition for viscoelastic properties (lot S) was reflected in slope *c*; thus, while the viscous slope increased in C1 (150%) relative to C0 after pasteurization, in lot S1 the increment (S1-S0) was considerably smaller (72.7%) (Table 4). This smaller increase of the *c*-slope (dissipated energy) in S1 suggests that NaCl helps to keep the network more cohesive by producing a degree of viscoelastic stabilization in the GM-protein matrix after heating. This specific stabilization is reflected by the similarity of the increase of the *a* (elastic) and *c* (viscous) slopes (S1-S0). Thus, the ratio of energy dissipated to energy stored was the same in S1 as in S0. This means that the bond strength in the S1 network after pasteurization was similar to that in S0, suggesting some structural stabilization.

This role is also reflected in the fact that WBC was greater in S1 than in C1 after pasteurization (Table 2), as noted above.

However, the response to pasteurization was different in lot O. The elastic slope (*a*) increased slightly (13%), and unlike in samples C1 and S1, the viscous slope (*c*) decreased (31%) relative to O0, with sample O1 registering the lowest values of both parameters (*a* and *c*) (Table 4). This different trend in O1 suggests that heating could partially disorganize the filler in the network due to a decrease of OBC (14%) (Section 3.2.), as indicated by the fact that the viscous slope was greatly reduced but crosslink density remained similar (similar *a* slope) to O0 sample. In general, heating reduces the interfacial tension between water and oil (Sahin & Sumnu, 2006), so that the capillary pressure diminishes, releasing liquids (oil and water). The oil released could therefore have had a lubricant effect on the gel network, which could explain the shallower viscous slope in lot O1 (Table 4).

Table 4. Influence of pasteurization and chilled storage on the parameters of linear fit from equations 1 and 2 four lots C, O and S.

	a (kPa)	*b* (Pa)	r^2	*c* (kPa)	*d* (Pa)	r^2
C0	6.128 ± 0.03	10.3 ± 1.1	0.997	1.071± 0.03	8.25 ± 1.0	0.92
C1	12.513± 0.01	2.97 ± 0.16	0.999	2.681± 0.03	7.97 ± 0.56	0.972
C21	13.847 ± 0.02	3.51 ± 0.24	0.999	2.241± 0.01	1.29 ± 0.11	0.997
O0	9.033 ± 0.06	10.3 ± 1.2	0.996	2.117 ± 0.04	6.38 ± 0.75	0.972
O1	10.198 ± 0.01	0.80±0.096	0.999	1.453 ± 0.01	1.12 ± 0.13	0.991
O21	15.870 ± 0.08	16.4 ± 1.5	0.997	3.641 ± 0.04	7.07 ± 0.72	0.987
S0	8.450 ± 0.02	4.97± 0.68	0.999	1.479 ± 0.02	5.83 ± 0.69	0.973
S1	14.965 ± 0.004	0.27 ± 0.071	0.999	2.554 ± 0.01	2.34 ± 0.17	0.995
S21	20.268 ± 0.07	45.5 ± 3.0	0.998	5.735 ± 0.04	26.8 ± 1.8	0.992

Values are given as linear fit parameters ±standard deviation of fitted parameters.

Physical interactions (e.g. ion-dipole, dipole-dipole and van der Waals forces) produce ordered systems, increasing the rigidity of the matrix; this is because although weak in nature, when combined they produce increased intermolecular association (steeper a slope) and consequently greater rigidity of the gel network (Lefebvre & Doublier, 2005). In addition, there was also a sizeable increase in slope c (124%) in lot S21, considerably greater than in slope a (35%) (Table 4). This suggests that chilled storage after pasteurization produced re-aggregation which was less ordered than the initial aggregation (S1). The result was a stronger but less cohesive GM-protein matrix, as evidenced by the fact that the viscous component increased more than the elastic component. This rheological trend is consistent with the significant decrease in WBC in S21 relative to S1 noted earlier and could be due to the greater proportion of dangling chains in the chilled network (S21).

However, in lot C the ordering effect associated with cooling is also apparent in the time stability observed during chilled storage, as reflected in the small increase of slope *a* (11%) and the decrease of slope *c* (16%) in C21 with respect to C1 (Table 4), which could be the consequence of a more cohesive (less dissipative) GM-protein network. This result could explain the slight increase of WBC in C21 relative to C1 noted earlier; thus, if physical interactions are reinforced, it is possible to enhance the ability to physically entrap water as capillary water and hydrogen-bonded water at low temperature (Kinsella, 1982).

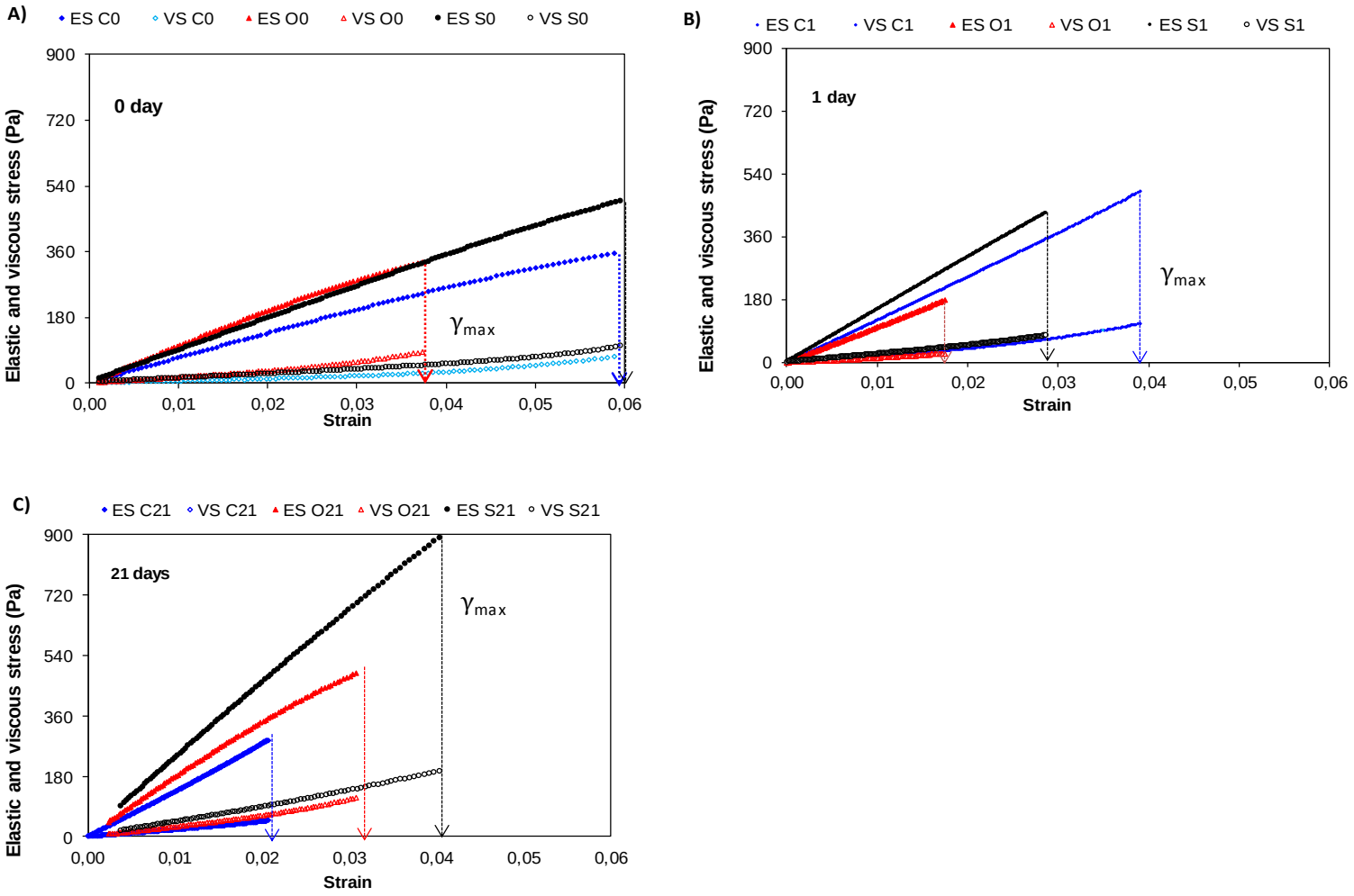

Figure 1. Linear Viscoelastic range in terms of the Elastic stress (ES) and viscous stress (VS) versus strain(γ) of three lots C, O, S at initial time (A), after pasteurization (B), and at 21 days (C) of chilled storage. Frequency=1Hz and T=25 °C.

The response of lot O was different, possibly because the decrease of OBC and subsequent reduction of volume fraction of oil in network in the course of chilled storage, producing some increase of crosslinking in the network as reflected in the pronounced increase of elastic slope (56%). This produced a less homogeneous structure, as evidenced by the considerable increase of the viscous slope (151%) in O21 relative to O1 (Table 4). Thus, low temperature (chilled storage) reduced the pore size and at the same time the interfacial tension (oil-water) augmented, raising the Laplace pressure (McClements, 2004) and thus helping to physically retain both fluids in the GM network. These changes may explain why the network in lot O retained an acceptable level of WBC, which was only slightly lower in O21 than in O1. This physicochemical state (O21) bears some physical resemblance to the state at zero time O0 (prior to pasteurization), which is in agreement with the CL data found in O21 and O0 as noted earlier (Table 2).

3.7. Puncture test

Fig. 2a and 2b show the influence of pasteurization and chilled storage on breaking force (BF) and breaking deformation (BD) respectively for the three lots. In terms of BF, lot C was practically unaffected by pasteurization; BF increased slightly then remained stable until the end of chilled storage (Fig. 2a), whereas there were significant changes ($p<0.05$) in lots O and S. In the case of lot O (sample O1), BF decreased significantly ($p<0.05$), which is consistent with the decrease of the viscous slope (stress sweeps) noted earlier. Considering that the fracture force involves the ability to transfer the breaking energy among polymer chains (Foegeding, González, Hamann & Case, 1994), if the viscous component is lower (lot O1), less energy will be dissipated as heat. This favours the interchange of breaking energy among filaments, and hence the gel network would break more readily, reducing BF (Fig. 2a). During chilled storage, BF remained practically stable, with no significant changes, over 21 days (Fig. 2a). In any case BF was lower in lot O than in lots C and S during pasteurization and chilled storage (Fig. 2a). On the other hand, a slight increase in BD (lot O1) (Fig. 2b) could be related to thermally-induced deformation from increased entropy caused by random chain motion (Foegeding et al., 1994).

In the case of lot S, BF increased sharply in S1 as a result of pasteurization (Fig. 2a) but remained stable throughout chilled storage. The considerable increase of BF in S1 relative to S0 indicates that a more compact network was formed after heating. This is consistent with the steeper elastic slope, which indicates a higher density of crosslinkings (higher connectivity) as discussed above in relation to stress sweeps (Fig. 1B). This suggests that NaCl could improve the functionality of the myofibrillar protein to some extent by producing a uniform colloidal dispersion that helps the proteins to develop a stable structure during thermal gelation. Thus the protein chains would bind more efficiently to the KGM network (KGM-protein-water system), producing a larger number of junction zones and consequently a stronger gel network. Such improved structural stability is also reflected in the small decrease of WBC and CL (Section 3.2). BF was greater than in lots C and O and was sustained throughout chilled storage. For its part, the considerable increase of BD in lot S1 (Fig. 2b) could be related to the fact that the viscous slope was steeper in S1 than in S0 (noted earlier in Fig. 1B), for if the network becomes more dissipative, it is more difficult for the breaking energy to be transferred mechanically among filaments (lot S1) (Herranz, Tovar, et al., 2012) More distance is therefore needed to reach the rupture point, hence the considerable increase of BD (Fig. 2b). These data indicate that lot S1 had a stronger and more deformable gel network than lots C1 and O1; however, BD decreased after 7 days of chilled storage (S7) to values similar to S0, increasing again (slightly) until the end of chilled storage (Fig. 2b). This indicates that salt produced some structural improvement in the "protein-GM-water" matrix, maintaining a similar overall degree of stiffness, i.e. a similar BF/BD ratio, during both pasteurization and chilled storage.

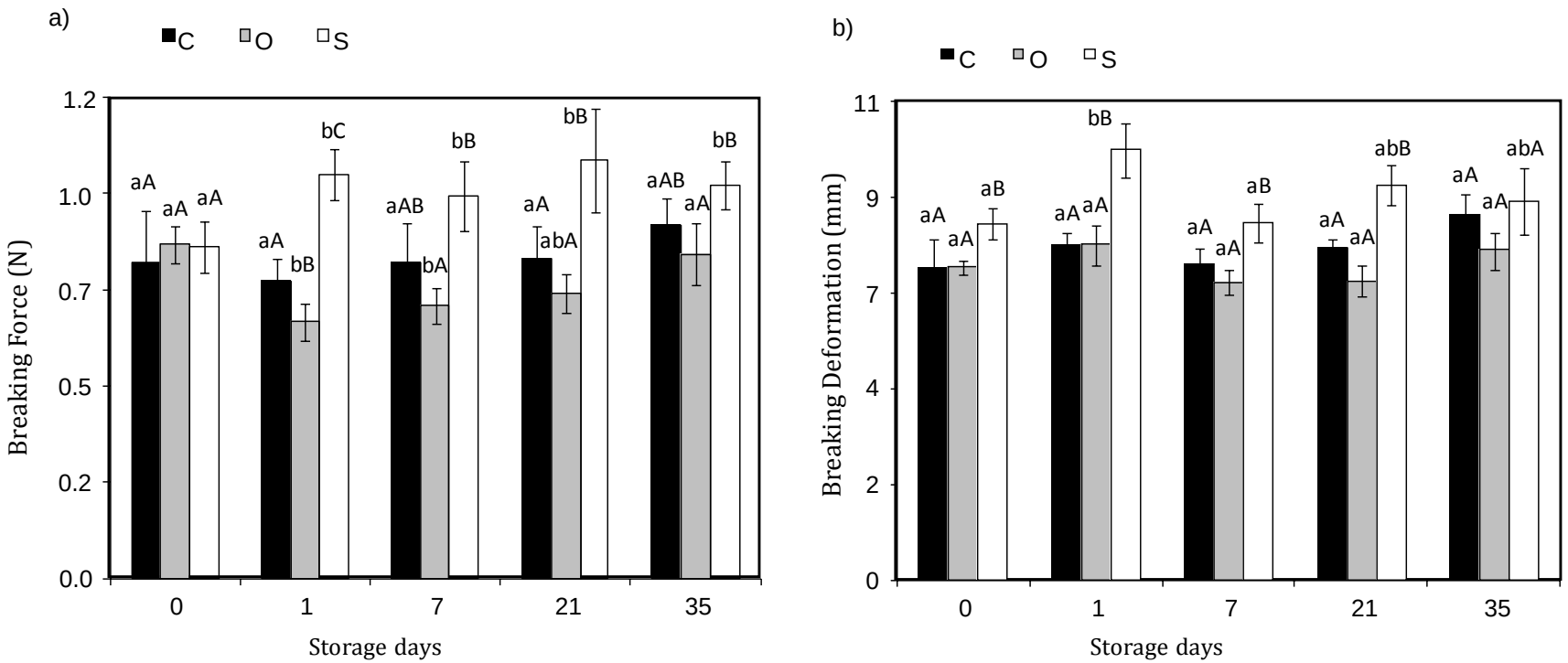

Figure 2. Influence of pasteurization and further chilled storage on breaking force (a) and breaking deformation (b) of three lots C, O, S of fish mince -glucomannan gels: control (C), 5% fish oil (O), 0.8% salt (S). T=25 °C. a-b Different small letters indicate significant differences among storage period (days) at a fixed sample (C, O and S) ($p < 0.05$). A-C Different capital letters indicate significant differences among samples C, O and S at the same storage period ($p < 0.05$).

3.8. Sensory evaluation

In the taste acceptance test (TAA) of recently pasteurized samples (C1, O1 and S1) lot C1 scored 5.5±0.8 and lot O1 4.2±0.8, while lot S scored significantly higher: 6.8±0.9. The reasons given by the panellists for these differences were that they found lot C1 drier than lots O and S, and conversely, they found lot O too soft and oily. On the other hand sample S was found tastier due to the presence of salt. These values remained roughly the same up to day 21 of chilled storage (data not shown). At day 35 the samples were not fit for oral testing because of unacceptable off-odours as noted earlier.

