

Inulin extraction from common inulin-containing plant sources

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ABSTRACT

Currently there is a growing interest from the food industry in obtaining inulin for its possible use in the elaboration of functional foods. A set of optimum extraction conditions was developed for the recovery of inulin plus fructo-oligosaccharides (FOS) from several common inulin-containing plant sources, like chicory roots (*Cichorium intybus* L.) and Jerusalem artichoke tubers (*Helianthus tuberosus* L.), as well as from novel sources like globe artichoke inflorescence (*Cynara cardunculus* L.) and its by-product. Optimal conditions for temperature (60–80 °C), time (20–60 min) and solvent to solid ratio (10–40 mL/g) were estimated in order to maximize inulin plus FOS extraction by using response surface methodology (RSM) with a Box-Behnken design. Inulin plus FOS were estimated colorimetrically by difference between total carbohydrate and reducing sugar contents for the optimization. Moreover, the profile of inulin and low molecular weight carbohydrates was studied in optimized plant extracts by HPLC. Inulin in raw samples and optimal extracts were further characterized by Fourier Transformed Infrared spectroscopy. According to response surface methodology model, optimal conditions for inulin plus FOS extraction depended on plant source and were achieved at a solvent to solid ratio of 27.8–37.4 mL/g, from 62–80 °C and a variable time of 22–60 min. The highest inulin plus FOS contents were achieved in chicory root (70.5 g/100 g dry weight) and Jerusalem artichoke tuber (81.1 g/100 g dry weight), and the lowest ones were attained in globe artichoke by-product (4.2 g/100 g). Nevertheless, its high availability and low cost would support this novel globe artichoke by-product as an alternative and valuable source of inulin and FOS for the food industry. At the same time their reuse as potential prebiotic ingredients would contribute to the circular economy.

1. Introduction

Inulin serves as a storage polymer in many plants of the *Asteraceae* (ex *Compositae*) family such as globe artichoke (*Cynara cardunculus* L. subsp. *scolymus* L. Hayek, formerly *Cynara scolymus* L.), chicory (*Cichorium intybus* L.), Jerusalem artichoke (*Helianthus tuberosus* L.), and elecampane (*Inula helenium*) (Apolinário et al., 2017; Barclay et al., 2012; Braz de Oliveira et al., 2011; Lingyun et al., 2007). Generally, inulin is known to have linear chains incorporating 2–60 fructose units linked by β -(2,1)-fructosyl-fructose bonds, with chain length and polydispersity depending on the plant species, maturity degree and

extraction process used (Guimaraes et al., 2018). This wide range of chain length makes inulin useful for a number of applications in different fields, most predominantly the food and pharmaceutical industries. Thus, inulin was used as a substitute for sucrose or fat (Paciulli et al., 2020; Leddomado et al., 2021) and as a prebiotic which influences the host by selectively stimulating the growth and/or activity of some microorganisms in the colon (Gibson et al., 2017).

Most of the commercially available inulin comes from chicory roots, the first major crop used for its industrial production (Apolinário et al., 2017), in which inulin content ranges from 42–76 g/100 g dw (Shoaib et al., 2016). Jerusalem artichoke tubers could also store high levels of

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inulin and fructo-oligosaccharides (FOS), amounting to 45–75,0 g/100 g dw (Judprasong et al., 2018; Singh et al., 2019).

There is also variation in the degree of polymerization (DP) among different plants, thus chicory root has 2–60 DP and Jerusalem artichoke 2–50 DP (Li et al., 2015; Meyer and Blaauwheod, 2009). Degree of polymerization can have an influence in the prebiotic effect and other health related properties of inulin and FOS (Li et al., 2015; Márquez-Aguirre et al., 2016). Considering that inulin recovery and degree of polymerization can be affected by the extraction process, the search to optimize the conditions from chicory roots and other inulin-rich crops is still under development and several procedures reported so far, mainly using water as extracting solvent. Thus, inulin from chicory roots was extracted with water at 80 °C for 1 h with constant shaking (Toneli et al., 2008). Other authors (Saengthongpinit and Sajjaanantakul, 2005) also used water at 80 °C for 1 h to extract inulin from Jerusalem artichoke tubers, followed by alcohol precipitation.

Besides, others (Laurenzo et al., 1999) use boiling water but for even less time (10–15 min) and (Lingyun et al., 2007) reported that the optimum conditions to achieve the highest recovery from this material are almost the same temperature (76.65 °C) but for a shorter time (20 min).

