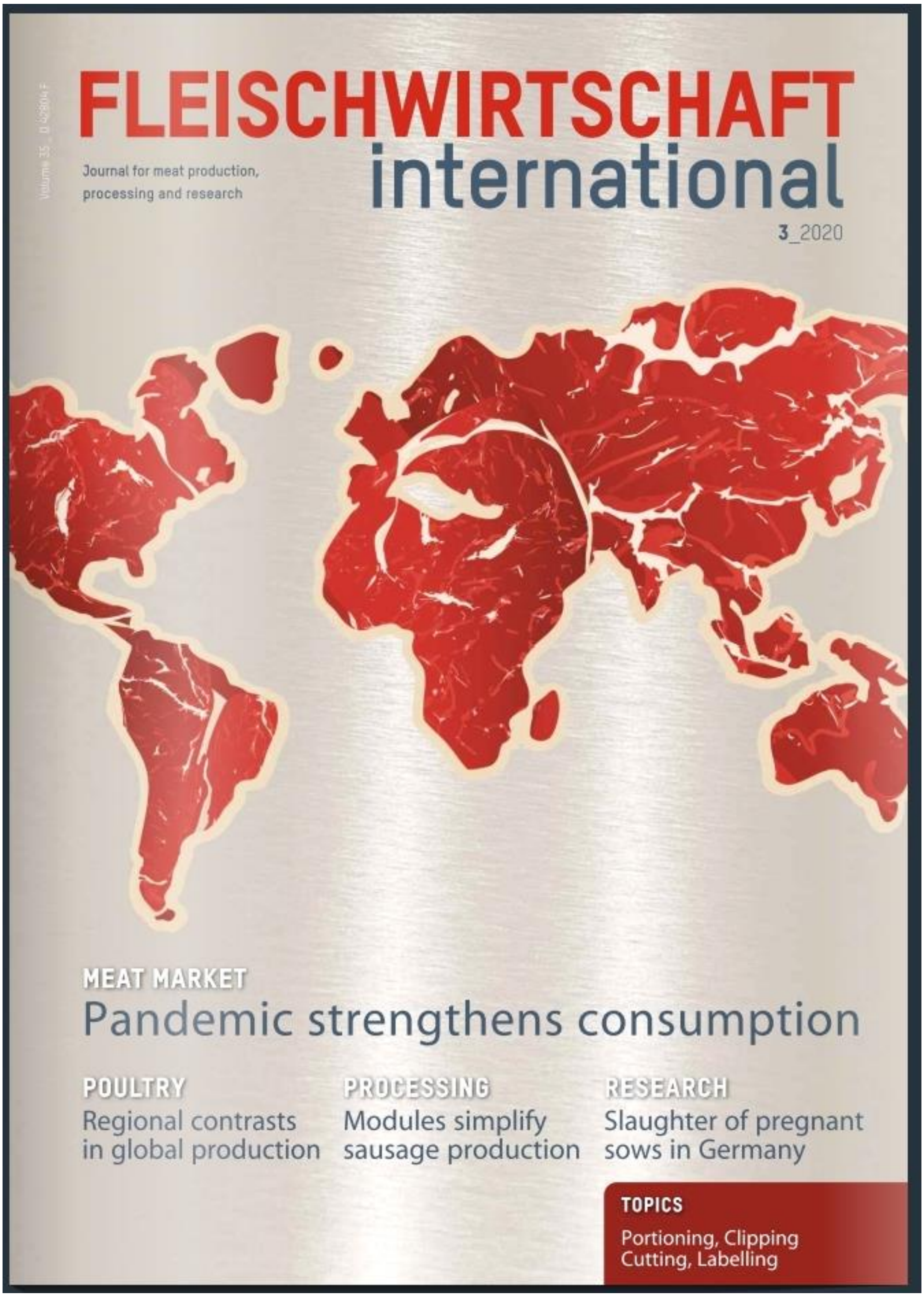
Montufar, K., Santos, C., Dublan, O., Selgas, M.D. and Fernández-León, M.F. (2020).Development of a ready-to-eat meat product. Investigations on rainbow trout (*Oncorhynchus mykiss*) meat and citrus fiber as functional ingredients. Fleischwirtschaft International, 3, 66-70.

https://english.fleischwirtschaft.de/digital-magazine/FLEISCHWIRTSCHAFT-international-32020



FLEISCHWIRTSCHAFT international 3_2020

Development of a ready-to-eat cooked meat product

Investigations on rainbow trout (*Oncorhynchus mykiss*) meat and citrus fiber as functional ingredients