The scores for texture acceptability (TEA) were very similar to those for taste acceptability, with lot S1 scoring best for texture attributes and lot O1 scoring lowest (data not shown). Moreover, the panellists found no significant changes in the texture acceptability of any of the lots over 21 days of chilled storage with respect to the scores awarded at day 1 after pasteurization (data not shown).

These results indicate that the addition of oil was not well accepted, probably because the structural rearrangements occurring during pasteurization and chilled storage (reported above) produced a slippery sensation and a soft texture. As noted above, this was reflected in the fact that BF was lower in O1 than in any other lot irrespective of time. It was also reflected in the decrease of OBC, which can impart an unexpected oily feel to restructured fish products, negatively affecting their acceptability. On the contrary, lot S scored best for both attributes (taste and texture), which may have been due to the stabilizing role of the salt. This would produce a more cohesive and compact network, which is consistent with the fact that BF was higher in S1-S21 than in the other samples as explained above. Finally, the dryness associated with lot C, especially just after pasteurization (C1), could be related to the greater decrease of WBC and CL in that lot and is consistent with thermo-induced strengthening of the GM matrix (Herranz, Borderias, Solas et al., 2012).

4. CONCLUSIONS

Heating (pasteurization) produced various structural responses in all three lots (C, O and S) depending on their composition. In control sample (lot C), pasteurization produced more network damage, evidenced by a greater decrease of WBC and giving a drier sensation.

In lot O, the filler in network became partially disorganized, releasing oil and consequently producing a lubricant effect that was reflected in a softer texture. The addition of salt (lot S) helped to form a uniform colloidal dispersion with the myofibrillar protein. This improved the connectivity in the gel network during gelation, producing firmer and more cohesive and homogeneous networks which reflect the stabilizing role of salt.

During chilled storage, the strengthening of the KGM network continued in lot S due to reinforcement of the polar crosslinkings. This is because networks tend to become more rigid, if slightly less homogeneous, at low temperatures. Lot C maintained the degree of rigidity achieved after pasteurization. And lastly, structural rearrangements of the filler due to the low temperatures were observed in the GM-protein network (lot O), so that the resulting networks were less homogeneous but better able to retain oil and water.

All these structural changes were also reflected in the physicochemical parameters and sensory analyses. The best scores in the sensory analyses were awarded to lot S just after pasteurization (day 1) and after a further 21 days of chilled storage.

ACKNOWLEDGEMENTS

The authors would like to thank the Spanish Ministry of Economy and Competitiveness for its support under Project AGL2011-24693, and the Consejo Superior de Investigaciones Científicas for Predoctoral Scholarship BES-2009-018321. The authors also wish to thank the Xunta de Galicia for its financial support under the Consolidation and restructuring program of competitive research units: Strategic Research Partnerships (2009/060).

REFERENCES

AOAC. (2000). Official method of analysis (17th ed.). Maryland, USA: Association of Official Analytical Chemists.

B.O.E. 25/02/2010 Official State Bulletin R.D 135/2010 approving the microbiological standards, limits on heavy metals content and analytical methods for determination of heavy metals for fishery products and aquaculture.

Borderias, A. J., Jimenez-Colmenero, F. & Tejada, M. (1985). Viscosity and emulsifying ability of fish and chicken muscle protein. Journal of Food Technology, 20(1), 31–42.

Chua, M., Baldwin, T. C., Hocking, T. J. & Chan, K. (2010). Traditional uses and potential health benefits of Amorphophallus konjac K. Koch ex N.E.Br. Journal of Ethnopharmacology, 128(2), 268–278.

Damodaran, S. (1997). Protein-stabilized foams and emulsions. In: Food Proteins and their Applications. New York: Inc

Damodaran, S. & Paraf, A. (1997). Food Proteins and their Applications. New York: Inc.

Eide, O. L. A., Borresen, T. & Strom, T. (1982). Minced Fish Production From Capelin (Mallotus villosus). A New Method for Gutting, Skinning and Removal of Fat from Small Fatty Fish Species. Journal of Food Science, 47(2), 347–349.

Fernández-Segovia, I., Camacho, M. M., Martínez-Navarrete, N., Escriche, I. & Chiralt, A. (2003). Structure and color changes due to thermal treatments in desalted cod. Journal of Food Processing Preservation, 27(6), 465–474.

Foegeding, E. A., González, C., Hamann, D. D. & Case, S. (1994). Polyacrylamide gels as elastic models for food gels. Food Hydrocolloids, 8(2), 125–134.

Gómez-Guillén, M. C., Montero, P., Hurtado, O. & Borderías, A. J. (2000). Biological characteristics affect the quality of farmed atlantic salmon and smoked muscle. Journal of Food Science, 65(1), 53–60.

González Canga, A., Fernández Martínez, N., Sahagún, A. M., García Vieitez, J. J., Díez Liébana, M. J., Calle Pardo, Á. P., Castro Robles, L. J. & Sierra Vega, M. (2004). Glucomannan: Properties and therapeutic applications. Glucomanano: Propiedades y aplicaciones terapéuticas, 19(1), 45–50.

He, S., Franco, C. & Zhang, W. (2013). Functions, applications and production of protein hydrolysates from fish processing co-products (FPCP). Food Research International, 50(1), 289–297.

Herranz, B., Borderias, A. J., Solas, M. T. & Tovar, C. A. (2012). Influence of measurement temperature on the rheological and microstructural properties of glucomannan gels with different thermal histories. Food Research International, 48(2), 885–892.

Herranz, B., Borderias, A. J., Solo-de-Zaldívar, B., Solas, M. T. & Tovar, C. A. (2012). Thermostability analyses of glucomannan gels. Concentration influence. Food Hydrocolloids, 29(1), 85–92.

Herranz, B., Solo-de-Zaldívar, B. & Borderias, A. J. (2013). Obtaining a restructured seafood product from non-functional fish muscle by glucomannan addition: first steps. Journal of Aquatic Food Product Technology, 22, 201–208.

Herranz, B., Tovar, C. A., Solo-de-Zaldívar, B. & Borderias, A. J. (2012). Effect of alkalis on konjac glucomannan gels for use as potential gelling agents in restructured seafood products. Food Hydrocolloids 27(1), 145–153.

ISO. (2008). Sensory analysis–Methodology–General guidance (UNE-ISO 6658). Geneva, Switzerland: International Organization for Standardization.

ISO. (2010). Sensory analysis–General guidance for the design of test rooms (UNE-ISO 8589). Geneva, Switzerland:International Organization for Standardization.

Kennedy, J., Wall, P., Storrs, M., Devoluy, M. C. & Cruveiller, P. (2007). Food safety challenges. In Safety Handbook: Microbiological Challenges (pp. 8-19). France: Bio-Mèrieux Education.

Kinsella, 1982, chapter 3 Food Proteins. edited by Fox, P.F. and Condon, J.J. Applied Science Publishers, LTD. England.

Lefebvre, J. & Doublier, J. L. (2005). Rheological behavior of polysaccharides aqueous systems. In S. Dumitriu (Ed.), Polysaccharides: Structural Diversity and Functional Versatility. (pp. 357-394). New York: Marcel Dekker.

Mc Clements, D. J. (2004). Role of hydrocolloids as emulsifiers in Foods. In Gums and Stabilisers for the Food Industry 12 edited by Williams, P.A. and Phillips, G.O. RSC, UK.

Mezger, T. G. (2006). The Rheology Handbook. Hannover, Germany: Vincentz Network GmbH & Co. KG (Chapter 8).

Pai, V. B. & Khan, S. A. (2002). Gelation and rheology of xanthan/enzyme-modified guar blends. Carbohydrate Polymers, 49, 207–216.

Rosnes, J. T., Skara, T. & Skipnes, D. (2011). Recent advances in minimal heat processing of fish: Effects on microbiological activity and safety. Food Bioprocess Technology, 4 (6), 883–848.

Sahin, S. & Sumnu, S. G. (2006). Physical Properties of Foods. New York: Springer Science+Business Media, LLC (Chapter 6).

Solo-de-Zaldívar, B., Tovar, C. A., Borderías, A. J. & Herranz, B. (2014). Effect of deacetylation on the glucomannan gelation process for making restructured seafood products. Food Hydrocolloid, 35, 59–68.

Solo-de-Zaldívar, B., Herranz, B., Borderías, A. J. & Tovar, C. A., (2014). Effect of freezing and frozen storage on restructured fish prototypes made with glucomannan and fish mince. Food Hydrocolloids, 41, 233–240.

Sperling, L. H. (2001). Introduction to physical polymer science (3th ed.). New York: Wiley-Interscience. John Wiley and Sons, Inc. (Chapter 3).

Steffe, J. F (1996) Rheological methods in food process engineering (2nd ed.). East Lansing: Freeman Press. (Chapter 5).

Zhang, H., Yoshimura, M., Nishinari, K., Williams, M. A. K., Foster, T. J. & Norton, I. T. (2001). Gelation behaviour of konjac glucomannan with different molecular weights. Biopolymers, 59(1), 38–50.

CAPÍTULO 3

APLICACIONES TECNOLÓGICAS: OBTENCIÓN DE REESTRUCTURADOS DE PESCADO A PARTIR DE GLUCOMANANO

DESCRIPCIÓN DE LA PATENTE: ES 2 363 291 CSIC (2011)

PRODUCTO ALIMENTICIO A BASE DE PESCADO Y GLUCOMANANO, Y PROCEDIMIENTO DE OBTENCIÓN

Borderías, A. J., Solo de Zaldívar, B., y Herranz, B.

Instituto de Ciencia y Tecnología de los Alimentos y Nutrición (ICTAN) Consejo Superior de Investigaciones Científicas (CSIC)

DESCRIPCIÓN

Producto alimenticio a base de pescado y glucomanano, y procedimiento de obtención.

La presente invención se refiere a un producto alimenticio cuya composición base comprende músculo de al menos una especie acuática animal y glucomanano, y su procedimiento de obtención.

ESTADO DE LA TÉCNICA ANTERIOR

El sector de la alimentación es uno de los que arroja un mayor producto industrial, dicha industria alimentaria tiene una gran cantidad de empresas dedicadas a la tecnología de los alimentos, es decir, a dedicadas a diferentes estudios de la cadena y procesado de los alimentos.

El autor Kawano, en la patente US6146684 describe la elaboración de productos a partir de músculo de pescado y glucomanano y, toma como premisa el hecho de que el producto no tenga sal añadida. Esta patente, aunque no lo específica en las reivindicaciones, forma el gel con un denominado álcali que es el hidróxido cálcico. La finalidad es, preferentemente, la elaboración de productos típicos de Japón como el "chikuwa" o tipos de geles de pescado similares al "kamaboko", en variantes de más o menos elasticidad y dureza.

Por otra parte, Musson y Prest en la patente US4894250 describe un método para elaborar un gel termoirreversible a partir de una combinación de goma xantana y glucomanano a pH preferentemente ente 6,5 y 8. La finalidad, siguiendo los ejemplos, sería la elaboración de productos cárnicos o puré de manzana.

En una tercera patente US5028445, cuyos autores, Wu y Suzuki, describen un método de elaborar carne de crustáceos a partir de surimi, gelificándolo, y añadiendo harina de konjac ("konnyaku") como un aditivo más para reforzar el gel formado por el surimi.

Por último, en la patente US4427704, Cheney et al. describen un método para la elaboración de geles y, basado en ellos, productos cárnicos análogos, entre otros productos proteicos de origen animal y/o vegetal, mezclando glucomanano, de diversas procedencias,

con carragenatos de forma que exista gelificación a pH inferior a 9.

DESCRIPCIÓN DE LA INVENCIÓN

La presente invención proporciona un producto alimenticio cuya composición base comprende músculo de al menos una especie acuática animal y glucomanano, a su vez, la presente invención también proporciona un procedimiento de obtención de dicho producto alimenticio.

Un primer aspecto de la presente invención se refiere a un producto alimenticio que comprende:

a.- Una fase acuosa de glucomanano, y
b.- músculo de al menos una especie acuática animal.

En una realización preferida el músculo de especie acuática animal procede de la industria pesquera y/o acuícola.

La relación entre la fase acuosa y el músculo de especie acuática animal está preferiblemente entre 10:90 y 90:10, y esta relación va a depender de la riqueza del músculo.

El glucomanano se obtiene preferiblemente del konjac (Amorphophallus Konjac), que es una planta cuya raíz contiene una fibra denominada glucomanano, la cual es usada en dietas para la pérdida de peso por su capacidad saciante del apetito.

En el caso de emplearse harina de konjac esta se encuentra preferiblemente con una riqueza de glucomanano del 90%.

Este glucomanano se encuentra en una realización preferida en un porcentaje en la disolución acuosa del 0,5 al 7% en peso.

La fase acuosa de glucomanano (a) además en otra realización preferida incluye hasta un 10% en peso de al menos un aditivo alimentario. Dicho aditivo alimentario es en una realización más preferida seleccionado de la familia de los emulgentes, estabilizantes, espesantes, gelificantes y cualquiera de sus combinaciones. En una realización más

preferida se selecciona dicho aditivo alimentario de la lista que comprende: almidón, goma xantana, kappa- carragenato, iota-carragenato y cualquiera de sus combinaciones. En el caso de adicionar goma xantana en una realización aún más preferida está en un porcentaje entre el 0,3 y el 3% en peso. Y si se emplea el carragenato como aditivo en una realización aún más preferida está en un porcentaje entre el 0,5 y el 2% en peso.

El músculo de la especie acuática animal preferiblemente procede de un subproducto de pescado, ya que el fin de la presente invención es el aprovechamiento de músculo de pescado, de cualquier especie pesquera, independientemente de la composición de dicho músculo.

Dicho músculo de especie acuática animal preferiblemente ha sido sometido a tratamientos que se selecciona de entre un tratamiento térmico, de ahumado, salado o marinado.

Además, este músculo preferiblemente comprende además sal (NaCl), una proteína funcional, aditivos alimentarios o cualquiera de sus combinaciones. Siendo el contenido en sal más preferiblemente menor al 15% en peso. Los aditivos alimentarios más preferiblemente se seleccionan de entre almidón, proteínas no musculares, sustancias aromatizantes, colorantes o cualquiera de sus combinaciones. Encontrándose dichos aditivos alimentarios aún más preferiblemente en un porcentaje menor al 10% en peso. Estos aditivos alimentarios en el caso de ser proteínas no musculares son aún más preferiblemente seleccionados de entre soja, clara de huevo, proteínas lácteas o cualquiera de sus combinaciones.

Un segundo aspecto de la presente invención se refiere a un procedimiento de obtención del producto alimenticio según se ha descrito anteriormente que comprende las etapas:

a.- homogenización del glucomanano en agua,
b.- adición a la mezcla obtenida en (a) del músculo de al menos una especie acuática animal (b),
c.- adición a la mezcla obtenida en la etapa (b) de una solución alcalina, hasta pH entre 9 y 12,

d.- conformado del producto alimenticio obtenido en (c),

e.- gelificación del producto obtenido en (d),

f.- neutralización del producto obtenido en (e) hasta pH entre 6,5 y 7,

g.- lavado del producto obtenido en (f) con agua.

En una realización preferida además se somete al producto obtenido en la etapa (g) a un tratamiento que se selecciona de entre refrigeración, congelación, marinado, ahumado, rebozado, empanado o cualquiera de sus combinaciones. Para de esta forma constituir productos en sí mismos o también puede ser incluidos en platos preparados precocinados.

La homogenización de la etapa (a) se realiza en agua preferiblemente en un intervalo de temperaturas entre 5 y 80 °C, y durante tiempos preferiblemente entre 30 min y 2 horas, dicha homogenización en una realización preferida se lleva a cabo en una homogenizadora o batidora.

Una de las ventajas de la presente invención consiste en poder aprovechar cualquier tipo de músculo sobrante en un proceso, con proteína sin ningún tipo de funcionalidad y, poder elaborar estructuras que traten de imitar o no, basándose en la capacidad de gelificar de forma termoestable del glucomanano e, incluir en la red formada por el gel, las partículas de pescado convenientemente particulado.