Globe artichoke also contains inulin as storage carbohydrate and it is cultivated principally in the Mediterranean basin, being Italy and Spain the main producers and consumers of artichoke. The amount of inulin in globe artichoke core ranges from 2–10 g/100 g dw with a wide polydispersity of 2–250 DP (Meyer and Blaauwheod, 2009; Singh et al., 2019). Its processing into parts is currently generating a large amount of waste, thus up to 77 % by mass of the whole globe artichoke is discarded by the food industry (Claus et al., 2015). Both, the edible part of artichoke inflorescence -known as core, head, receptacle or capitulum- and its by-products formed by the external bracts, stalks, and leaves, containing many useful bioactive compounds known for their beneficial effects in the human body (Lattanzio et al., 2009). Globe artichoke by-product from the canning industry is reported to contain 10 g/100 g dw inulin (Ruiz-Cano et al., 2014).

Wastes from globe artichoke are currently discarded by the food industry. In this regard, globe artichoke core, and more especially its main by-product, could represent a potential alternative source for inulin extraction, because of its cheaper cost and higher availability with a huge potential to make value-added products.

Previous works on the extraction process of inulin from different plant sources, reported that the most important factors affecting inulin recovery and quality are temperature, time, and the ratio of solvent volume to solid (Lingyun et al., 2007; Khuenpet et al., 2017; Ruiz-Aceituno et al., 2016; Rubel et al., 2018).

Therefore, the main aim of this work was to develop a set of optimum conditions for inulin extraction from several inulin-containing plants, including the most traditional sources such as chicory roots and Jerusalem artichoke tubers, as well as an alternative and novel source of inulin such as the by-product of Spanish globe artichoke. Variables such as temperature and time extraction, and solvent to solid ratio were optimized for the extraction of inulin plus FOS by using Response Surface Methodology (RSM) with a Box-Behnken design.

2. Materials and methods

2.1. Materials

Chicory root (*Cichorium intybus* L.) var. “orchies”, was collected in Gomezserracin, Segovia, Spain. Jerusalem artichoke tubers (*Helianthus tuberosus* L.) var. “rosa”, were acquired at a local market. Roots and tubers were cleaned and peeled. Globe artichoke (*Cynara cardunculus* L.) var. Blanca de Tudela was kindly provided by “Alcachofas de España” (Murcia, Spain). Inflorescences from fresh globe artichoke were manually separated into two parts, the edible one composed by the inner and tender bracts of the core (also named receptacle, head or capitulum), and the non-edible one formed by the outer and harder bracts and stalks

that constitute the by-product. All samples were sliced, freeze-dried (Telstar model Lyo Quest freeze-dryer) and milled to pass through a 1 mm sieve. Freeze-dried samples, with approximately 0.2 % residual moisture content, were stored for further use in a dry container, protected from direct light at room temperature.

Commercial standard of Inulin from chicory root (Sigma-Aldrich I-2255) was obtained from Sigma-Aldrich Chemicals (Alcobendas, Madrid, Spain). Other commercial standards such as Inulin (Raftiline® HP-Gel) and Oligofructose (Raftilose® P95) were provided by ORAFTI España S.L. (Barcelona, Spain). Stachyose, raffinose, sucrose, fructose, glucose, and 3,5-dinitrosalicylic acid (DNS) reagent were obtained from Sigma-Aldrich (USA). Cellobiose was purchased from Merck (Germany). Anthrone reagent was supplied by Panreac (Spain). All other reagents were of analytical grade.

2.2. Inulin plus FOS extraction

2.2.1. Procedure

The extraction process was carried out under different conditions of temperature, time, and ratio of solvent to solid according to the below experimental design. In brief, one gram of powdered freeze-dried samples was weighed into 50 ml falcon tubes and different volumes of water (10–40 mL) were added as extracting solvent. Extraction was performed at 60 °C–80 °C for 20–60 min. After each extraction, the tubes containing the samples were allowed to cool down at room temperature and then centrifuged at 3300 x g for 15 min to remove the insoluble residue. Supernatant was filtered through Whatman cellulose filter paper (3 mm). The filtered solution was then concentrated in a rotatory evaporator at 45 °C and then adjusted to 25 ml final volume. An aliquot of each sample extract was freeze-dried to calculate recovery percentage. The remaining extracts were stored at –18 °C until further analyses were performed.