By K. Montúfar, C. Santos, O. Dublán, M.D. Selgas and M.F. Fernández-León

The objective of the present study has been the development of new reduced-fat cooked sausages manufactured with rainbow trout-meat and citrus fiber as substitute for pork fat and as a source of PUFA n-3. The composition, technological and sensory properties and shelf life were studied. With the trout addition, a significant fat reduction (25–67%) was obtained as well as a change in the lipidic profile with an increase of polyunsaturated fatty acids in relation to conventional sausages. Although color and texture changes were detected instrumentally, the sausages obtained a high sensory evaluation being the odor the characteristic that received the lowest score. The newly cooked trout sausages were microbiological and sensorially stable during chilled storage with a shelf life of at least 45 days.

KEYWORDS

›› Rainbow trout
›› Cooked meat sausages
›› Citrus fiber
›› Polyunsaturated fatty acid

According to the WHO (2013), many of the non-communicable diseases (NCDs) can be reduced through diet; decreasing sodium, free sugars and fats are the essential objectives. An important component of processed meat products is fat, which may have a percentage of up to 30% in this products, due to its technological and sensory properties (Choi et al., 2010, 2014). However, not only can the amount of fat present health risks but also its content in saturated fatty acids and cholesterol that are associated with the NCDs (Choi et al., 2009; Choi et al., 2010). Therefore, there is an increasingly growing interest in the consumption of low-fat foods.

One of the most interesting technological strategies to manufacture most healthy foods is the improvement of the fatty acid profile. This implies the partial or total substitution of animal fat with other fats more in line with current nutritional guidelines, i.e. improving the polyunsaturated:saturated fatty acid or the PUFA n6:n3 ratio (Calder, 2006; Kolanowski et al., 2007; Harlioglu, 2012).

Soluble dietary fiber is one of the healthy ingredients that has received more scientific attention for reducing the fat content in meat products because its consumption has been demonstrated to have beneficial health effects (Verma and Banerjee, 2010; Li and Komarek, 2017). In this way, numerous insoluble and soluble fibers from cereals, vegetables or fruit have been successfully used in meat products (García et al., 2007; Viuda-Martos et al., 2010; Carvalho et al., 2019).

Worldwide fish consumption has gradually increased over the years and rainbow trout (*Oncorhynchus mykiss*) has become very popular because of its tender, less oily flesh and nutty flavor, and its nutritive value due to its PUFA content, especially n-3. It would therefore be interesting to study if the incorporation of trout can be technically feasible in order to make the lipidic profile of meat products healthier. The objective of the present work has been the development of new reduced-fat cooked sausages manufactured with rainbow trout-meat as a substitute of pork fat and as a source of PUFA n-3 as well as soluble fiber obtained from citrus fruit. The final composition, the technological and sensory properties as well as the shelf life have been studied.

This work supports the meat production from aquaculture species, which mixed with the meat pork would allow the manufacture of new healthier meat products, the consumption of which could be extended worldwide.

Material and methods

Sausage manufacture

Trout, beef meat and pork back fat were bought in the local market. The trouts were filleted and the thorns were removed. The fillets were kept at −18 °C until use.

Two batches were performed according to a Mortadella formula developed at the Chemistry Faculty of the Autónoma University (Mexico). One batch (TS) was manufactured with trout (%): 52.0 trout lean meat, 17.5 beef lean meat, 21 pork back fat, 7 ice and 2.5 of commercial mixture of spices and additives (Procavi, Arganda del Rey, Madrid). Another batch (TFS) was manufactured with a reduced pork back fat amount and citrus fiber was added. The amount of components was adjusted and the final formula was (%): 57 trout lean meat, 20 beef lean meat, 5.5 pork back fat, 13 ice, 1 citrus fiber and 3.5 of the mixture of spices and additives.

The citrus fiber (Vilher S.A., Minnesota, USA) contains about 33% of soluble dietary fiber. The fiber was added to the meat batters mixed with the spices. A control batch was also manufactured according to a commercial formula: 55% pork meat, 30% pork fat, 10% ice water and 5% of the spice mixture mentioned above. The cooked sausages were manufactured in duplicate according to the procedure of Soto et al. (2014). After the heat treatment, the sausages were sliced (2 mm thickness), vacuum-packaged and stored at 4 °C during 45 days.