Preferiblemente el músculo de la especie acuática animal se encuentra picado. En el caso de hacerse la disolución en caliente, ésta se hará más rápidamente, aunque será necesario su posterior enfriamiento, especialmente si se quiere, que en el producto resultante, el pescado deba tener aroma y color de pescado crudo. Con la disolución de glucomanano o la harina de konjac, se deberá obtener un producto translúcido y continuo, libre de grumos o porciones de color 50 lechoso.

La solución alcalina que se puede emplear en la etapa (c) puede ser cualquier sustancia alcalina de uso alimentario que sea capaz de subir el pH a valores entre 9 y 12. La solución alcalina empleada en la etapa (c) es preferiblemente NaOH o KOH. Y más preferiblemente la solución alcalina está en una concentración total en peso de entre el 5 y el 20%. En esta etapa se pueden adicionar los aditivos alimentarios según se han descrito anteriormente, en las proporciones indicadas por los fabricantes.

En una realización preferida el conformado de la etapa (d) se realiza por extrusión o moldeado, para formar fibras o bien porciones de anchura menor a 3 cm.

Una vez estructurada la masa de producto alimenticio con la forma definitiva que se le quiere otorgar, en otra realización preferida se procede a la gelificación de la etapa (e) se realiza a un intervalo de temperaturas entre 5 y 80 °C, para que gelifique se deja a esta temperatura durante tiempos entre 1 a 6 horas. Se gelifica calentando cuando no importe que el producto final tenga color y aroma a pescado cocido, mientras que no se superen los 30 °C se quiere conseguir un producto con apariencia y aroma a pescado crudo.

Una vez gelificado el producto con la textura deseada, según el producto que se quiera elaborar se procede a la neutralización del mismo, para ello se emplea preferiblemente una solución tampón, en dicha solución tampón se introduce por inclusión el producto alimenticio, siendo más preferiblemente la solución tampón de fosfato. Y más preferiblemente el tiempo de neutralización es entre 2 y 24 horas. La proporción de producto-tampón empleado es de aproximadamente 1:10 y la temperatura a la que se lleva a cabo es preferiblemente entre 2 y 20 °C.

Dada la elevada capacidad de ligar agua por parte del glucomanano, se procesan productos de hasta un 90% de los mismos, con lo que podrían considerarse productos dietéticos.

Una vez lavado el producto se escurre y se envasa convenientemente por técnicas conocidas por cualquier experto en la materia. El producto elaborado puede ser sometido a refrigeración, congelación o a cualquier otro tratamiento tecnológico como el marinado y el ahumado. En cualquier caso, los sabores correspondientes a estos tratamientos tecnológicos pueden ser introducidos también en la etapa (c), incorporando los aromas correspondientes, como se describió anteriormente.

A lo largo de la descripción y las reivindicaciones la palabra “comprende” y sus variantes no pretenden excluir otras características técnicas, aditivos, componentes o pasos. Para los expertos en la materia, otros objetos, ventajas y características de la invención se desprenderán en parte de la descripción y en parte de la práctica de la

invención. Los siguientes ejemplos se proporcionan a modo de ilustración, y no se pretende que sean limitativos de la presente invención.

EJEMPLOS

A continuación, se ilustrará la invención mediante unos ensayos realizados por los inventores, que pone de manifiesto la especificidad y efectividad de un procedimiento de obtención de un producto alimenticio en base de músculo 25 de especie acuática animal y glucomanano.

Ejemplo 1: *Elaboración de un filete de pescado a partir de "serrín de pescado"*

Se toma harina de konjac y se disuelve en agua, en una proporción del 3%, añadiéndose 3% de almidón de patata y, se introduce en una homogeneizadora tipo "cutter" a vacío que tenga posibilidad de calentamiento. La homogeneización se hace a 80 °C durante 30 min, a velocidad media, en que la dispersión de glucomanano estará translúcida y sin grumos. Posteriormente, esta masa se enfría a 5 °C y, se mezcla con músculo de pescado procedente del aserrado de bloques congelados (serrín de pescado), cuya proteína está totalmente agregada y, por tanto, su capacidad de gelificación es mínima. Las dos masas, de glucomanano y almidón en agua, y de pescado, se homogenizan durante 1 minuto con una solución de NaOH 0,8 N, en una proporción del 10% respecto al total de la masa. Inmediatamente, la masa se distribuye en moldes paralepipédicos de 0,6 cm de espesor. Dichos moldes completamente cubiertos, para evitar desecación superficial, se ponen 1 hora a 30 °C y justo a continuación 4 horas a 5-10 °C. Una vez que el producto ha gelificado, se sumerge en tampón fosfato pH 5, 10 veces su volumen, y se tiene 12 horas a 5 °C. Transcurrido este tiempo el producto alcanzará un pH alrededor de 6,8. Las láminas se desmoldan y se unen paralelamente, vertiendo entre ellas una suspensión acuosa que contenga un 0,5% de carbonato cálcico y 1 % de transglutaminasa microbiana.

Finalmente, este "sándwich" se corta en láminas transversalmente, que se asemejan a filetes de pescado con miotomos y mioseptos.

Ejemplo 2: *Sucedáneo de rodajas de tentáculo de pulpo a partir de "cabeza" o de otros subproductos procedentes de pulpo cocido*

Se toma glucomanano procedente de konjac, en un porcentaje del 3% en solución acuosa, a la que se añade un 2% de almidón de patata y, se homogeniza durante 10 min a 80 °C en una homogeneizadora tipo "cutter" a vacío. Por otra parte, se toman cabezas de pulpo cocidas, separadas de los tentáculos y, que constituyen un subproducto en este tipo de procesado, se pican en picadora de carne con tamaño de orificio de alrededor de 0,5 cm. Este músculo picado se lava con agua y se escurre en prensa de tornillo. Posteriormente, se le añade un aroma a pulpo y óxido de titanio al 1% para blanquearlo. Una parte de este músculo, bien picado en la homogeneizadora "cutter", se mezcla con otra parte de la dispersión acuosa del glucomanano y, se homogenizan las dos masas juntas a vacío durante 3 min. A continuación, se adiciona, aproximadamente, un 10% de solución acuosa 1N de KOH, controlando que el pH llegue a un valor alrededor de 10 y, se homogeniza a vacío durante 1 min. El producto se incluye en moldes cilíndricos de alrededor de 3 cm de diámetro y se deja asentar durante 3 horas a 30 °C y, posteriormente, 2 horas a 5 °C. Al cabo de este tiempo, la masa gelificada se extrae del cilindro y se corta en finas rodajas de alrededor de 3 mm. Dichas rodajas, se sumergen en tampón fosfato pH 5, 10 veces su volumen, durante 12 horas hasta que el producto alcance un pH alrededor de 6,5.

Ejemplo 3: *Carne de cangrejo a partir de pescado picado, procedente de recortes del procesado de filetes de merluza, y dispersión de glucomanano*

Se toma glucomanano de konjac, en un porcentaje del 3% en solución acuosa, a la que se añade un 1% de almidón de patata y, se homogeniza durante 20 min a 80 °C en una homogeneizadora "cutter" a vacío. Por otro lado, se toma pescado picado congelado, de baja capacidad funcional, y se lava en 4 veces su volumen de agua a 3 °C agitando durante 15 min. El pescado se pasa a través de una prensa de tornillo, para quitar el agua sobrante y dejarlo con una humedad inferior al 80%. El pescado picado, lavado y escurrido, con un 2% de almidón añadido y suficiente aroma a cangrejo, se mezcla en la cutter a vacío con la solución de glucomanano en una relación de 5:1 durante 1 minuto. Se añade la cantidad de NaOH 1N suficiente para llevar la masa a pH 10,5. La masa se introduce en una máquina formadora- extrusionadora, con una boquilla de múltiples taladros, para confeccionar fibras, y con una envoltura que las recubre. El producto resultante es un cilindro de 1 cm de diámetro aproximado, relleno de fibras paralelas. Pequeños fragmentos de 1,5 cm de largo se introducen en una solución de tampón fosfato pH 6 y, se mantienen dentro de dicha

solución durante 5 horas. El producto se lava con agua fría, se escurre y se envasa para ser posteriormente pasteurizado.

Ejemplo 4: *Anilla de calamar a partir de surimi de calamar de baja calidad funcional y de dispersión de harina de konjac*

Se parte de una solución de harina de konjac en agua, en una proporción de 4% a la que se añade un 1% de almidón de patata y posteriormente, surimi de calamar gigante (Dosidicus gigas) en una proporción de dos partes de solución de harina y almidón por una de surimi. Se mezclan en una homogeneizadora "cutter" a vacío durante 3 min. Después, se adiciona suficiente solución de KOH 0,8 N, para elevar el pH de la masa a 10,8, agitándose durante 1 minuto. A continuación, se obtienen anillas mediante una formadora de anillas de calamar, las cuales se mantendrán a 30 °C durante 2 horas seguidas de 3 horas a 5 °C para su completa gelificación. Una vez gelificadas, dichas anillas se introducen en un baño con tampón fosfato de pH 6 durante 12 h. Finalmente, las anillas se rebozan, pudiendo ser 30 congeladas o no, y se envasan.

Ejemplo 5: *Análogo a filetes de salmón ahumado a partir de subproductos musculares procedentes de raspas o recortes de filetes de salmón y/o trucha*

Se toma glucomanano procedente de harina de konjac y, se disuelve en agua en una proporción del 2 %, añadiéndose 2 % de almidón de patata y, se introduce en una homogeneizadora tipo cutter a vacío que tenga posibilidad de calentamiento. La homogeneización se hace a 80 °C durante 20 min, a velocidad media, obteniéndose una dispersión de glucomanano translúcida y sin grumos. Posteriormente, esta masa se enfría a 5-10 °C y, se mezcla en una proporción 1:1 con músculo de pescado picado, extraído de raspas o de recortes de filetes de salmones y/o truchas cuyo contenido de proteínas miofibrilares es insuficiente para que se produzca la gelificación del producto por sí sola. Este pescado picado se homogeniza con sal (2-6%) y aroma a humo, o a salmón ahumado, en la proporción recomendada por el fabricante. Las dos masas, de glucomanano y almidón en agua, y de pescado con sal y aroma, se homogenizan durante 1 minuto con una solución de NaOH 1,0 N, en una proporción del 10% respecto al total de la masa, obteniendo un pH entre 12 y 13. Inmediatamente después, la masa se distribuye en moldes paralepipédicos de 0,6 cm de espesor. Dichos moldes completamente cubiertos, para evitar desecación

superficial, se ponen durante 1 hora a 30-40 °C y seguidamente 4 horas a 3-10 °C. Una vez gelificado el producto se sumerge en tampón fosfato pH 5-6 (10 veces su volumen) durante 12 horas a 5 °C. Transcurrido dicho tiempo, el producto alcanzará un pH aproximado de 6,8. A continuación, las láminas se desmoldan y se unen paralelamente vertiendo entre ellas, una suspensión acuosa que contenga un 0,5% de carbonato cálcico y un 1% de transglutaminasa microbiana. Después, este "sándwich" se corta transversalmente en láminas que se asemejarán a filetes de pescado con sus miotomos y mioseptos. Este producto tendrá la ventaja para determinadas poblaciones, respecto del salmón ahumado real, de poder contener menores proporciones de sal y una menor cantidad de grasa, lo que además producirá menor fatiga sensorial. Por otra parte, el producto podrá tener la forma deseada en función del molde utilizado en la gelificación, lo cual puede ser de utilidad, por ejemplo, en el caso de que la elaboración sea para hacer sándwiches con tamaños determinados de pan.

REIVINDICACIONES

1. Producto alimenticio que comprende:
   a. Una fase acuosa de glucomanano, y
   b. músculo de al menos una especie acuática animal.
2. Producto alimenticio según la reivindicación 1, donde el músculo de especie acuática animal procede de la industria pesquera y/o acuícola.
3. Producto alimenticio según cualquiera de las reivindicaciones 1 ó 2, donde la relación entre la fase acuosa y el músculo de especie acuática animal está entre 10:90 y 90:10.
4. Producto alimenticio según cualquiera de las reivindicaciones 1 a 3, donde el glucomanano es procedente de la harina de konjac.
5. Producto alimenticio según la reivindicación 4, donde el glucomanano se encuentra como harina de konjac en una riqueza superior al 90%.
6. Producto alimenticio según cualquiera de las reivindicaciones 1 a 5, donde el glucomanano se encuentra en un porcentaje en la disolución acuosa del 0,5 al 7% en peso.
7. Producto alimenticio según cualquiera de las reivindicaciones 1 a 6, donde la fase acuosa de glucomanano (a) además incluye hasta un 10% en peso de al menos un aditivo alimentario.

8. Producto alimenticio según la reivindicación 7, donde el aditivo alimentario es de la familia de emulgentes, estabilizantes, espesantes, gelificantes y cualquiera de sus combinaciones.
9. Producto alimenticio según la reivindicación 8, donde el aditivo alimentario se selecciona de la lista que comprende: almidón, goma xantana, kappa-carragenato, iota-carragenato y cualquiera de sus combinaciones.
10. Producto alimenticio según la reivindicación 9, donde la goma xantana está en un porcentaje entre el 0,3 y el 3 % en peso.
11. Producto alimenticio según la reivindicación 9, donde el carragenato está en un porcentaje entre el 0,5 y el 2 % en peso.
12. Producto alimenticio según cualquiera de las reivindicaciones 1 a 11, donde el músculo (b) procede de un subproducto de pescado.
13. Producto alimenticio según cualquiera de las reivindicaciones 1 a 12, donde el músculo (b) ha sido sometido a tratamientos que se seleccionan de entre un tratamiento térmico, de ahumado, salado o marinado.
14. Producto alimenticio según cualquiera de las reivindicaciones 1 a 13, donde además el músculo (b) comprende sal (NaCl), una proteína funcional, aditivos alimentarios o cualquiera de sus combinaciones.
15. Producto alimenticio según la reivindicación 14, donde el contenido en sal es menor al 15% en peso.
16. Producto alimenticio según la reivindicación 15, donde los aditivos alimentarios se seleccionan de entre almidón, proteínas no musculares, sustancias aromatizantes, colorantes o cualquiera de sus combinaciones.
17. Producto alimenticio según la reivindicación 16, donde los aditivos alimentarios están en un porcentaje menor al 10% en peso.
18. Producto alimenticio según cualquiera de las reivindicaciones 16 ó 17, donde las proteínas no musculares se seleccionan entre soja, clara de huevo, suero lácteo o cualquiera de sus combinaciones.
19. Procedimiento de obtención del producto alimenticio según cualquiera de las reivindicaciones 1 a 18, que comprende las etapas:

    a.- homogenización del glucomanano en agua,

b.- adición a la mezcla obtenida en (a) del músculo de al menos una especie acuática animal (b),

c.- adición a la mezcla obtenida en la etapa (b) de una solución alcalina, hasta pH entre 9 y 12,

d.- conformado del producto alimenticio obtenido en (c),

e.- gelificación del producto obtenido en (d) a temperaturas entre 5 y 80 °C,

f.- neutralización del producto obtenido en (e) hasta pH entre 6,5 y 7,

g.- lavado del producto obtenido en (f) con agua.

20. Procedimiento según la reivindicación 19, donde además se somete al producto obtenido en la etapa (g) a un tratamiento que se selecciona de entre refrigeración, congelación, marinado, ahumado, rebozado o cualquiera de sus combinaciones.
21. Procedimiento según cualquiera de las reivindicaciones 19 ó 20, donde el músculo (b) se encuentra triturado.
22. Procedimiento según cualquiera de las reivindicaciones 20 a 21, donde la solución alcalina de la etapa (c) es NaOH o KOH.
23. Procedimiento según la reivindicación 22, donde la solución alcalina está en una concentración total en peso20 de entre el 5 y el 20%.
24. Procedimiento según cualquiera de las reivindicaciones 19 a 23, donde el conformado de la etapa (d) se realiza por extrusión o moldeado.
25. Procedimiento según cualquiera de las reivindicaciones 19 a 24, donde la gelificación de la etapa (e) se realiza a un intervalo de temperaturas de entre 5 y 80 °C.
26. Procedimiento según cualquiera de las reivindicaciones 19 a 25, donde la neutralización de la etapa (f) se realiza por inclusión en una solución tampón.
27. Procedimiento según la reivindicación 26, donde la solución tampón es de fosfato.