2.2.2. Experimental design

The inulin plus FOS extraction from common inulin-containing plant sources was optimized by RSM using a Box-Behnken design (number of central points = 3). Temperature (X_1), time (X_2), and solvent to solid ratio (X_3) were included as independent variables using the following values: X_1 from 60 to 80 °C; X_2 from 20 to 60 min and X_3 from 10:1 to 40:1 ml/g (Table 1) and inulin plus FOS (Y) used as a dependent variable (Table 2). Experimental ranges for the factors were based on previous reports on inulin extraction from different plant sources (Lingyun et al., 2007; Khuenpet et al., 2017; Rubel et al., 2018; Ruiz-Aceituno et al., 2016).

A second order polynomial was used for the mathematical modeling of the relationship between the dependent and independent variables (Eq. 1). The inulin plus FOS content at optimal conditions, estimated using second order polynomials, was validated experimentally. The RSM experiments were designed and analysed using a trial Design Expert software (Stat-Ease, Inc., USA).

$$Y = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i=2}^3 \sum_{j=1+1}^3 \beta_{ij} X_i X_j + \varepsilon \quad (1)$$

Table 1

Real and coded values of independent variables in response surface models.

Independent variables	Real values	Coded values
X_1 : Temperature (°C)	60	–1
	70	0
	80	+1
	20	–1
X_2 : Time (min)	40	0
	60	+1
	10:1	–1
	25:1	0
X_3 : Solvent to solid ratio (v/w)	40:1	+1

Table 2

Box-Behnken design to optimize inulin extraction from plant material.

Source	Chicory root				Jerusalem artichoke tuber				Globe artichoke by-product				Globe artichoke core			
	SS	MS	F-value	P-value	SS	MS	F-value	P-value	SS	MS	F-value	P-value	SS	MS	F-value	P-value
Model	223.0	24.8	143.3	<0.0001	1546.0	220.9	20.3	0.0004	13.2	2.6	12.9	0.0007	102.8	20.6	14.9	0.0004
X ₁ -Temperature	27.3	27.3	157.9	<0.0001	524.6	524.6	48.2	0.0002	4.4	4.4	21.6	0.0012	4.9	4.9	3.6	0.0906
X ₂ -Time	39.9	39.9	230.9	<0.0001	119.3	119.3	11.0	0.0129	0.7	0.7	3.3	0.1008	0.6	0.6	0.4	0.5278
X ₃ -Solvent to solid ratio	61.5	61.5	356.0	<0.0001	605.0	605.0	55.6	0.0001	1.8	1.8	9.0	0.0151	65.2	65.2	47.3	<0.0001
X ₁ X ₂	3.1	3.1	17.7	0.0084	66.7	66.7	6.1	0.0425								
X ₁ X ₃	24.5	24.5	141.7	<0.0001	110.3	110.3	10.1	0.0154								
X ₂ X ₃	14.9	14.9	86.0	0.0002												
X ₁ ²	1.4	1.4	7.9	0.0377	73.8	73.8	6.8	0.0352	5.8	5.8	28.3	0.0005	16.5	16.5	12.0	0.0072
X ₂ ²	21.8	21.8	126.2	<0.0001												
X ₃ ²	24.8	24.8	143.2	<0.0001	54.8	54.8	5.0	0.0598	0.7	0.7	3.3	0.1039	13.4	13.4	9.7	0.0124
Residual	0.9	0.2			76.2	10.9			1.8	0.2			12.4	1.4		
Lack of Fit	0.7	0.2	2.1	0.3371	65.0	13.0	2.3	0.3281	1.8	0.3	9.2	0.1020	4.8	0.7	0.2	0.9641
Pure Error	0.2	0.1			11.2	5.6			0.06	0.03			7.6	3.8		
R ²			0.9961				0.9530				0.8772				0.8924	
Adjusted R ²			0.9892				0.9060				0.8090				0.8327	
Predicted R ²			0.9509				0.7590				0.7558				0.7558	

where Y was response or dependent variable, X₁, X₂, and X₃ were independent variables, while β_0 , β_i , β_{ii} and β_{ij} are the intercept, linear, quadratic, and interaction coefficients, respectively. The residual is denoted as ϵ .