Sausage composition

AOAC procedures (2011) were used to quantify protein, water and ash; the fat content was determined according to Hanson and Olley (1963). Carbohydrates were estimated by difference. Total calories (kcal) were calculated using the Atwater coefficients. The caloric value of fiber was considered to be 3 kcal per g (manufacturer information). Energy values (kJ) were calculated as 4.184 kJ×kcal (FAO, 2003).

Physico-chemical analysis

The water activity and pH were determined according to Soto et al. (2016). The carotenoid content was determined using organic solvents (acetone:hexane 4:6, v:v) (El-Hadidy and Salam, 2017) and the optical density of the extracts was measured in a spectrophotometer at different wavelengths (663, 645, 505 and 453 nm) (Beckman Coulter DU, Nyon, Switzerland). The carotenoid amount was estimated according to the Nagata and Yamashita (1992) equations. Six determinations were performed on every batch.

Instrumental analysis

Color measurements were performed using a tristimulus colorimeter (Minolta Chroma Meter Mod. CR-400 Minolta Co. Osaka, Japan) using the color space CIE L*a*b*. The determined parameters were L*, a*, b*, Hue Angle (tonality) and Saturation Index (Chroma). Measurements were performed

Received: 6 January 2020 | reviewed: 3 July 2020 | revised: 7 July 2020 | accepted: 7 July 2020

using eighteen slices for each batch, at room temperature, a D-65 illuminant source and fresh-cut.

A Textural Profile Analysis (TPA) was performed using a Stable Micro System texturometer (Mod. TA.XT 2i/25, Stable Micro System, London). For this purpose, the central cores (1 cm high and 2.5 cm in diameter) of ten slices of each batch were used, which were compressed twice to 50% of their original height. Hardness, cohesiveness, springiness, adhesiveness and chewiness were determined.

Fatty acids quantification

The fat extraction was performed with chloroform/methanol and methyl esters of fatty acids according to Calvo et al. (2017). The identification and quantification were performed by chromatography in gas phase according to Hoz et al. (2004), using a Konik gas chromatograph (KNK-3000-HRGC) with a flame ionization detector. Four determinations were performed on each batch.

Microbiological analysis

Total viable counts were determined during storage. For this purpose, 10 g of each batch were homogenized with 90 ml of sterile physiological serum, serially diluted, plated on Plate Count Agar (Oxoid, Basingstoke, UK) and incubated at 32 °C for 48 h. Microbial analyses were carried out in triplicate in each batch and day of sampling.

Sensory analysis

Forty untrained assessors were selected according to their habits, acquaintance with the sausages to be analyzed and their sensitivity together with the ability to accurately reproduce the evaluation of the samples. A hedonic test was carried out using a non-structured 10 point scale (0 = dislike extremely and 10 = like extremely) to rate odor, color, taste, texture and overall acceptability. Two sessions per day were conducted in individual booths under white lights. Two slices of each sample (2 mm high approximately) were served to the assessors at room temperature. Unsalted crackers and water were also provided to rinse the palate between samples.

Statistical analysis

For statistical studies IBM SPSS Statistics TS, Version 22, software was used (SPSS Inc. Chicago, IL, USA, 2013). Data were expressed as means ±SD. Mean values were analyzed by Student's test at $p \leq 0.05$.

Results and discussion

Sausage composition

The proximate composition of different batches is shown in Table 1. The data obtained reflect the changes in the sausage formulations. Higher protein and water content were observed in trout added batches which could be a consequence of the trout proteins and their higher water content as well as the amount of ice added. Similar results have been reported in cooked sausages manufactured with different fish species (Chuapoehuk et al., 2001; Izquierdo, 2007; Dinçer and Çakly, 2010).

The fat reduction was significant ($p \leq 0.05$) in TS and TFS batches in relation to the control, and it was close to 25.7% and 66.9% respectively; consequently, the energy value decreased by 17.41% in TS and 44.55% in TFS. No significant differences were observed in carbohydrates and ash content. The fat reduction is in line with those of other authors. Choi et al. (2016) obtained up to 30.34% less energy in reduced-fat chicken sausages enriched with apple fiber. Adibelli and Serdaroglu (2017) reported a 35% reduction in energy values in frankfurters formulated with apricot pomace fiber. Al-Bulushi et al. (2013) also observed a significant fat reduction, close to 30%, in cooked sausages manufactured with crimson snapper.