28. Procedimiento según la reivindicación 26 ó 27, donde el tiempo de neutralización es de entre 2 y 24 horas.

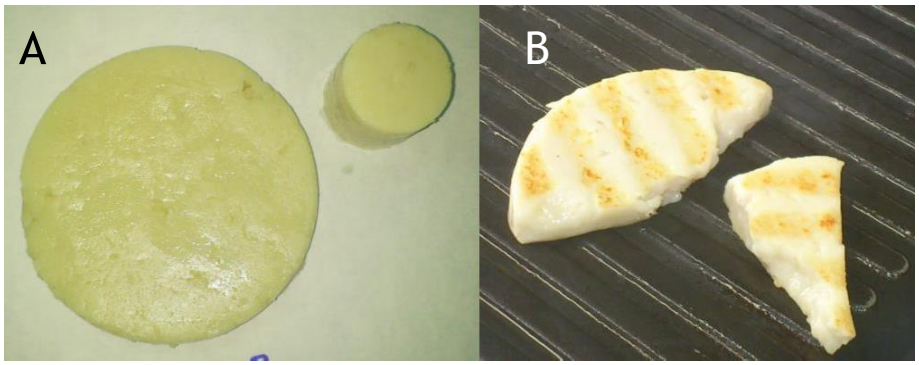

Figura 1. Producto pesquero reestructurado a partir de glucomanano (GM) elaborado según la patente descrita (Ejemplo 1). (A) Crudo (B) A la plancha.

NUEVOS PRODUCTOS REESTRUCTURADOS A PARTIR DE MÚSCULO DE PESCADO PICADO Y GLUCOMANANO

TRABAJO Nº1.- Elaboración de análogos de pescado- glucomanano tipo "fibras".

TRABAJO Nº2.- Elaboración de análogos de pescado con fibras de pescado- glucomanano incluidas en una matriz de Kamaboko.

TRABAJO Nº3.- Elaboración de análogos de pescado con miotomos y mioseptos.

TRABAJO Nº4.- Elaboración de análogos de pescado tipo" rabas".

Como ya se explicó en la introducción, la gelificación termoestable del GM permite reutilizar y reestructurar diversos subproductos de la industria pesquera como el "serrín" generado en los procesos de corte de grandes bloques de pescado congelado, o restos de atún cocido en la industria del enlatado. En general, se podría aprovechar cualquier tipo de músculo sobrante en un proceso, que ha podido dejar su proteína sin ningún tipo de funcionalidad y, poder elaborar estructuras que traten de imitar productos frescos o cocinados, basándose en la capacidad de gelificar de forma termoestable del GM, con lo que las partículas de pescado convenientemente particulado se podrían incluir en la red formada por dicho gel. Esta tecnología podría ponerse a punto también para pescados grasos para los que la reestructuración con alginato o transglutaminasa es limitada. Una ventaja de estos productos reestructurados adicionados de GM es que al incluir más agua que el músculo normal, puede actuar como elemento saciante bajo en calorías. Por otro lado, estos reestructurados ofrecen la posibilidad de incluir ingredientes funcionales, como el aceite de pescado, rico en ω- 3 y/o aceites vegetales ricos en ácido linoleico conjugado (CLA).

De cara a la puesta en el mercado de los reestructurados es necesario establecer las condiciones de procesado idóneas. Los reestructurados de pescado con GM pueden ofrecerse al consumidor en forma de producto fresco, pasteurizado o congelado, que permitan mantener la calidad nutritiva y sensorial lo más parecida a los alimentos tradicionales.

A continuación, se presentan a modo de ejemplo, algunos trabajos tecnológicos llevados a cabo para el desarrollo de prototipos de productos pesqueros reestructurados, que pueden resultar análogos a los productos tradicionales. Algunos de ellos se llevaron a cabo siguiendo los ejemplos propuestos en la Patente ES 2 363 291 CSIC, anteriormente descrita.

La Figura 2 representa esquemáticamente el proceso de obtención de diversos prototipos de pescado-GM

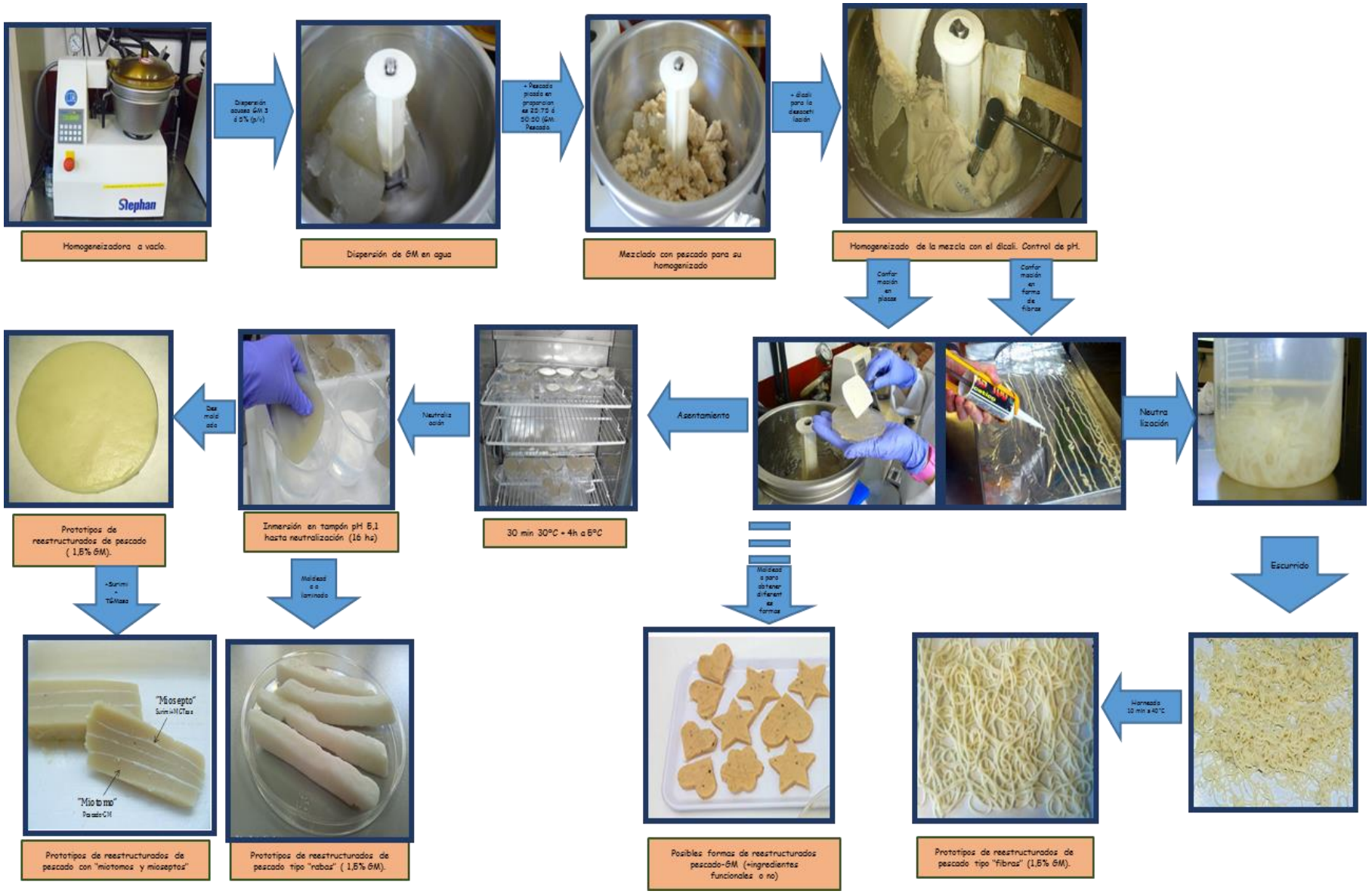

Figura 2.- Esquema de la elaboración de diversos tipos de reestructurados de pescado

TRABAJO Nº1.-

ELABORACIÓN DE ANÁLOGOS DE PESCADO-GM TIPO "FIBRAS"

A partir del músculo de pescado procedente del serrado de bloques congelados y GM se elaboran reestructurados de pescado tipo "fibras" mediante la gelificación del GM inducida por álcali.

Se procedió como se describe a continuación:

1. Se prepararon dispersiones acuosas de glucomanano (AGD) a una concentración total del 3% (p/v) utilizando agua destilada a 80 °C. Se homogeniza en una máquina de homogenización a vacío "Stephan" (Stephan Machinery GmbH & Co., Hameln, Alemania) durante 30 min y se dejó enfriar a temperatura ambiente.

2. A continuación, se mezclaron en la homogenizadora a vacío (Stephan) la proporciones de AGD al 3% y pescado picado ("serrín") (66:33) (p/p) para obtener una concentración final de GM de 1,2%. La homogenización se realizó a menos de 30 °C durante 5 min.

3. Seguidamente, se llevó la mezcla a diferentes pH para inducir la gelificación del GM. Se probaron las siguientes opciones:

Opción 1: Gelificación por inmersión en baño de KOH 1N. Se introduce la masa en una pistola de silicona y se deja caer la fibra sobre un vaso de precipitado con base y agitación continua, de este modo se pretende gelificar por difusión de la base en AGD- pescado.

Opción 2: Gelificación por adición de KOH en Stephan hasta alcanzar un pH 10,8. Se adicionan pequeñas cantidades KOH 0,6N hasta alcanzar pH 10,6- 10,8. Se introduce la masa en la pistola de silicona y se elaboran las fibras sobre una superficie no adherente. Se facilita la difusión de la base para la desacetilación y gelificación del GM introduciendo las fibras en horno "Rational" (Rational Combi-Master CM6, Alemania) durante 10 min a 40 °C, utilizándose con calor seco.

Opción 3. Gelificación por adición de KOH en Stephan hasta alcanzar un pH 11,6. Se adicionan pequeñas cantidades KOH 0,6N hasta alcanzar pH 11,6-11,8. Se introduce la masa en la pistola de silicona y se elaboran las fibras sobre una superficie no adherente. Se facilita difusión de la base para la desacetilación y gelificación del GM introduciendo las fibras en horno Rational (10 min a 40 °C) con calor seco.

Opción 4. Gelificación por adición de KOH en Stephan hasta alcanzar pH 12,3. Se adicionan pequeñas cantidades KOH 0,6N hasta alcanzar pH 12,0- 12,3. Se introduce la masa en la pistola de silicona y se elaboran las fibras sobre una superficie no adherente. En este caso no es necesaria la aplicación de calor.

Figura 3.-Reestructurados de pescado -GM tipo fibras (pH 11,6- Opción 3)

La opción 1 fue descartada, ya que los reestructurados que se obtuvieron no tenían la firmeza adecuada, posiblemente por la baja difusión del álcali en la masa pescado-GM, que no facilitó la desacetilación de éste y con ello la formación del gel.

La opción que mejores características de textura tuvo fue la obtenida a pH 11,6-11,8 (Opción 3) (Figura 3). A este pH se obtiene una desacetilación de 95- 100% (Solo-de-Zaldivar, Herranz, y Borderias, 2012), que permite la formación del gel de GM incluyendo de forma eficaz al pescado en la red formada por el hidrocoloide dando lugar a "fibras" de pescado que se podrían consumir cocinadas (cocidas, a la plancha, etc), o se podrían incluir en una masa de surimi, por ejemplo, para obtener reestructurados de pescado con aspecto fibroso. El producto reestructurado así elaborado, también podría someterse a métodos de conservación convencionales como congelación, refrigeración, ahumado, pasteurizado, esterilizado, etc. Y se podría elaborar con cualquier otro músculo de pescado picado, incluso con especies grasas como el salmón.

TRABAJO Nº2.-

ELABORACIÓN DE ANÁLOGOS DE PESCADO CON FIBRAS DE GM INCLUIDAS EN UNA MATRIZ DE KAMABOKO

Se elaboró el Kamaboko que será la matriz donde se incluirán las fibras de pescado-GM desarrolladas en el Trabajo nº1, del siguiente modo:

1. Surimi flying Fish (75% humedad)
2. Agua/hielo (15%)
3. NaCl (2%)
4. Homogenización mecánica a vacío. 8-10 min a T< 12 °C
5. Gelificación: 30 min a 90 °C
6. Baño de agua / hielo.

Las fibras de pescado-GM gelificadas a pH 11,6 y 12,2 se incluyeron en la masa de Kamaboko en las siguientes proporciones:

a) 40% fibra pH 11,6 + 60% surimi
b) 30% fibra pH 11,6 + 70% surimi
c) 30% fibra pH 12,4 + 70% surimi

Figura 4.- Fibras pescado-GM

Los reestructurados tipo fibras de pescado- GM (Figura 4) se dispusieron en una matriz de Kamaboco (de dos modos, unos al azar y otros ordenadas longitudinalmente en la misma dirección). Las placas se envasan a vacío y se sumergen en baño de agua a 90 °C, 30 min.

De esta manera se obtuvieron reestructurados con aspecto y textura diferente, unos con las fibras al azar (Figura 5A), y otro con las fibras direccionadas (Figura 5B), que podrían ser análogos a tipos de músculo de pescado más fibroso y/o irregular, como por ejemplo el músculo de cangrejo y langosta.

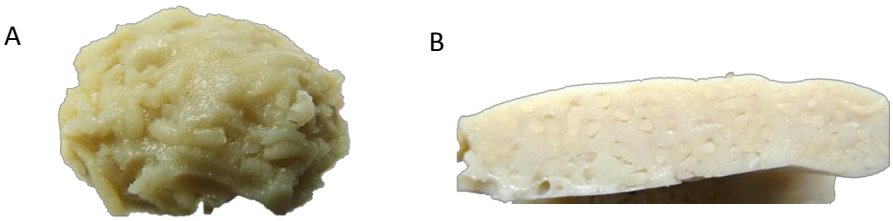

Figura 5.-Análogos de músculo de pescado fibroso (A)Fibras al azar (B)Fibras direccionadas longitudinalmente.

Otra forma de presentar los reestructurados es en formato embutidos y/o salchichas de pescado y constituye una forma atractiva de presentar el pescado que ya está teniendo éxito en el mercado europeo (Gallardo y López- Benito, 1978). Supone una forma fácil y cómoda de consumo de pescado y además atractiva para la población infantil. También permiten incluir ingredientes, funcionales o no que mejoren sus características organolépticas. La facilidad de conservación de este tipo de productos, ya sea en congelación o en envases estériles y herméticos también es un factor conveniente para su puesta en el mercado con una calidad óptima. Para emular un embutido tipo salchicha que además tenga una textura más parecida al músculo de determinados pescado o mariscos, se embute en tripa artificial la mezcla de fibras pescado-GM con surimi como matriz:

Se mezclan en un recipiente y posteriormente se embuten en las siguientes proporciones:

a) 30% fibra pH 11,6 + 70% surimi (Figura 6).
b) 30% fibra pH 12,4 + 70% surimi

Se sumergen en baño de agua a 90 °C 30 min.

Figura 6.- Embutido tipo salchicha elaborado con fibras de pescado-GM y surimi (30:70 (p/p))

TRABAJO Nº3.-

ELABORACIÓN DE ANÁLOGOS DE PESCADO CON MIOTOMOS Y MIOSEPTOS

Siguiendo la invención "Conformación de miotomos o mioseptos en productos pesqueros reestructurados" (Borderías, Carballo, y Moreno Conde, 2008), se elaboraron análogos de pescado simulando la estructura del músculo con miotomos y mioseptos (Figura 7).