2.3. Total carbohydrate, reducing sugar and inulin content by colorimetric methods

Total carbohydrates and reducing sugars were determined in raw materials and extracts. Total carbohydrate content was determined at 630 nm according to the anthrone method (AOAC, 2007), using a reference curve ($y = 0.0359 + 7.71x$; $r^2 = 0.9963$) with fructose as a standard (0–100 µg/mL). Reducing sugars were quantified at 540 nm by the dinitro-salicylic acid method as described formerly (Miller, 1959), using a reference curve ($y = -0.0078 + 0.136x$; $r^2 = 0.996$) with fructose as a standard (0–2 mg/mL). Both colorimetric methods were miniaturized and adapted to a Synergy HTX Absorbance Microplate Reader (BioTek Co., USA). In order to optimize inulin extraction, the amount of inulin plus FOS was estimated by difference between total carbohydrate and free reducing sugar contents (Apolinário et al., 2014). Soluble solids obtained during the extraction process were freeze-dried and then weighed to calculate recovery (%).

2.4. Inulin and low molecular weight carbohydrates by HPLC

Identification and quantification of inulin and low molecular weight carbohydrates (LMWC) in the plant extracts obtained under optimized conditions was conducted by liquid-chromatography analysis using an Agilent 1100 Series HPLC, equipped with a refractive index detector (RID) on a Rezex™ ROA-Organic Acid H+ (8%), 300 mm x 7.8 mm column, protected with a Carbo-H 4 x 3.0 mm ID security guard cartridge (Phenomenex España, Madrid, Spain). Ultrapure Milli-Q water (Milli-Q Integral 5 Water Purification System from Millipore) acidified with 2.5 mM H₂SO₄ was used as mobile phase, at a flow rate of 0.4 mL/min. The column was maintained in a thermostatic oven at a constant temperature of 25 °C. Both standards and samples were filtered through 0.45 µm syringe filters for aqueous solutions and 5 µL volume injected into the HPLC. Inulin and different low molecular weight carbohydrate standards (DP4= stachyose, DP3= raffinose, DP2= cellobiose, DP1= sucrose, DP1= fructose, and glucose) were injected in triplicate at various concentrations (1.0, 0.5 and 0.25 mg/mL) and used for calibration. Sample extracts were diluted in ultrapure water (5 mg/mL), filtered and then injected. Regression standard curves were obtained for concentration versus area (mV x s). Peaks in chromatograms of samples

were identified by coincidence of their retention times with available LMWC standards and they were quantified by comparison of their areas with the corresponding calibration curves (Condezo-Hoyos et al., 2015).

2.5. FTIR-ATR spectra acquisition

The method for acquisition of Fourier Transform Infrared spectra with an attenuated total reflectance sampling accessory (ATR) was previously reported (Gómez-Ordóñez and Rupérez, 2011). Briefly, the FTIR spectra of freeze-dried and ground raw samples, optimized inulin extracts, and inulin standards were recorded using the PerkinElmer® Spectrum™ 400 FT-IR/NIR spectrometer (Perkin Elmer Inc., Tres Cantos, Madrid, Spain) in mid-IR mode, equipped with a Universal ATR sampling device containing diamond/ZnSe crystal. Spectra were acquired and then processed with the Spectrum software version 10.6.1. The spectra were scanned at room temperature in transmission mode over the wave number range of 4000–650 cm⁻¹, with a scan speed of 0.20 cm/s, and 30 accumulations at a resolution of 4 cm⁻¹. Triplicates of each sample were averaged to get an average spectrum. A background spectrum of air was scanned under the same instrumental conditions before each series of measurements.

2.6. Statistical analysis

All determinations were performed at least in triplicate. Data were expressed as mean values ± standard deviation. The statistical software used GraphPad (GraphPad Prism 6.1, San Diego, CA, USA). Comparison of means was performed by one-way analysis of variance (ANOVA) with a significance level of $P < 0.05$. Tukey's test was used for multiple comparisons. The Design Expert trial version 12 software (Stat-Ease, Inc., U.S.A) was used for constructing the regression model, designing the Box-Behnken and predicting the optimal parameters.