Physico-chemical properties

The a_w values were similar in TS and TFS batches (0.987±0.03 and 0.984±0.001, respectively) and significantly different to the control (0.968±0.04). pH values were 6.60±0.18 for control, and 6.75±0.08 for TS batches and, in the TFS ones, the values decreased slightly (6.55±0.10), probably due to the pH of the citrus fiber which ranged between 3.28-3.91 (manufacturer communication). Viuda-Martos et al. (2010) observed a similar pH decrease in conventional cooked sausages with substitution of fat with orange fiber and essential oils.

The carotenoid content was similar in TS and TFS batches (2.82±0.14 and 2.98±0.63 µg/g, respectively) and no carotenoids were detected in the control batch. It is known that the characteristic pink trout meat color is related to the deposited carotenoids, mainly astaxanthin, coming from feed because the trout is unable to synthesize them (Büyükçapar et al., 2007). Rahman et al. (2016) reported that the salmon color appears in rainbow trout with a diet supplemented only with 0.05% of a commercial supplement containing 10% of astaxanthin. The carotenoid content of the fiber had minimal influence. The physico-chemical parameters were similar at the end of storage.

Color

Color measurement is an important parameter in cooked sausages because a bright and characteristic pink color has a better association for consumers (Dinçer and Çakly, 2010). Significant differences were observed in the color parameters of the three batches (Tab. 2). Lightness was higher in TS and TFS batches than in the control. These results disagree with Cáceres et al. (2006) and García et al. (2007) who indicated that L* decreased along with fat reduction in cooked sausages. In our work, the difference observed can be a consequence of the higher water content in the trout meat added to these batches. Differences were also observed between TS and TFS batches with TFS showing the lower value.

The highest redness was detected in the control batch and it was also significant different between TS and TFS batches with TS showing the lower value. This could be due to the different spice content more than to the carotenoid content. This result could also be due to the reactions between the nitrite and the polyphenols of the citrus fiber which favor the nitrosomyoglobin formation as Fernández-López et al. (2008) reported. Özvural et al. (2009) also described that parameter a* increased in Frankfurters with added spent barley fiber. Consequently, the citrus fiber content can also influence the a* parameter in TFS.

Composition

Tab.1: Proximate composition of experimental cooked sausages (%)

Batches	Moisture	Protein	Fat	Carbohydrates	Ash	Energy value (kj)
Control	56.91±0.40[c]	11.85±0.17[c]	22.12±3.80[a]	7.10±0.51[a]	1.59±0.50[a]	1150.51[a]
TS	62.79±1.19[b]	12.30±1.11[b]	16.53±1.40[b]	6.89±0.66[b]	1.50±0.06[b]	950.31[b]
TFS	69.23±0.37[a]	13.73±0.56[a]	7.32±0.47[c]	8.11±1.29[a]	1.61±0.14[a]	637.85[c]

Data are mean±standard deviation; Different letters in the same column indicate significant differences (p≤0.005)

Source: Selgas et al. FLEISCHWIRTSCHAFT international 3_2020

Color indices

Tab. 2: Color parameters (CIEL*a*b*) of experimental sausages

Batches	L*	a*	b*	Hue Angle	Saturation index
Control	65.86±0.46[b]	13.49±0.24[a]	9.20±0.29[b]	34.25±1.17[c]	18.15±1.92[c]
TS	78.44±0.51[a]	8.04±0.23[c]	17. 17±0.44[a]	64.81±2.31[a]	34.35±1.16[a]
TFS	71.56±0.52[b]	l l.09±0.05[b]	16.35±0.29[a]	55.77±2.11[b]	29.56±0.96[b]

L*: 0 = black, 00 = white; a*: –60 = green, +60 = red; b*: –60 = blue, +60 = yellow;
Hue Angle calculated as tan-1(b*/a*); Saturation Index calculated as (a*2+ b*2)[0.5]
Data are mean±standard deviation; Different letters in the same column indicate significant differences (p≤0.005)

Source: Selgas et al. FLEISCHWIRTSCHAFT international 3_2020

Texture

Tab. 3: Textural properties of the experimental sausages

Batches	Hardness (N)	Cohesiveness (ratio)	Springiness (cm)	Adhesiveness (N s)	Chewiness (N cm)
Control	42.25±3.00[a]	0.63±0.03[a]	0.65±0.01[b]	–0.28±0.11[b]	17.30±1.31[b]
TS	27.79±1.19[b]	0.69±0.08[a]	0.82±0.27[a]	–0.42±0.30[a]	15.72±0.60[b]
TFS	42.69±1.65[a]	0.64±0.13[a]	0.81±0.30[a]	–0.48±0.12[a]	22.13±1.25[a]