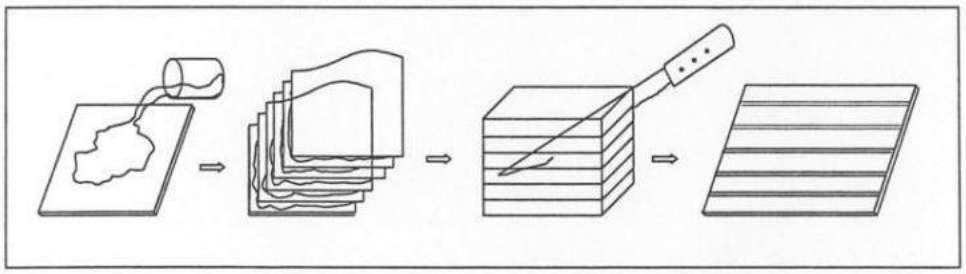

Figura 7.- Representación esquemática del proceso de elaboración de análogos de pescado (miotomos y mioseptos) Fuente: "Conformación de miotomos o mioseptos en productos pesqueros reestructurados"

El aspecto que aportan las características láminas que representan los miotomos y los mioseptos es un valor añadido para la aceptación de los productos reestructurados de pescado. La elaboración de estos análogos, mejora sus características comerciales. El proceso de reestructurado y conformación permite que después del cocinado se distingan los mioseptos y miotomos propios del pescado cocinado. El producto así elaborado, podrá someterse a métodos de conservación convencionales como congelación, refrigeración, ahumado, pasteurizado, esterilizado, etc. En este caso los miotomos son elaborados a partir de reestructurados de pescado-GM elaborados del siguiente modo:

1. Se preparan dispersiones acuosas de glucomanano (AGD) a una concentración total de polisacáridos del 3% (p/v) utilizando agua destilada a 80 °C. Se homogeniza en una máquina de homogenización mecánica a vacío (Stephan Machinery GmbH & Co., Hameln, Alemania) durante 30 min y se deja enfriar a temperatura ambiente.

2. Se mezcla, en el homogeneizador mecánico a vacío, la proporciones de AGD al 3% y pescado picado ("serrín") necesarias para obtener una concentración en el producto final del 3% de GM. Durante 5 min a menos de 30°C

3. Se adicionan pequeñas cantidades de KOH 0.6N hasta alcanzar pH 11,6-11,8. La masa se introduce en placas Petri (o similar) y se envasan a vacío. Para facilita la difusión de la base y con ello la desacetilación y gelificación del GM, las placas se introducen en un horno con calor seco (10 min a 40 °C).

Los mioseptos se preparan a partir de una masa de surimi con MTGasa elaborada de la siguiente forma:

1. Surimi flying Fish (75% humedad)
2. Agua/hielo (15%) 3. 0,1% TiO2
3. 3% MTGasa (Activa WM, Ajinomoto Co, Inc, Kawasaki, Japa)
4. Homogenización a vacío en Stephan (Stephan Machinery GmbH & Co., Hameln, Alemania) durante 8-10 min. evitando que la temperatura supere los 12 °C.

A la masa de surimi se le añade la MTGasa (3%) y el TiO2 (0,1%) que va a aportar blancura a la mezcla, y se unta directamente sobre las placas de pescado-GM. Se mantienen en refrigeración durante 16 h aproximadamente envasadas al vacío para favorecer el contacto y facilitar su unión.

La MTGasa adicionada al surimi, en contacto directo con el reestructurado de pescado-GM cataliza la formación de enlaces ε-(γ- glutaminil) lisina entre las proteínas de ambos componentes (Moreno, Carballo, y Borderías, 2008), permitiendo que los análogos de miotomos y mioseptos permanezcan unidos y puedan ser cocinados (cocidos, ahumados, a la plancha) sin que se destruya la estructura creada (Figura 8).

Figura 8.- Análogo de pescado con miotomos y mioseptos

TRABAJO Nº4.-

ELABORACIÓN DE ANÁLOGOS DE PESCADO TIPO" RABAS".

Una opción de moldeado o formación de reestructurados son los tipos "raba de calamar o pota".

Para su elaboración se siguen los pasos descritos en el trabajo nº1 hasta obtener una masa homogénea de AGM y músculo de pescado picado, en este caso "serrín". La masa se lleva a pH 12,2 mediante la adición de KOH 0,5N, la gelificación del GM a este pH da como resultado un gel más firme y parecido a la textura del músculo del calamar (Figura 9). Estos análogos de raba de pota o calamar podrían ser enharinado y fritos al estilo de la fritura "andaluza

Figura 9.- Análogos de rabas de pota o calamar elaboradas con músculo de pescado picado y GM

REFERENCIAS

Borderías, A. J., Carballo, J., y Moreno Conde, H. M. (2008). Conformación de miotomos o mioseptos en productos pesqueros reestructurados.

Gallardo, J. M., y López-Benito, M. (1978). Fabricación de salchichas de pescado.

Moreno, H. M., Carballo, J., y Borderías, A. J. (2008). Influence of alginate and microbial transglutaminase as binding ingredients on restructured fish muscle processed at low temperature. Journal of the Science of Food and Agriculture, 88(9), 1529-1536.

Solo-de-Zaldívar, B., Herranz, B., y Borderías, J. (2012). First steps in using glucomannan to make thermostable gels for potential use in mince fish reestructuration. International Journal of Food Engineering. 8(1).

IV.-DISCUSIÓN DE RESULTADOS

La idea central de este apartado se basa en el análisis concreto de cada una de las propiedades **físico-químicas**, **mecánicas** y **reológicas** estudiadas en los trabajos ya publicados. Se analizarán por un lado los geles de GM y, por otro, los reestructurados de pescado-GM. Cada propiedad se discutirá individualmente siguiendo un método común, que consta de cuatro partes: la **primera**, se centra en la optimización del procesado para la obtención de geles de GM (3% y 5%), según el tipo de álcali utilizado (KOH y NaOH) y sus concentraciones (0,6N y 1N) a temperatura constante (25 °C). En la **segunda**, se estudiará el efecto del grado de desacetilación del GM en cada una de las propiedades, en función de la concentración inicial de las dispersiones acuosas (3% y 5%),

En la **tercera** parte, se analizará la termoestabilidad de todos estos geles (calentados a 50 °C, 70 °C y 90 °C durante 20 min), atendiendo a la concentración de GM (3% y 5%), tipo de álcali y su concentración.

La **cuarta** parte de esta discusión, estudiará el comportamiento de cada propiedad, en los prototipos de pescado reestructurados frescos, congelados y pasteurizados. Este estudio finalizará siguiendo la evolución de cada propiedad durante el almacenamiento del prototipo, de acuerdo con el tratamiento: congelado (almacenamiento a -20 °C), y pasteurizado (almacenamiento en refrigeración).

Las propiedades fisicoquímicas discutidas en este apartado, son: capacidad de retención de agua (**CRA**), pérdida de agua por cocinado (**PAC**), capacidad de retener aceite (**CRAc**) y color (concretamente, luminosidad, L^*). Las propiedades mecánicas mediante ensayos de punción: fuerza de rotura (**FR**), y deformación de rotura (**DR**). Las propiedades viscoelásticas obtenidas por reometría dinámica, que permiten obtener los parámetros reológicos que definen el intervalo viscoelástico lineal: amplitud máxima de tensión (σ_{max}), amplitud máxima de deformación (γ_{max}). También los parámetros viscoelásticos característicos del intervalo lineal: módulo de almacenamiento elástico (G'), módulo de pérdida viscoso (G''), factor de pérdida ($tan\delta$), y módulo complejo (G^*).

El estudio de las características microestructurales**,** se realizará mediante **microscopía electrónica**. Se llevará a cabo el análisis comparativo de la microestructura de los geles seleccionados por tener características fisicoquímicas, mecánicas y reológicas más adecuadas para obtener los reestructurados de pescado.

Por último, se presentarán los resultados de los **recuentos microbiológicos** de aerobios totales de los reestructurados antes y después del proceso de pasteurización, y a lo largo de su conservación durante 35 días en refrigeración. Además, se describirán los resultados obtenidos en un **análisis sensorial** de los reestructurados, cocinados a la plancha después de la pasteurización.

1. CAPACIDAD DE RETENCIÓN DE AGUA (CRA)

1.1. Capacidad de retención de agua (CRA) y pérdida de agua por cocinado (PAC) en los geles de GM:

La medida de la CRA y su interpretación es esencial para entender la estructura del gel de GM y del sistema proteína (pescado)– polisacárido (GM), y, en consecuencia, el comportamiento mecánico y reológico.

Respecto a los geles de GM al 3% (p/v), los resultados de la CRA en función del tipo y concentración de álcali utilizado para la desacetilación y consecuente gelificación del GM, indican que al gelificar con álcali de baja concentración (0,6N) los geles elaborados con NaOH poseen un valor de CRA ligeramente superior, aunque no es significativo, respecto a los geles elaborados con KOH. Cuando se emplea álcali de mayor concentración (1N), la CRA de los geles elaborados con NaOH fue significativamente más alta que en los elaborados con KOH (1N).

Este hecho se reflejó también en un estudio complementario que se realizó, sobre la pérdida de agua por cocinado (PAC). En este caso, fueron los geles elaborados con NaOH (0,6 y 1 N) los que presentaron menos PAC, respecto a los elaborados con KOH (0,6 y 1 N).

Este comportamiento de los geles de GM se podría explicar considerando que los iones Na^+ al tener menor radio, puede alojar más moléculas de agua en sus esferas de hidratación que los iones K^+, confiriendo al gel mayor habilidad para retener agua. Además, en la serie lipotrópica de Hofmeister, (serie que ordena los cationes de mayor a menor capacidad de hidratación), el Na^+ se clasifica con mayor capacidad de hidratación que el K^+.

Cuando se trata de geles de mayor concentración de GM (5%(p/v)) se analizan solamente dos geles: los elaborados con KOH 0,6N y con NaOH 1N y no se observaron

diferencias significativas en CRA entre ellos, ya que la diferencia en la capacidad de hidratación que presentan los cationes Na^+ y K^+ se ve apantallada por la mayor concentración de GM.

A continuación, se estudió la evolución de la CRA en función del grado de desacetilación incrementando la cantidad de álcali (KOH 0,6N), en dispersiones acuosas de GM (AGD) a 3% y 5% (p/v). Para geles del 3% de GM, a medida que aumenta el grado de desacetilación, aumenta CRA, siendo significativa la diferencia en el intervalo entre 75 y 95% de desacetilación (pH 9,8 y 10,8). Para geles de 5% GM se observó una tendencia similar, siendo significativa la diferencia en un intervalo de desacetilación mayor, entre 58 y 91% (pH 10,7 y 11,0). Los dos geles alcanzan valores similares de CRA con grados de desacetilación diferentes, 75% de desacetilación para el gel con un 3% de GM y 58% para el del 5% de GM. Este hecho indica que geles con mayor concentración de GM tienen mayor CRA a menor grado de desacetilación.

Los geles elaborados con KOH 0,6N o NaOH 1N, fueron estudiados a 25 °C, 50 °C, 70 °C y 90 °C, observándose su termoestabilidad tanto al 3% como al 5% (p/v) de concentración de GM. Para el gel del 3% el comportamiento de la CRA con la temperatura depende esencialmente del tipo de álcali utilizado. Así, mientras que con KOH 0,6N la CRA aumenta entre 25 °C y 50 °C, con NaOH 1N disminuye. Sin embargo, para los geles calentados a 50 °C y 90 °C no se encontraron diferencias significativas en la CRA en función del álcali utilizado. Los datos obtenidos para los geles calentados a 70 °C no se publicaron porque no presentaron diferencias significativas respecto a los geles de 50 °C , en ninguna de las propiedades físico químicas. Los geles elaborados con NaOH 1N, a 25 °C retienen más agua con respecto a los obtenidos con KOH 0,6N. La cantidad de dipolos de agua atrapados electrostáticamente en la red por enlaces ion-dipolo es mayor cuando se utiliza NaOH 1N, debido al menor tamaño de los cationes Na^+ (mayor valor de la relación carga/radio), y a su mayor concentración. Cuando se somete el gel a un calentamiento a 50 °C y 90 °C, estas interacciones electrostáticas se debilitan, por lo que la presión mecánica asociada a la centrifugación que conlleva el método de determinación de la CRA, podría liberar más agua que estuviera retenida físicamente. Sin embargo, los geles elaborados con KOH 0,6N, al tener el catión K^+ menor relación carga /radio (menor intensidad de campo eléctrico), y estar en menor concentración, es menos hidrofílico. De ahí que el agua retenida en la red de GM gelificado con KOH es menos sensible térmicamente, lo que hace al gel más hidro-

estable con la temperatura, y la CRA a 50-90 °C es ligeramente mayor (no significativamente).

A alta concentración de GM (5%) no se encontraron diferencias significativas en CRA entre los geles elaborados con KOH 0,6N y NaOH 1N estudiados a 25 °C, 50 °C y 90 °C, poniendo de manifiesto de nuevo que, a mayores concentraciones de GM, el efecto electrostático se ve apantallado por el mayor número de cadenas de polisacárido.

1.2. Capacidad de retención de agua (CRA), pérdida de agua por cocinado (PAC) y capacidad de retener aceite (CRAc) en los prototipos reestructurados de pescado -GM:

La elaboración de los reestructurados de pescado se llevó a cabo partiendo de AGD al 5% (p/v), a la que se adiciona el pescado picado homogeneizado previamente en una proporción 25:75, GM: pescado (p/p). A los prototipos se les adiciona sal al 0,8% (lote S), o aceite de pescado rico en ω-3 al 5% (lote O), y se induce la desacetilación para la gelificación del GM con KOH 0,6N hasta pH 11- 11,1. Una vez neutralizados, se obtienen los prototipos con un 1,25% de GM final, y se estudia la influencia sobre la CRA de estos ingredientes (sal y aceite), en los prototipos frescos frente a un lote control (lote C) sin ingredientes añadidos.

La CRA aumentó significativamente en el prototipo con sal respecto al control y a la muestra con aceite. La presencia de iones Na^+ procedentes de la sal (NaCl), incrementa el agua retenida electrostáticamente en la red (Schut, 1976), y en consecuencia la CRA. En relación a las pérdidas de agua por cocción (PAC), el lote O fue el que presentó una mínima PAC con respecto al control y al lote S. La presencia de aceite en la red de pescado-GM formando emulsión en los huecos de la red, compacta la estructura completa a temperatura alta, atrapando las moléculas de agua más eficientemente por interacciones hidrofóbicas, e incluso reforzando la emulsión aceite-proteína-agua lo que justifica la mínima PAC.

Con el fin de estudiar los efectos que tienen los métodos tradicionales de conservación de alimentos sobre los prototipos de pescado-GM, éstos se congelaron y se mantuvieren en congelación a -20 °C. Transcurridos 30, 90 y 150 días de almacenamiento, las muestras se descongelaron a 5 °C y se realizó una caracterización completa a 25 °C. Se utilizaron como controles las muestras de los correspondientes lotes que se habían mantenido en refrigeración durante 24h.

Se observó que todos los prototipos presentaron una disminución significativa de la CRA, y un incremento significativo de las PAC al ser congelados. Comportamiento que se mantuvo durante los 30 , 90 y 150 días de almacenamiento en congelación, aunque para los lotes S y O entre los 30 y 90 días las diferencias no fueron significativas ni en CRA ni en PAC.

La pérdida de CRA y el aumento de la PAC fue máximo para el lote con sal transcurridos los 150 días de almacenamiento en congelación. Las moléculas de agua que estaban retenidas electrostáticamente por los cationes Na^+ y el agua libre en los poros de la red, es susceptible de formar cristales de hielo durante la congelación y el almacenamiento en estado congelado, lo que deshidrata la red de pescado–GM disminuyendo en consecuencia su CRA e incrementando su PAC con respecto al control. El lote O mantuvo los valores de PAC durante el almacenamiento en congelación, lo que refuerza la idea de la estabilidad que produce la emulsión aceite-proteína-agua en la red atrapando las moléculas de agua al aplicar calor.

También se estudió la influencia de la pasteurización en la CRA de los tres tipos de prototipos: lote control (C) (sin ingredientes), con sal (lote S) y con aceite de pescado (lote O) sometidos a un tratamiento de pasteurización (80 °C durante 20 min). La influencia de los ingredientes adicionales en los prototipos pasteurizados se estudió tras 1, 7, 21 y 35 días en almacenamiento a 5 °C, frente al lote control (C). Tras ser pasteurizados, la CRA decreció significativamente en todos ellos presentando los prototipos que contenían aceite y sal una menor disminución de CRA respecto al control.