3. Results and discussion

3.1. Inulin and total carbohydrate in raw plant material

As a useful approach for the optimization during extraction, Inulin plus FOS were estimated colorimetrically by difference between total carbohydrate and reducing sugar contents. Inulin amount plus FOS (g/100 g dw) in raw plant material, estimated by difference from colorimetric data, was the highest in chicory root (75.90 ± 1.46) and Jerusalem artichoke (74.50 ± 2.44), as expected, followed by globe artichoke core (17.9 ± 0.75) and globe artichoke by-product ($6.20 \pm$

0.39). When inulin plus FOS content is estimated by difference between total carbohydrate and reducing sugar, besides inulin, other non-reducing sugars -such as low DP FOS and sucrose (DP 2) - are also accounted for. Determination of other non-reducing sugars, mainly sucrose, cannot be excluded when inulin plus FOS are estimated by difference. The presence of inulin is related to the ability of plants belonging to the *Asteraceae* (ex *Compositae*) family to store inulin and oligofructans as storage carbohydrates (Barclay et al., 2012). According to the literature reports, the content of inulin (g/100 g dw) in descending order is as follows: 76 chicory, 73 Jerusalem artichoke, 18 globe artichoke core and 10 globe artichoke by-product (Ruiz-Cano et al., 2014; Shoaib et al., 2016; Singh et al., 2019). Our values for raw plant materials are similar to those reported by these authors. Variation in inulin content could be due to different physiological processes that occur in each plant tissue to synthesize and store carbohydrates, being roots and tubers more efficient than fruits in inulin synthesis and storage (Saengthongpinit et al., 2005). Other factors that may affect inulin content are plant cultivar, cultural practices, geographic location, harvest date and storage time (Apolinário et al., 2014). In descending order, total carbohydrate content (g/100 g dw) found in raw material were: chicory root (80.43 ± 1.46), Jerusalem artichoke tuber (76.60 ± 2.44), globe artichoke core (29.00 ± 0.75) and globe artichoke by-product (17.51 ± 0.39).

3.2. Optimization of inulin extraction from plant material by using response surface methodology

3.2.1. Analysis of the models

Inulin plus FOS (I + FOS) extraction from raw plant materials was optimized using an RSM Box-Behnken experimental design (Tables 1 and 2). For chicory root, the ANOVA regression model ($P < 0.0001$), lack-of-fit ($P < 0.3371$) and adjusted R^2 (0.9892) confirmed that a full quadratic model produced a good estimation of the I + FOS (Table 3). Similarly, another quadratic model gave a good prediction of the amount I + FOS extracted from Jerusalem artichoke tuber ($P = 0.0004$; lack of fit $P = 0.3281$; adjusted $R^2 = 0.9060$) but the terms X_2 , X_3 , and X_2^2 were excluded to reach that adjusted R^2 minus predicted R^2 will be lower than 0.2 (Table 3). For globe artichoke by-product and core, the reduced quadratic models allowed to predict adequately I + FOS that excluded the interaction terms and X_2^2 . Thus, $P = 0.0007$; lack of fit $P = 0.1020$; adjusted $R^2 = 0.8090$ were obtained for the globe artichoke by-product and $P = 0.0004$; lack of fit $P = 0.9641$; adjusted $R^2 = 0.8327$ for the globe artichoke core (Table 3). The quadratic regression models that related I + FOS content with the temperature, time, and solvent to solid ratio for selected plant material are shown in Eq.s (2–5).

Chicory root:

$$Y = 26.445 + 0.797X_1 + 0.092X_2 - 0.138X_3 - 0.004X_1X_2 + 0.0165X_1X_3 - 0.006X_2X_3 - 0.0061X_1^2 + 0.061X_2^2 - 0.0116X_3^2 \quad (2)$$

Jerusalem artichoke tuber:

$$Y = -99.417 + 5.374X_1 + 1.237X_2 - 1.017X_3 - 0.0204X_1X_2 + 0.035X_1X_3 - 0.045X_1^2 - 0.017X_3^2 \quad (3)$$

Globe artichoke by-product:

$$Y = -63.871 + 1.824X_1 - 0.015X_2 + 0.126X_3 - 0.013X_1^2 - 0.0019X_3^2 \quad (4)$$

Globe artichoke core:

$$Y = 99.612 - 2.872X_1 - 0.014X_2 + 0.612X_3 + 0.021X_1^2 - 0.008X_3^2 \quad (5)$$

3.2.2. Effect of temperature, time and solvent to solid ratio on inulin extraction

Results suggested that inulin + FOS extraction was significantly

Table 3

ANOVA of response surface quadratic model for the extraction of inulin plus FOS from plant materials.