Data are mean±standard deviation; Different letters in the same column indicate significant differences (p≤0.005)

Source: Selgas et al. FLEISCHWIRTSCHAFT international 3_2020

The yellowness was very similar in TS and TFS batches and both differed significantly (p≤0.05) from the control batch. It is possible that the carotenoid content has a higher influence in this parameter than in redness. The carotenoid content is responsible for the color of the trout meat. Remember that salmon color is a mixture of white, pink and orange, the latter being the predominant color (ColorMine.org, 2019) and, consequently, it can be the responsible for the b* increase. The results agree with López-Vargas et al. (2013) and Fernández-López et al. (2007) who indicated that the color changes also depend on the meat type used. In relation to the possible influence of the citrus fiber, Yilmaz and Dağlioğlu (2003) reported that the b* parameter increased in meatballs with added oat bran and García et al. (2002) described a similar finding when adding orange fiber in reduced-fat cooked sausages. However, our results are in disagreement with those authors and it could be due to the low amount of fiber added, only 1%. No significant differences in color parameters were observed during 45 days of storage.

Texture profile analysis

The main difference was observed in hardness which was significantly lower in the TS batch (Tab. 3) than in the TSF or control batches. These results indicate that trout meat makes the final sausage softer than the control, but the presence of fiber may be able to reverse this change. Cardoso et al. (2008) and Dinçer and Çakly (2010) also reported a softening in sausages manufactured with South African hake and rainbow trout fish, respectively. However, Moreira et al. (2002) reported that in emulsified sausages manufactured with fish fillets, the characteristics of the final product depends on the type of fish added. Chewiness behaves as hardness because it is a secondary parameter depending on it. Springiness and adhesiveness were similar in the batches of added trout and both differ significantly from the control one. This could be due to the high water amount, which is a food component that influences these parameters.

Several authors have reported the influence of the dietary fiber on the texture of cooked sausages such as Özvural et al. (2009) for Frankfurters with added barley fiber or Fernández-Ginés et al. (2003) for Bologna sausages with added citrus fiber. Cofrades et al. (2000) reported that probably the soluble fiber interacts with proteins and fat yielding a more stable network that modifies the texture of the meat product.

The fiber amount added also has an influence on texture. García et al. (2007) reported the use of 1.5–3% of orange fiber in reduced-fat cooked sausages as adequate, and Viuda-Martos et al. (2010) 0.5-1.0% of citrus fiber in bologna sausages. In our work, only a 1% citrus fiber was used. No changes on textural properties during storage were observed, which is in agreement with Fernández-Ginés et al. (2003) who reported that time and storage conditions did not change the texture properties of bologna sausages with added citrus fiber.

Quantification of fatty acids

The results are shown in Table 4. Although it has been described that factors such as size, age, water quality, region or season have an influence on the trout fat composition (Danabas, 2011; Vranić et al., 2011), the predominant FA in rainbow trout are always unsaturated fatty acids (UFA). In the rainbow trout used in this work, monounsaturated fatty acids (MUFA) constituted the majority UFA fraction (62.86%), with oleic acid being the predominant one (45.85% of the total MUFA). Polyunsaturated acids (PUFA), constituted 37.14% of the UFA fraction. The fatty acid profile in the TS and TFS batches seems to be directly related to the meat trout added.

The SFA and UFA content was very similar in the three batches (p≥0.05) but the UFA profile changed. So, in the batches with trout, the MUFA content decreased significantly in relation to the control one while that of PUFA was significantly higher. The PUFA n-6 content was similar in the three batches but the PUFA n-3 increased in TS and TFS with a significant difference in relation to the control batch and they are, consequently, responsible for the observed change in the PUFA fraction.