Así, después de la pasteurización la red del gel del lote O experimenta probablemente un reordenamiento estructural, fortaleciéndose las interacciones hidrófobas. El aceite añadido podría formar un "sistema complejo de emulsión" que actuaría como una fase *filler* (relleno) en la matriz de proteína-GM, que ayudaría a retener agua en la red, lo que justificaría una disminución menor de CRA, respecto a control. En el caso del lote S, el mayor número de iones Na^+ creó una mayor densidad de carga electrostática, lo que reforzó las interacciones ion-dipolo con los dipolos permanentes del agua, mejorando la capacidad de retención de agua del lote S respecto al lote C, tras la pasteurización.

A lo largo del periodo de almacenamiento la CRA se mantuvo durante los 21 días, excepto la muestra con sal (S) en la que se observó un decrecimiento significativo entre 1 y 21 días. Pero, en general, incluso en el día 21 de almacenamiento en refrigeración después

de la pasteurización, la CRA de todos los lotes estaba por encima del 75%, lo que indica una alta estabilidad de los reestructurados durante el almacenamiento. En el día 35, hubo una disminución significativa de CRA en los tres lotes, pero particularmente en los lotes C35 y S35 (~ 16%), en comparación con C21 y S21, respectivamente, lo que indica un daño estructural irreversible en ambos geles. Sin embargo, esta disminución de CRA fue considerablemente menor (~ 5%) en la muestra O35 que en C35 y S35, lo que muestra el papel estabilizador del aceite en la red.

Las PAC se redujeron en los productos al ser pasteurizados pero no fue significativo para los lotes O y S y sí para el lote C, en consonancia con los cambios estructurales que se ponen de manifiesto con la disminución de la CRA. Paralelamente, a lo largo del periodo de almacenamiento, la PAC aumentó en mayor medida en el lote S con respecto al control C y al lote O. Esto es debido, sobre todo, al ion Na^+ que inmoviliza fuertemente el agua en torno a sí, deshidratando la matriz pescado-GM y, en consecuencia, desestabilizando parcialmente la estructura de la matriz del gel.

Los lotes que contenían aceite fueron sometidos a la medida de la capacidad de retener aceite (CRAc). El mecanismo para la CRAc combina el atrapamiento físico de las micro-gotas de aceite en los capilares de la red de gel, con la capacidad de la red para unir el aceite mediante fuerzas atractivas como van der Waals, e interacciones hidrofóbicas entre grupos no polares de las proteínas de pescado (no funcionales) y el aceite. Estas uniones evitan la agregación de las micro-gotas de aceite y, en consecuencia, estabilizan la emulsión. La temperatura de pasteurización (80 °C) hace que el aceite fluya más fácilmente en la red (menor viscosidad) y, al mismo tiempo, reduce la tensión interfásica, induciendo una disminución significativa en la CRAc al ser pasteurizados. El sistema de emulsión formado en el lote O no se mantuvo estable durante el almacenamiento, y la CRAc se redujo. A bajas temperaturas (refrigeración), las emulsiones se vuelven termodinámicamente inestables y las fases tienden a separarse parcialmente con el tiempo.

2. COLOR (Luminosidad, *L**)

La medida de la luminosidad (*L**) y la interpretación de los resultados mediante la escala CIELab de los geles y los reestructurados de pescado, es esencial para la aceptación del producto y la percepción de su calidad, además da idea del estado estructural y reológico de los geles.

2.1. Color en los geles de GM:

Analizando en primer lugar la influencia de la concentración y el tipo de álcali en el color de geles de GM al 3% (p/v). Tanto a alta como a baja concentración de álcali (0,6N y 1N) los geles elaborados con NaOH presentaron valores de *L** significativamente mayores que los elaborados con KOH. Como se indicará más tarde, los geles elaborados con NaOH dan lugar a estructuras con mayor capacidad de empaquetamiento, más compactas y por tanto reflejan difusivamente, mejor la luz.

Cuando nos fijamos en geles con mayor concentración de GM (5% (p/v)), no se encontraron diferencias significativas en *L** entre los elaborados con NaOH y KOH, ni a alta ni a baja concentración de álcali. Al aumentar la concentración de GM aumenta la densidad de cadenas y enlaces cruzados, las "zonas de unión" (que actúan como centros difusores de luz) son más gruesas, la estructura es más compacta y continua, por esto tiene mayor capacidad de difundir la luz dando valores más elevados de *L**.

A alta concentración de GM (5% (p/v)), se determinó que la mayor *L** se obtenía también en geles elaborados con NaOH, pero no se encontraron diferencias significativas en función de la concentración de este álcali (0,6N y 1 N). Los geles elaborados con KOH 1N tampoco mostraron valores de L* significativamente diferentes a los de los geles elaborados con NaOH 1N.

Posteriormente, se estudió la evolución de *L** en función del grado de desacetilación utilizando cantidades crecientes de álcali (KOH 0,6N), sobre dispersiones acuosas de GM (AGD) al 3 y al 5% (p/v). Para geles 3% de GM, a medida que aumenta el grado de desacetilación aumenta *L**, siendo significativa la diferencia entre los geles con 26 y 75% de desacetilación. Para geles de GM (5%) también se observó la misma tendencia al incrementar el grado de desacetilación, siendo en este caso, significativa la diferencia entre 91% y 100% de desacetilación del GM. Se observó un valor máximo de *L** en los geles con mayor grado de desacetilación, tanto para los del 3% como para los del 5% de GM, este efecto es consecuencia de una mayor densidad de red, con mayor número de centros difusores de luz, lo que incrementa la luminosidad del gel.

Con el objetivo de estudiar la influencia de la temperatura y del álcali en el color, concretamente en la luminosidad (*L**), se analizan los geles obtenidos con KOH 0,6N y NaOH 1N elaborados con un 3% y un 5% (p/v) de GM a 25 °C, 50 °C y 90° C.

Los resultados mostraron que a baja concentración de GM (3%), los geles elaborados con KOH 0,6N presentaron valores significativamente menores de *L** a 25°C. Al ser calentados a 50 °C, tienen menor luminosidad que los elaborados con NaOH, lo que concuerda con la menor CRA. Al calentar los geles de 50 °C a 90°C aumentó débilmente *L**, tanto cuando se utilizó KOH 0,6N como NaOH 1N. Cuando la base utilizada es NaOH 1N la *L** tiende a aumentar ligeramente entre 25 °C y 50 °C, siendo significativo el aumento entre 25 °C y 90 °C, debido probablemente a la compactación de la microestructura de los geles.

2.2. Color en los prototipos reestructurados de pescado-GM:

La luminosidad (*L**) para la merluza fresca ronda valores cercanos a 47,96 (Fuentes-Zaragoza, Pérez-Álvarez, y Sánchez-Zapata, 2009), mientras que los reestructurados con GM frescos y sin ingredientes adicionales estudiados en este trabajo, superan este valor alcanzando valores de luminosidades entre 60 y 68. La *L** está influida por muchos parámetros, entre ellos los procesos de picado del músculo, la composición del reestructurado y la CRA. Los productos de pescado picado son más luminosos y con mayor CRA, debido a las roturas musculares, e incorporación de pequeñas burbujas de aire. En la red formada por este sistema, el agua potenciaría la reflexión difusa de la luz, lo que justificaría el incremento de *L** (Perez-Alvarez y Fernandez-Lopez, 2006)

Cuando al músculo picado se le adiciona AGD en la mezcla inicial, se homogeniza y posteriormente se añade la cantidad adecuada de álcali para la gelificación, se obtienen reestructurados de pescado con mayor valor de *L**, que el propio pescado fresco. Este efecto se ha observado también en los geles de surimi, al adicionar GM aumenta *L** (Park, 1996). Los geles de GM tienen valores de *L** próximos a 50, en oposición a la opacidad del músculo de pescado, y aportan luminosidad al reestructurado.

Los ingredientes adicionales (sal y aceite) añadidos a los reestructurados también influyen en *L**. El lote O (5% de aceite) fue el que presentó mayor valor de *L** respecto al lote C y al lote S (0,8% NaCl), este resultado estaría de acuerdo con Pérez-Alvarez et al., (1998), los cuales indican que la presencia de grasas en los reestructurados ayude a difundir

la luz aumentando la luminosidad. Además, se pone de manifiesto que el lote O posee una estructura más compacta, con agregados más grandes, que reflejan la luz con mayor intensidad. Igualmente, el lote S tiene una mayor luminosidad que el lote C, con la adición de sal se aumenta la fuerza iónica (Hammer, 1992), y el sistema proteína de pescado- GM retiene mayor cantidad de agua aumentando la luminosidad.

Cuando los prototipos se congelaron se redujo significativamente *L**, en los tres lotes de forma similar, hecho que se podría relacionar con la posible formación de estructuras pseudo-cristalinas (deshidratadas), producidas por la congelación, que reducirían la capacidad de reflejarla luz. Después, en los tres lotes, *L** se mantuvo prácticamente constante durante el almacenamiento a -20 °C a los 30, 90 y 150 días, siendo mayor el valor de *L** en el lote O hasta los 150 días. Este hecho realza la estabilidad estructural que produce el aceite emulsionado dentro de la red GM-pescado y el posible efecto antioxidante del aceite de pescado "Omevital" añadido, que contiene ácido eicosapentaenoico (EPA), ácido docosahexaenoico (DHA) y una combinación de α-tocoferoles como antioxidantes.

Tras la pasteurización, *L** se mantuvo prácticamente constante en los prototipos con aceite (O) y sal (S). Sin embargo, en el gel control (C), *L** incrementó, este efecto podría ser indicio de un mayor nivel de compactación reticular que favorece la difusión de la luz. La reorganización de las moléculas de agua después del tratamiento térmico, modifica la red de gel, mostrando un aspecto más brillante (Tabilo-Munizaga & Barbosa-Cánovas, 2004). A lo largo del periodo de almacenamiento (1-21 días), para los geles O y S, *L** se mantuvo contante. Sin embargo, en el gel control se observó decrecimiento significativo de *L**, lo que indicaría el daño estructural que se produce en la red pescado-GM durante el tiempo de refrigeración tras ser pasteurizado el producto.

3. FUERZA DE ROTURA (FR) Y DEFORMACIÓN DE ROTURA (DR).

3.1. FR y DR en los geles de GM:

A baja concentración de álcali (0,6N), los geles elaborados con NaOH poseen valores de FR y DR inferiores a los geles elaborados con KOH. Sin embargo, a concentraciones elevadas (1N) ocurre lo contrario. La mayor rigidez que presentaron los geles con NaOH 1N se podría explicar teniendo en cuenta el fortalecimiento de las interacciones catión-dipolo (Na^+—H_2O), que produce mayor grado de orden electrostático, dando lugar a una nueva fase

de agua (semi-sólida), constituida por una red intermolecular de enlaces de hidrógeno (Damodaran, 1997). No obstante, aunque FR aumenta notablemente en los geles elaborados con NaOH 1N, su DR es similar a la de los restantes geles. Este resultado parece indicar que la rigidez de los geles depende fuertemente del orden dipolar impuesto por los cationes Na^+ a los dipolos de agua, y a las cadenas de GM desacetiladas, las cuales, se ven frenadas favoreciendo una mayor compactación intermolecular.

A elevada concentración de GM (5%), no se encontraron diferencias significativas debidas al tipo y concentración de álcali en los valores de FR entre los cuatro geles, hecho que manifiesta el papel preponderante del GM en la fortaleza estructural del retículo polimérico, incrementando la densidad de red, es decir el número de cadenas elásticamente activas (Sperling, 2001). Es natural que el mayor número de asociaciones GM-GM apantalle el efecto estructural asociado al orden dipolar entre los cationes Na^+ y K^+ y los dipolos de agua, y también con los grupos OH disponibles en las cadenas de GM.

El menor valor de DR para los geles elaborados con KOH 1N, refleja geles frágiles y con zonas de unión menos estables (Leksrisompong, Lanier y Foegeding, 2012).

Para geles de GM al 3%, a medida que aumenta el grado de desacetilación, FR aumenta, mientras que DR no cambia. Este hecho, manifiesta un notable incremento en la firmeza del gel, que se podría relacionar con el aumento de la conectividad entre las cadenas de GM. Al incrementarse el grado de desacetilación, se extienden las zonas intermoleculares de unión a nuevos fragmentos poliméricos y a más cadenas de GM, en distintas posiciones, pero manteniendo propiedades mecánicas similares en las "zonas de unión" (Clark y Ross-Murphy, 1987), ya que DR no cambia (Renkema, 2004).

Para geles de GM al 5% se observó una tendencia similar en ambos parámetros FR y DR. Siendo naturalmente en este caso, los valores de FR más elevados debido a la mayor densidad de red, consecuencia de la mayor densidad de enlaces cruzados.

Los geles elaborados con KOH 0,6N o NaOH 1N, fueron estudiados a 25 °C, 50 °C y 90 °C, observándose que al aumentar la temperatura del gel, hay un aumento pronunciado de la FR en ambas concentraciones de GM (3% y 5%). Además, la FR de los geles elaborados con NaOH es significativamente mayor a 50 °C y 90 °C que en las muestras elaboradas con KOH. Naturalmente, una concentración más alta de GM (5%) aumentó considerablemente

la FR, ya que la concentración creciente de GM favoreció la interconexión de las cadenas moleculares, al promover la formación de un mayor número de zonas de unión, lo que produce redes de gel más cerradas.

Para los valores de DR a 25 °C, se observa una disminución no significativa en geles hechos con KOH 0,6N frente a los elaborados con NaOH, particularmente a baja concentración de GM (3%). Además, la dependencia de DR con la temperatura fue mayor para los geles del 3% de GM, ya que para los del 5%, los valores se mantuvieron prácticamente constantes con el aumento de la temperatura.

Estos datos FR y DR pueden resumirse mediante el parámetro K_f (constante de fractura; FR/DR), que proporciona una medida de la rigidez del gel con respecto a la concentración de álcali y GM a cada temperatura. Cuando se utiliza NaOH 1N, los valores de K_f son más altos en ambas concentraciones de GM, lo que indica geles más firmes a cualquier temperatura. En este ensayo se vio que la relación entre K_f y la temperatura de tratamiento del gel es proporcional (independientemente de la concentración de álcali y GM), lo que sugiere una red unida permanentemente. Aunque no es de naturaleza covalente, la matriz del gel se comporta como si fuese una red químicamente reticulada (Lapasin y Pricl, 2013). Hecho que concuerda con el carácter termoestable de estos geles de GM (Nishinari y Zhang, 2004).

3.2. FR y DR en los reestructurados de pescado -GM:

Los valores de FR fueron iguales para los tres lotes a tiempo inicial (1 día de conservación a 5 °C): control (C1), con sal (S1) y con aceite (O1). Sin embargo, DR es más elevado en el prototipo con sal, respecto al control, y la muestra con aceite. Los cationes Na^+ en el gel S1 incrementan el agua retenida electrostáticamente en la red y, en consecuencia, aumenta la CRA. Al mismo tiempo, los cationes fortalecen las interacciones electrostáticas ion–ion, e ion–dipolo que afectarían a las cadenas de GM y de proteína. Ambas, podrían incrementar el grado de asociación intermolecular al inmovilizar fragmentos de ambos polímeros que dificultan la transmisión del "*crac*" durante la propagación de la rotura (Sperling, 2001). En consecuencia, se precisaría una distancia de penetración mayor para extender la rotura por toda la red.

Debido a la congelación (30 días a -20 °C) el prototipo con sal (S30) experimentó el máximo incremento de FR, mientras que DR apenas aumentó. Este resultado indica incremento de dureza en el gel S30 respecto a S1. Efecto que concuerda con la pérdida de CRA y el mayor incremento de PAC en el gel S30. Así, al perder capacidad de ligar agua después de la congelación, la matriz se deshidrata (se concentra), y se endurece, sin alterar casi las propiedades mecánicas de las zonas de unión, que conservan su capacidad de deformación, también durante el periodo de almacenamiento a -20°C. De forma similar, aunque en menor cuantía, en los geles O30 y C30 también se incrementó su rigidez después de la congelación, es decir, FR incrementó, mientras que DR apenas cambió.