As a consequence, the n-6:n-3 ratio fell from values from 9.3 in the control batch to 5.2 and 5.6 in the TS and TFS ones, respectively. EFSA (2010) proposes not to set specific values for the n-6:n-3 ratio as available data are insufficient to recommend a ratio independent of absolute levels of intake which should be the subject of nutrient and/or food based dietary guidelines. However our data agree with the German-Austrian-Swiss rec-

Fatty acids

Tab. 4: Fatty acid composition (%) of the experimental batches

Fatty acids	Control	TS	TFS	Trout
SFA	44.81±1.35[a]	43.44±1.96[a]	41.38±3.76[a]	19.63±0.21
UFA	56.19±1.40[a]	56.56±1.01[a]	58.53±2.01[a]	80.37±0.85
MUFA*	89.69± 1.92[a]	73.08±0.49[b]	68.44±1.35[c]	62.86±0.53
PUFA*	15.27±0.65[b]	26.92±2.46[a]	31.54±5.11[a]	37.14±0.31
n-6**	86.25±2. 84[a]	83.24±3.02[a]	84.82±5.94[a]	58.29±0.22
n-3**	9.27±0.08[b]	16.06±0.61[a]	15.12±0.75[a]	20.06±0.08
n-6:n-3	9.3	5.2	5.6	2.9

SFA: Saturated fatty acids; UFA: Unsaturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; * Expressed as percentage of UFA **Expressed as percentage of PUFA;
Data are mean±standard deviation; Different letters in the same column indicate significant differences (p≤0.005)

Source: Selgas et al. FLEISCHWIRTSCHAFT international 3_2020

Sensory

Tab. 5: Sensory properties of the experimental batches

Batches	Odor	Color	Texture	Taste	Overall acceptability
Control	7.12±0.82[a]	7.47±1.42[a]	7.65±2.24[a]	7.65±0.97[a]	7.33±1.10[a]
TS	6.32±0.26[b]	7.51±0.19[a]	7.50±0.23[a]	6.24±0.25[a]	6.49±0.23[b]
TFS	6.07±0.46[b]	7.28±0.45[a]	7.82±0.46[a]	6.18±0.49[b]	6.30±0.42[b]

Data are mean±standard deviation; Different letters in the same column indicate significant differences (p≤0.005)

Source: Selgas et al. FLEISCHWIRTSCHAFT international 3_2020

ommendations (D-A-CH, 2008) that recommend a ratio of linoleic acid (n-6) to alpha-linolenic acid (n-3) of 5:1.

Mesophilic aerobic content

The microbial counts of the three batches evolved in a similar way during the storage and no significant differences were observed (data not shown). The initial values 10^1-10^2 CFU/g at Day 0 increased to counts between 10^5-10^6 CFU/g after 15 days of storage. From this day, the counts were kept at the same levels until the end of storage (45 days). The mesophilic microbiota is derived from the manipulation associated with the slicing and packaging operations. These results indicate that the trout addition does not have an influence on the microbial load of the sliced product. Patsias et al. (2006), Nowak et al. (2007) and Feng et al. (2013) described similar results in different types of cooked sausages. Nevertheless, the Commission Regulation (EC) No 2073/2005 established a maximum load of 10^6 CFU/g as a limit of security. Our experimental sausages are below these values and they would have a shelf life of at least 45 days refrigeration.

Sensorial analysis

The results obtained on the sensory properties are shown in Table 5. In general terms, and independently of the data obtained in the instrumental assays, the scores awarded by the panelists to the color and texture were very similar (p≤0.05) in the three experimental sausages.

On the contrary, significant differences were observed in the odor and taste of the TS and TSF samples when they were compared with the control batch, the latter being the one obtaining the highest scores (p≤0.05). This may be related to the presence of meat trout that could give a fishy smell. This fact is also reflected in the overall acceptability. Nevertheless, the scores achieved in the three batches were higher than 5, the medium value of 10 points of the hedonic scale, which indicates that the panelists notice a change in both sensory parameters, but they do not reject the product. In this way, Chuapoehuk et al. (2001) indicated that sausages manufactured with catfish meat and surimi had a high acceptability when prepared with 10% pork fat. Cortez-Vega et al. (2013) also reported a good acceptability in frankfurters containing mechanically deboned chicken meat mixed with surimi.

It is well known that fiber is a good fat replacer but, as it has been mentioned above, its effect on the sensory properties depends on the amount added. In our study, the addition of only a 1% of citrus fiber does not significantly change the sensory properties. Therefore, the manufacture of meat products using meat of aquatic origin and fiber could be an alternative for the consumption of healthy meat products.

Conclusion

The mixture of skinless trout, citrus fiber, pork back fat and lean beef can be used to obtain new healthier cooked sausages. They have a better nutritional profile compared to conventional sausages because of a reduction of the energy value and a more recommended polyunsaturated fatty acid content, without sacrificing their sensory quality and shelf-life.

Acknowledgements

Postgraduate grant of Consejo Nacional de Ciencia y Tecnología (CONACYT), México. Research Group Complutense University N° 920276 (Ref. GR3/14), Spain.

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