Durante el almacenamiento a -20 °C (90–150 días), los valores de FR en el lote O fueron más altos que a los 30 días, con valores similares entre ambos puntos de muestreo (O90–O150). Este hecho indica que, durante el almacenamiento en estado congelado, ha podido ocurrir cierta reordenación en la fase *filler,* y en la matriz pescado–GM que incrementó la dureza del gel. Sin embargo, en los lotes S90–S150 y C90–C150, FR se mantuvo sin cambios significativos respecto al valor de 30 días.

Después de la pasteurización, en el lote O la disminución de FR fue significativa mientras que DR incrementó débilmente (O1). Al contrario, el prototipo que contenía sal (S1), presentó un incremento importante en FR y DR respecto al producto fresco (S0) y respecto a la muestra control pasteurizada (C1). En la muestra control, la pasteurización no alteró significativamente los parámetros FR y DR. Los valores altos de FR y DR en el lote S1 (pasteurizado), indican que el gel incrementó su firmeza paralelamente con su deformabilidad, hecho que manifiesta la mejora estructural (estabilidad energética), que aporta la sal a la matriz pescado–GM. En el lote S, durante el almacenamiento en refrigeración, FR se mantuvo mientras que DR decreció, indicando que se conserva similar firmeza en la red pero con zonas de unión menos flexibles y/o deformables (Renkema, 2004). Hecho coherente con el decrecimiento significativo de CRA y con el incremento de PAC entre 1 y 21 días, que produciría estructuras más concentradas por la pérdida de agua en la red.

4. COMPORTAMIENTO DE LOS PARÁMETROS VISCOELÁSTICOS (σ_{max}, γ_{max}, tanδ, G', G'', S y n)

4.1. Parámetros viscoelásticos en los geles de GM:

A partir de los ensayos oscilatorios a frecuencia constante se dedujo que a baja concentración de álcali (0,6N), los geles elaborados con NaOH poseen valores de σ_{max} y γ_{max} más bajos que los geles elaborados con KOH. Sin embargo, a concentraciones elevadas (1N) ocurre lo contrario, siendo los valores de σ_{max} y γ_{max} significativamente más altos en geles elaborados con NaOH. La mayor fortaleza energética de los enlaces en la red de GM usando NaOH 1N, se observa en los valores más bajos de $tan\delta$, hecho que concuerda con su mayor CRA.

El carácter transitorio de la red inducido por cizalla oscilatoria, se percibe en los espectros mecánicos, en particular en el máximo incremento de G'' al disminuir la frecuencia angular (ω) en el gel elaborado con NaOH 1N . Hecho que indica que la matriz de GM es más densa, con más uniones de mayor espesor, que podrían formar zonas micro-cristalinas originando cierto estado vítreo en la red (Sperling, 2001). Este efecto contribuye a la formación de una red heterogénea y, por tanto, más frágil, sobre todo cuando la cizalla es más prolongada (bajas frecuencias), lo que conlleva un reordenamiento estructural en las "zonas de unión". Ese reordenamiento absorbe energía, que se gasta en los diferentes cambios estructurales, pero que no se almacena en la red, por eso incrementa la componente viscosa, reduciendo su elasticidad neta al disminuir ω. Este efecto se vio confirmado en los parámetros que proporcionan los ensayos de carga y recuperación, experimentos realizados a escalas de tiempo más elevadas que los oscilatorios; en particular, en los valores más altos del exponente n (menor conectividad de red), y más bajos del porcentaje de elasticidad. Comparando ambos álcalis, es el KOH 0,6N, el que origina geles de GM (3%) algo más flexibles y homogéneos, con mayor conectividad y porcentaje de elasticidad, lo que les hace estructuralmente más estables.

A elevada concentración de GM (5%), tampoco se encontraron diferencias significativas provocadas por el tipo y concentración de álcali, en los valores de las amplitudes del rango lineal siendo σ_{max} notablemente más elevado y γ_{max} menor que en geles de GM al 3%. Resultado que muestra el aumento lógico del nivel de estructuración y compactación molecular, que denota una mayor densidad de red.

Los geles elaborados con KOH 0,6N, experimentan el menor incremento de G'' a baja ω, de lo que se deduce una mayor estabilidad energética de los enlaces durante el tiempo de cizalla (Steffe, 1996). Este resultado favorable, también se encontró en los experimentos de carga y recuperación, ya que los valores de fuerza de gel (S), conectividad de red (n), y porcentaje de elasticidad, fueron máximos para los geles de GM al 5% elaborados con KOH 0,6N.

Para geles de concentración de GM al 3%, a medida que aumenta el grado de desacetilación aumenta σ_{max}, y γ_{max}, y disminuye $tan\delta$. Este hecho, indica un notable incremento en la fortaleza del gel, que podría deberse al aumento del número de interacciones por enlaces de hidrógeno entre fragmentos de GM, que se extienden por la red, debido a la progresiva desacetilación. A baja concentración de GM, el incremento del pH va tejiendo un retículo polimérico más elástico (menor $tan\delta$), más flexible y homogéneo (mayor γ_{max}), y estructuralmente más estable (mayor σ_{max}).

Paralelamente, se observó un incremento en los valores del módulo G' en todo el rango de frecuencias (10–0,1Hz), junto con una disminución de G'' en el rango de altas frecuencias (10–1Hz). En conjunto, al aumentar el grado de desacetilación, el sistema gana estabilidad energética gracias a la asociación de nuevas cadenas de GM por enlaces de H, que "congela" cinéticamente la estructura incrementando el valor de G' sobre G'', dentro del marco característico de los geles físicos (esencialmente transitorios), y por tanto, dependientes de la frecuencia (Lapasin y Pricl, 2013). Las redes de GM ganan solidez porque se incrementa el orden orientacional fortalecido por enlaces de H entre los fragmentos de GM que forman las zonas de unión (Larson, 1999).

En los geles de GM al 5% también se observó un decrecimiento de $tan\delta$ con el aumento del grado de desacetilación, acentuado entre la muestras con 23% y 58% de desacetilación, rango donde el efecto estabilizante de los enlaces de hidrógeno entre las cadenas de GM, alcanza su punto álgido (mínimo $tan\delta$). Así, en este intervalo (23-58%) se nota un "*crecimiento exponencial*" en el grado de estructuración de la red, en la que la asociación entre cadenas se intensifica, incrementando el número y la fortaleza energética de las zonas de unión; la red se vuelve más compacta con agregados de mayor tamaño y, en consecuencia, γ_{max} disminuye intensamente .

Este resultado se corresponde con los máximos valores de *G'* entre 10 y 0,1 Hz y los mínimos de *G''*, que manifiesta la mayor capacidad de almacenar energía de las redes de GM al 5%, a desacetilación máxima, en concordancia con la máxima CRA que mostraron estos geles.

De los ensayos de carga–recuperación también se deduce que la fuerza de gel aumenta (aumenta *S*) mientras que el porcentaje de elasticidad disminuye, al aumentar el grado de desacetilación, de forma similar a los geles de GM al 3%.

La diferencia principal entre los geles a las dos concentraciones de GM, es que, aunque en ambas al aumentar la desacetilación se incrementa la densidad de red, lo hacen de modo diferente. A la concentración de GM al 3% la estructura se fortalece formando geles débiles (sin regiones micro-cristalinas), por ser menor el espesor de las "zonas de unión", y en consecuencia menor grado de compactación a esta concentración de GM (3%), por eso la red se hace más flexible (aumenta γ_{max}). En cambio, en los geles del 5% GM, la agregación alcanzó el nivel suficiente para formar pequeños micro-cristales que hacen más heterogénea una estructura formada por "zonas de unión" más rígidas, con menor capacidad de curvatura (Renkema, 2004) y, por tanto, con menor flexibilidad conformacional (decrece γ_{max}). A 5% GM la red es más compacta (aumenta *G**) y más estable (mayor σ_{max}). Debido a la compactación y al mayor contenido energético de la estructura reticular, es de esperar una mejor capacidad de retención de agua en capilares, o a modo de relleno en los huecos de la red, lo que justificaría los valores máximos de CRA.

A 3% de GM, la fuerza de gel expresada en términos de *G** se mantiene con NaOH 1N mientras que el elaborado con KOH 0,6N se ve un incremento a 90 °C, manteniéndose en el rango 25-70 °C.

En los geles de GM 5%, al incrementar la temperatura entre 25 y 50 °C la fuerza de gel disminuyó significativamente independientemente del álcali utilizado (NaOH 1N y KOH 0,6 N), manteniéndose constante en el rango 50-90 °C.

En relación con la flexibilidad conformacional no se observaron diferencias destacables entre las distintas temperaturas para ambos geles al 3% y 5% GM para ambos álcalis.

4.2. Parámetros viscoelásticos en los reestructurados de pescado– GM:

En el lote O1 el valor de σ_{max} fue similar respecto al control, sin embargo γ_{max} fue menor, lo que indica que se ha formado una red más compacta y, por tanto, menos flexible, más firme y heterogénea debido al mayor grado de empaquetamiento molecular debido al contacto entre varias fases (oleica, proteica, acuosa), por eso, es más sensible a la cizalla oscilatoria (menor γ_{max}). Esto se podría relacionar, por una parte, con el menor contenido de agua en la red del gel O1, y, por otra, con la co-existencia de dos unidades estructurales básicas: una fase *filler* constituida por el aceite y unida, formando una emulsión, a la matriz sólida: agua–proteína (pescado)–GM. También podría existir cierta proporción de agua en los poros y capilares de la red, formando una nueva fase *filler*, dispersa. Las distintas fases pueden formar una red de gel continua, pero más heterogénea e irregular que la del lote control (C1), lo que podría explicar el mayor incremento del parámetro G_0'' (20%) en O1 *vs* C1, comparado con el menor incremento de G_0' (11%) de O1 *vs* C1.

Estos resultados son consistentes con los valores más altos de *n'* y *n''* encontrados en O1 *vs* C1. Es razonable que una red compuesta en la que pueden co-existir varias fases, tenga módulos viscoelásticos más dependientes de la frecuencia, ya que toda interfase implica una región límite (Sahim y Sumnu, 2006), que conlleva una zona de discontinuidad estructural. Eso explicaría que sea más sensible a la tensión armónica de frecuencia variable, y en consecuencia ambos exponentes *n'* y *n''* aumentan en O1 respecto a C1. Paralelamente, también se percibe este efecto estructural de la interfase en la mayor sensibilidad a la cizalla, (menor γ_{max}) comentada previamente.

Estos resultados también se reflejan en los parámetros obtenidos en los ensayos de carga y recuperación. Por una parte, en el lote O1 se observó un fuerte incremento de la fuerza de gel (aumenta *S*) con respecto a C1, y por otra, el incremento del exponente de relajación (*n*), indica un menor grado de conectividad entre los elementos estructurales que componen la red, hecho coherente con la "*discontinuidad*" interfásica, cuya superficie límite de contacto, reduce la estabilidad reticular (Sahim y Sumnu, 2006), por tanto, el tiempo de vida media de las zonas de unión se reduce (*n* aumenta) en O1 respecto a C1.

La adición de sal (S1), incrementó significativamente ($p<0,05$) σ_{max} e incrementó débilmente γ_{max} respecto al gel control (C1), indicando que la sal mejoró el acoplamiento GM-proteína, debido al incremento del número de interacciones electrostáticas. Éstas no

solo ordenan la disposición relativa de las moléculas de GM y proteína, sino que mejoran la capacidad de retener agua en la matriz. El resultado se traduce en una mayor estabilidad estructural en el prototipo, conservando el grado de flexibilidad en la matriz pescado-GM respecto al gel control. La mejora del acoplamiento GM-proteína se refleja en el incremento del grado de solidez, tal y como se desprende del menor valor de G_0'' en S1 respecto a C1, y podría explicar los valores más elevados de CRA en el lote S1 *vs* C1.

En el prototipo control (C) se incrementó la rigidez después de la congelación, es decir aumentó G^*, mientras que γ_{max} se mantuvo como en el producto conservado en estado refrigerado. Asimismo, en el prototipo con sal (S), la congelación también incrementó G^* mientras que los valores de las amplitudes σ_{max} y γ_{max} apenas variaron. Estos resultados indican un aumento de la dureza del gel en ambos prototipos, consecuencia de la deshidratación que sufre la matriz tras la congelación, tal y como se observó en el decrecimiento de CRA para el gel C30 *vs* C1 y más intensamente para S30 *vs* S1.

El efecto de la deshidratación por congelación, también se refleja en el comportamiento de los parámetros viscoelásticos en los espectros mecánicos. En particular, para el prototipo S30 la congelación provocó mayor incremento de G_0' (90%) que de G_0'' (67%), mientras que ese porcentaje es notablemente menor en C30 *vs* C1, donde G_0' aumentó un 58% mientras que G_0'' un 39%.

Análogamente, en los ensayos de carga y recuperación, también se observó un incremento de la fuerza de gel, mayor en el lote S30 (96%) respecto a S1, mientras que en C30 *vs* C1 fue menor (16%). Además, el exponente de relajación (n) aumentó un 25% en S30 *vs* S1, hecho que manifiesta cierto daño estructural provocado por la congelación, pues la red pierde conectividad (aumenta n), mientras que en el lote C permaneció constante.

Estos resultados concuerdan con el fuerte incremento de FR y con la máxima reducción de CRA experimentada en S30 *vs* S1 en relación con los otros dos lotes (C30/C1 y O30/O1). En los lotes S y C, el endurecimiento de la red asociado a la congelación, tiene en común la conservación de las propiedades mecánicas de las zonas de unión, en la matriz pescado-GM, las cuales, mantienen similar grado de flexibilidad conformacional ($\sim\gamma_{max}$) incluso durante el almacenamiento a -20 °C.

El lote con aceite (O) presentó diferencias respecto a los otros dos, en los parámetros viscoelásticos (σ_{max}, γ_{max}, G^*) del rango lineal, que mantuvieron el mismo valor en O30 respecto a O1. De los espectros mecánicos se observó un ligero decrecimiento de los exponentes n' y n'', hecho que indica que tras la congelación se mantuvo prácticamente la estructura inicial; paralelamente, se observó un pequeño incremento de G_0' (33%) y G_0'' (14%) en O30 *vs* O1.

Durante el almacenamiento a -20°C (lotes C30–C150), los valores de G^* se mantuvieron sin cambios significativos entre 30–90 días. Sin embargo a 150 días, se produjo una especie de *colapso estructural* debido al fuerte decrecimiento en σ_{max} y γ_{max} (C150), indicando que la matriz "pescado-GM" ha perdido en gran parte la estabilidad y flexibilidad conformacional que tenía inicialmente, sin embargo, se incrementó su firmeza. Esto se podría explicar debido al reordenamiento estructural inducido por el crecimiento del tamaño de los cristales de hielo tras 150 días a -20°C, que podría contribuir a la formación de nuevas "zonas de unión" más heterogéneas, más gruesas y por tanto, menos flexibles y más sensibles a la cizalla (menor γ_{max}). De este modo, la red se hace más rígida (mayor G^*) y, conformacionalmente, menos estable (menor σ_{max}). Resultado que también se refleja en el incremento de los parámetros G_0' y G_0'' (espectros mecánicos) y de fuerza de gel (S) en C150. Este efecto también se relaciona con el decrecimiento de CRA, e incremento de PAC en C150 respecto a C30.

En la muestra con sal (S150) las amplitudes del IVEL (σ_{max} y γ_{max}) se mantuvieron similares a S30; también se observó un aumento en la rigidez del gel considerando los ensayos oscilatorios (barridos de tensión y frecuencia), de los que se dedujo un aumento de cualquier parámetro reológico relacionado con la fuerza de gel (G^*, G_0', G_0'') en S150 *vs* S30.

Sin embargo, en el lote O los valores de las amplitudes de tensión (σ_{max}) y de deformación (γ_{max}) aumentaron en O150 *vs* O30. La rigidez del gel también incrementó en O150 *vs* O30 pero en menor cuantía si comparamos las muestras C150–S150 *vs* C1–S1 respectivamente. De este resultado se deduce el particular papel estabilizador del aceite en la matriz pescado-GM, en donde la fase *filler* podría "amortiguar" reordenamientos en las zonas de unión, asociados al crecimiento de cristales de hielo durante el almacenamiento a -20°C, manteniendo la flexibilidad de las "zonas de unión" tras la congelación, incluso en el gel O150 *vs* O30.

En general, la influencia de la pasteurización se notó en la reducción de γ_{max} en los tres lotes (C1, O1 y S1) y, particularmente, en los lotes S1 y C1, en los que se incrementó la rigidez con respecto a los respectivos prototipos frescos (S0 y C0). Este hecho indica que el calentamiento asociado a la pasteurización, reforzó el acoplamiento intermolecular en la matriz pescado-GM, que se puede fortalecer durante el calentamiento por interacciones hidrofóbicas, formando *super-estructuras* más compactas (lotes S1 y C1), que son más rígidas (Renkema, 2004), tal y como se observa en los incrementos de las pendientes elásticas y viscosas. Este resultado es coherente con el mayor decrecimiento de la CRA de C1 *vs* C0.

El papel estabilizante de la sal (S1) se evidencia en el similar incremento del carácter elástico respecto al viscoso (menor que en el lote control) después de la pasteurización, y por tanto, la matriz pescado-GM, mantiene el mismo grado de viscoelasticidad que en el prototipo antes de la pasteurización (S0). Este resultado es consistente con el incremento de la rigidez (incremento de FR) en el lote S1 (ensayo de penetrometría mecánica). Asimismo, la estabilidad energética que aporta la sal a la matriz pescado-GM después de la pasteurización, también se vio reflejada en el incremento de DR después de la pasteurización.

Lo contrario ocurre en la muestra control (lote C1), donde el incremento de la propiedad viscosa (150%) es más alto que la elástica (104%), lo que incrementa la capacidad disipativa de la red debido a la pérdida de zonas de unión elásticamente activas (menor densidad de red) en el "entramado "agua-pescado-GM". Este hecho es consistente con la menor CRA (11%) del lote C1 vs C0, y podría relacionarse con la rotura de enlaces de hidrógeno en la estructura reticular.

El comportamiento reológico del lote O fue notablemente diferente a los otros dos, porque la pasteurización apenas alteró su componente elástica, en cambio redujo considerablemente la viscosa. Este comportamiento podría ser consecuencia del reordenamiento estructural asociado al calentamiento que podría mejorar la interacción de la emulsión (fase *filler*) y la matriz pescado-GM. Es razonable que, tras el calentamiento, se libere cierta cantidad de aceite retenido en los huecos de la red, lo que incrementaría la fluidez del gel, de ahí la disminución de la resistencia viscosa. Este hecho es consistente con el comportamiento de las propiedades mecánicas tras la pasteurización, en particular con el notable decrecimiento de FR y débil incremento de DR y podría evidenciar la capacidad

lubricante del aceite difundido por la red, tal y como se deduce de la notable reducción de la resistencia viscosa.

Con respecto al almacenamiento en estado refrigerado (1–21 días), después de ser pasteurizados los tres prototipos, en el gel S21 destaca el incremento de la componente elástica (35%) y mucho más acusado en la componente viscosa (125%). Estos resultados indican que se ha producido un refuerzo estructural irregular, desordenado y disipativo ya que se ha incrementado el carácter viscoso sobre el elástico de la red. Este hecho es coherente con el decrecimiento de DR en S21 *vs* S1, que expresa menor capacidad de plegamiento en la estructura (Renkema, 2004). Todo ello, en consonancia con el decrecimiento significativo ($p<0,05$) de CRA observado entre 1 y 21 días, ya que el agua en la red también podría actuar como "*cemento*", unida por enlaces de hidrógeno al resto de la matriz pescado–GM (Walstra, 2003). Si se pierde agua, se reduce el número de enlaces físicos intermoleculares, y en consecuencia, disminuye la capacidad de almacenar energía de la red (Sperling, 2001), tal y como se muestra en el incremento neto de la resistencia viscosa.

Sin embargo, en el lote C con el tiempo de refrigeración 1-21 días, se observó un débil refuerzo en la estructura (se incrementa débilmente la componente elástica y decrece la viscosa), ya que las bajas temperaturas fortalecen las interacciones polares, lo que ayudó a retener agua entre 1-21 días y a cohesionar la "red completa". Este hecho evidencia la parte de agua enlazada en la red al incrementarse el número de interacciones físicas, lo que también es coherente con la mayor pérdida de agua por cocción, ya que el calor rompe los enlaces de hidrógeno y debilita las interacciones dipolares (Damodaran y Paraf, 1997).

En el caso del lote O, el efecto del tiempo de refrigeración fue contrario al del lote C, tal vez porque la pérdida de aceite debido a la pasteurización alteró la estructura que, tras los 21 días de almacenamiento en estado refrigerado, sufre reformas sin pauta definida, por razón de proximidad. Como resultado se origina una red en O21 menos estable energéticamente, porque ha incrementado la componente viscosa (151%), en mayor proporción que la elástica (56%).

5. MICROESTRUCTURA

El estudio de la microestructura de los geles y los cambios que se producen en ella mediante microscopía electrónica de barrido (SEM) ofrece información acerca de los parámetros de textura (Stanley, Gill, deMan, & Tung, 1976).

Las micrografías de los geles con un 3% y un 5% (p/v) de GM, muestran redes bien definidas, esponjosas y con huecos homogéneamente distribuidos en la matriz. La microestructura de los geles de GM al 3% a 25 °C elaborados con KOH 0,6N fue más homogénea y menos densa que la elaborada con NaOH 1N, donde se originó una red más compacta, con una estructura más firme. El mayor empaquetamiento molecular se corresponde con el incremento de los módulos $G´$ y $G´´$ (espectros mecánicos) en los geles obtenidos con NaOH 1N respecto a los obtenidos con KOH 0,6N. Se observaron densos filamentos enredados en la estructura que origina una red más heterogénea ("hecha a trazos") y de menor elasticidad. Este hecho es consistente con el incremento del módulo G'', más acusado en el rango de bajas frecuencias a 0,1 Hz respecto al gel elaborado con KOH 0,6N.

Para los geles de GM al 3%, al aumentar el grado de desacetilación (del 75% al 95%) la estructura de la red se observó más continua y con mayor conectividad intermolecular, con los huecos distribuidos más homogéneamente, dando una estructura más esponjosa. La microestructura de los geles, pasa de tener unidades estructurales gruesas para los geles con menor grado de desacetilación (75%), a tener una estructura más continua y mejor definida cuando el grado de desacetilación aumenta (95%). Lo que podría relacionarse con un mayor grado de solidez (valores más altos de los módulos $G´$ y más bajos de G'' en todo el intervalo de frecuencias y con una mayor flexibilidad, porque a 3% GM el nivel de agregación reticular no es suficiente para "vitrificar" la red, de ahí el aumento de γ_{max} con el grado de desacetilación.

Del mismo modo, en el caso de los geles de GM al 5%, se observó una red más compacta cuando el grado de desacetilación es mayor (91%), encontrándose una microestructura con mayor asociación intermolecular entre cadenas de GM y, en consecuencia, con mayor densidad de red. Este empaquetamiento molecular podría originar el grosor suficiente en las "zonas de unión" para que se forme cierta estructura vítrea, dándole una peculiar rigidez a la estructura, que hace la red más frágil, tal y como se observa en el decrecimiento de γ_{max} al incrementar el porcentaje de desacetilación.

Cuando la red elaborada con KOH fue calentada a 90 °C, se observó una microestructura más compacta y densa, lo que se corresponde con el incremento notable del módulo $G´$ y la disminución simultánea de $G´´$ entre 25 y 90 °C a 1 Hz. Hecho que se traduce en un aumento neto del grado de elasticidad del gel, en concordancia con los huecos de menor tamaño observados en la microestructura, lo que favorecería la retención de agua por capilaridad resultado consistente con el incremento de la CRA observada. Cuando el álcali fue NaOH, el calentamiento de los geles produjo una microestructura más heterogénea e irregular que podría formar huecos más grandes, que reducirían la resistencia viscosa en la red, tal y como se desprende del notable decrecimiento de G'' observado a altas y bajas frecuencias a 90 °C respecto a 25 °C. Esta estructura más irregular tendría menor CRA a 90 °C.

6. ANÁLISIS MICROBIOLÓGICO Y SENSORIAL.

Además de análisis de las propiedades físico-químicas, mecánicas y reológicas de los reestructurados de pescado-GM se llevó a cabo un análisis microbiológico y sensorial de las muestras con sal y con aceite de pescado rico en ω-3, teniendo como control el reestructurado sin ingredientes adicionales.

Se parte de reestructurados de pescado con una carga microbiológica total de aerobios mesófilos inferior a $4x10^4$ ufc/g. Las recomendaciones oficiales españolas (RD 135/2010 BOE 25/02/2010) indican que los productos de la pesca frescos, refrigerados, y congelados no deben superar las 10^6 ufc/g de microorganismos aerobios mesófilos.

Los resultados mostraron que, como era de esperar, la pasteurización produjo una disminución notable del recuento de microorganismos totales (3 unidades logarítmicas) en todos los lotes. Estos conteos se mantuvieron prácticamente estables durante los 7 días de almacenamiento refrigerado en los tres lotes, pero en el día 21 los lotes C (C21) y O (O21) mostraron un crecimiento bacteriano inesperado, incrementándose en 5 unidades logarítmicas los recuentos de aerobios totales, siendo ya inaceptables ($5x10^6$ ufc/g). Sin embargo, en el lote S no es hasta los 35 días de almacenamiento cuando se produce el incremente en el recuento hasta 10^6 ufc/g. Esto refleja el papel limitante de la sal en el crecimiento microbiano.

Considerando el deterioro de todas las muestras después de 35 días de almacenamiento refrigerado, (C35, O35 y S35), se decidió realizar el análisis sensorial únicamente en las muestras de los tres lotes (C, O y S) a 1, 7, y 21 días de almacenamiento refrigerado después de la pasteurización y cocinadas a la plancha. Estas pruebas se realizaron según la norma UNE-ISO 6658 (ISO, 2008).

Se realizaron pruebas para determinar la aceptación del sabor y de la textura. En la prueba de aceptación del sabor de las muestras recién pasteurizadas (C1, O1 y S1), el lote S obtuvo una puntuación mayor (6,8 ±0,9 puntos sobre 9), seguido del lote C y por último el lote O que obtuvo una puntuación de (4.2±0.8). Así, los panelistas encontraron que el lote C1 estaba más seco que los lotes O1 y S1 y, por el contrario, el lote O1 gustó menos por ser demasiado aceitoso. Por otro lado, la muestra S fue catalogada como más sabrosa debido a la presencia de sal. Estos valores se mantuvieron aproximadamente iguales hasta el día 21 de almacenamiento refrigerado.

Las puntuaciones para la aceptabilidad de la textura fueron muy similares a las de la aceptabilidad del sabor, con la mejor valoración para el lote S1, y la puntuación más baja del lote O1. Además, los panelistas no encontraron cambios significativos en la aceptabilidad de la textura de ninguno de los lotes durante más de 21 días de almacenamiento refrigerado con respecto a las puntaciones otorgadas el día 1 después de la pasteurización.

Estos resultados indican que la adición de aceite no fue bien aceptada, probablemente porque los reordenamientos estructurales que se producen durante la pasteurización, y el almacenamiento refrigerado (anteriormente comentados), produjeron una sensación pastosa y una textura demasiado suave. Esta percepción es coherente con el hecho de que BF era más bajo en O1 que en cualquier otro lote, independientemente del tiempo. La sensación aceitosa de los productos pesqueros reestructurados también se reflejó en la disminución de CRAc, afectando negativamente su aceptabilidad. Por el contrario, el lote S obtuvo la mejor puntuación para ambos atributos (sabor y textura), lo que podría relacionarse con el papel estabilizador de la sal. Ésta, contribuiría a la formación de una red más cohesiva y compacta, lo que es consistente con el hecho de que FR fuera más alto en S1-S21 que en las otras muestras. Finalmente, la sequedad asociada con el lote C, especialmente justo después de la pasteurización (C1), podría estar relacionada con la mayor disminución de CRA y PAC en ese lote, y es consistente con el fortalecimiento termoinducido de la matriz de GM.

REFERENCIAS

Clark, A. H., & Ross-Murphy, S. B. (1987). Structural and mechanical properties of biopolymer gels. En Biopolymers (pp. 57-192). Springer.

Damodaran, S. (1997). Protein-stabilized foams and emulsions. Food Proteins and their Applications.New york: Inc.

Damodaran, S & paraf, A (1997). Food Proteins and their Applications.New york: Inc.

Hammer, G. F. (1992). Sustancias aditivas y aditivos. Tecnología de los embutidos escaldados. Editorial Acribia, Zaragoza.

Lapasin, R., & Pricl, S. (2013). Rheology of Industrial Polysaccharides Theory and Applications. Springer Verlag.

Leksrisompong, P. N., Lanier, T. C., & Foegeding, E. A. (2012). Effects of heating rate and pH on fracture and water-holding properties of globular protein gels as explained by micro-phase separation. Journal of food science, 77(2), E60-E67.

Nishinari, K., & Zhang, H. (2004). Recent advances in the understanding of heat set gelling polysaccharides. Trends in Food Science & Technology, 15(6), 305-312.

Park, J. W. (1996). Temperature-tolerant fish protein gels using konjac flour. Journal of Muscle Foods, 7(2), 165-174.

Pérez-Alvarez, J. A., Fernández-López, J., Sayas-Barberá, M. E., & Cartagena-García, R. (1998). Caracterización de los parámetros de color de diferentes materias primas usadas en la industria cárnica. Eurocarne, 63, 115-122.

Peréz-Alvarez, J. A., & Fernandez-Lopez, J. (2006). Chemistry and biochemistry of color in muscle foods. Food biochemistry and food processing, 337-350Renkema, J. M. S. (2004). Relations between rheological properties and network structure of soy protein gels. Food Hydrocolloids, 18(1), 39-47.

Schut, J. (1976). Meat emulsions. Food emulsions, 385-458.

Sperling, L. H. (2006). Introduction to physical polymer science (Vol. 78). Wiley Online Library.

Stanley, D. W., Gill, T. A., deMan, J. M., & Tung, M. A. (1976). Microstructure of rapeseed. Canadian Institute of Food Science and Technology Journal, 9(2), 54-60.

Tabilo-Munizaga, G., & Barbosa-Cánovas, G. V. (2004). Color and textural parameters of pressurized and heat-treated surimi gels as affected by potato starch and egg white. Food research international, 37(8), 767-775.

V.-CONCLUSIONES

Tras haber desarrollado el objetivo principal de la presente Memoria consistente en el **diseño y la obtención de productos reestructurados a base de músculo de pescado picado, utilizando glucomanano (GM) como gelificante**, es posible llegar a las siguientes conclusiones:

1. El glucomanano tiene una extraordinaria capacidad de formar geles termoestables mediante desacetilación alcalina utilizando KOH 0,6N.

2. Los geles de glucomanano elaborados con KOH 0,6N a partir de una dispersión acuosa del 3% y 5% de glucomanano, presentan una gran capacidad de retención de agua, bajas pérdidas de agua por cocción, y alta luminosidad. Estas características se mantienen después de los periodos de conservación en refrigeración.

3. Los geles elaborados con KOH 0,6N presentan propiedades mecánicas y viscoelásticas adecuadas para la reestructuración del músculo de pescado, finamente picado, con escasa funcionalidad.

4. Es posible obtener productos pesqueros reestructurados con características funcionales, aspecto de pescado fresco, y mínimamente procesados, mediante gelificación en frío del glucomanano con músculo de pescado picado en una proporción 25:75 (p/p).

5. La red tridimensional de gel correspondiente al prototipo, puso de manifiesto un adecuado acoplamiento intermolecular entre el polisacárido (glucomanano) y la proteína de pescado.

6. La adición de ingredientes adicionales como aceite de pescado rico en ω-3, permite la elaboración de reestructurados con carácter funcional.

7. La conservación en congelación a -20 °C durante al menos 150 días, de los prototipos reestructurados, preserva sus propiedades físicas, manteniendo el acoplamiento reticular establecido entre el GM y la proteína de pescado.

8. El proceso de pasterización (80 °C, 20min) permite almacenar en refrigeración los reestructurados de pescado-glucomanano hasta 21 días, conservando sus características físicas y organolépticas.

9. Con esta tecnología, es posible elaborar diversos prototipos de productos pesqueros con diferentes formatos: frescos, congelados, pasteurizados, marinados y/o ahumados.