Run	Coded independent variables			Inulin plus FOS (g/100 g dw)			
	X_1	X_2	X_3				
				CR	JAT	GAP	GAC
1	-1	-1	0	62.38 ± 0.29	80.32 ± 0.24	2.13 ± 0.15	12.46 ± 0.30
2	-1	+1	0	68.84 ± 0.02	77.87 ± 1.78	1.33 ± 0.14	12.08 ± 0.20
3	-1	0	-1	60.21 ± 0.50	73.61 ± 1.69	1.04 ± 0.20	8.26 ± 0.32
4	-1	0	+1	60.38 ± 0.50	78.02 ± 0.73	2.55 ± 0.05	14.01 ± 0.32
5	0	0	0	65.41 ± 0.50	74.14 ± 1.56	4.01 ± 0.14	13.73 ± 1.01
6	0	0	0	65.21 ± 0.36	78.23 ± 3.23	3.89 ± 0.32	10.15 ± 1.11
7	0	-1	-1	58.30 ± 0.29	65.79 ± 1.72	3.90 ± 0.01	6.92 ± 0.79
8	0	+1	-1	66.38 ± 0.82	56.42 ± 1.33	2.86 ± 0.02	5.31 ± 0.88
9	0	-1	+1	68.13 ± 0.06	81.12 ± 1.51	3.87 ± 0.01	11.89 ± 0.23
10	0	+1	+1	68.50 ± 0.26	80.84 ± 3.46	3.33 ± 0.69	13.12 ± 0.88
11	0	0	0	65.84 ± 0.87	78.25 ± 1.35	4.22 ± 0.01	10.60 ± 0.51
12	+1	-1	0	67.53 ± 0.29	71.15 ± 5.23	3.58 ± 0.14	15.07 ± 0.61
13	+1	+1	0	70.49 ± 0.29	52.36 ± 2.47	3.62 ± 0.01	13.65 ± 0.43
14	+1	0	-1	59.25 ± 0.29	48.06 ± 0.89	1.96 ± 0.14	10.04 ± 0.72
15	+1	0	+1	69.32 ± 1.44	73.47 ± 0.61	3.84 ± 0.01	14.34 ± 0.52

FOS = fructo-oligosaccharides; CR = chicory root; JAT = Jerusalem artichoke tuber; GAP = Globe artichoke by-product; GAC = Globe artichoke core.

affected by the temperature for chicory root (F-value = 157.9, $P < 0.0001$), Jerusalem artichoke tuber (F-value = 48.2, $P = 0.0002$), globe artichoke by-product (F-value = 21.6, $P = 0.0012$) (Table 3). Opposite, temperature had not a significant influence on inulin + FOS extraction for globe artichoke core (F-value = 3.6, $P = 0.0906$) (Table 3). The response surface also demonstrated the relationship between temperature and inulin + FOS extraction from selected plant material (Fig. 1). An increase in extraction temperature usually improves recovery of carbohydrates; however, the use of very high temperatures can frequently result in an increase of undesirable co-extracted materials (Ruiz-Aceituno et al., 2016). Moreover, the quadratic effect of temperature on I + FOS extraction could be possibly due to hydrolysis and release of simple sugars occurred upon extraction (Ávila Núñez et al., 2012).

On the other hand, the extraction time had only a significant influence on the I + FOS extraction for chicory root (F-value = 230.9; $P < 0.0001$) and Jerusalem artichoke tuber (F-value = 11.0; $P < 0.0129$) (Table 3). This influence of extraction time was also shown in the response surface plots (Fig. 1A and B). Moreover, solvent to solid ratio had strong influence on the I + FOS extraction for all selected plant material: chicory root (F-value = 356.0; $P < 0.0001$), Jerusalem artichoke tuber (F-value = 55.6; $P < 0.0001$), globe artichoke by-product (F-value = 9.0; $P = 0.0151$) and globe artichoke core (F-value = 47.3; $P < 0.0001$) (Table 3).

Finally, a ratio of 40:1 (v/w) gave the highest recovery for all samples except for chicory, followed by temperature range (70–80 °C, except for Jerusalem artichoke tuber 60–70 °C), and time interval (20–40 min, except for chicory 60 min) (Table 2 and Supplementary material 1). In spite of its good recovery, total carbohydrate and inulin contents were lower in globe artichoke by-product than in the other

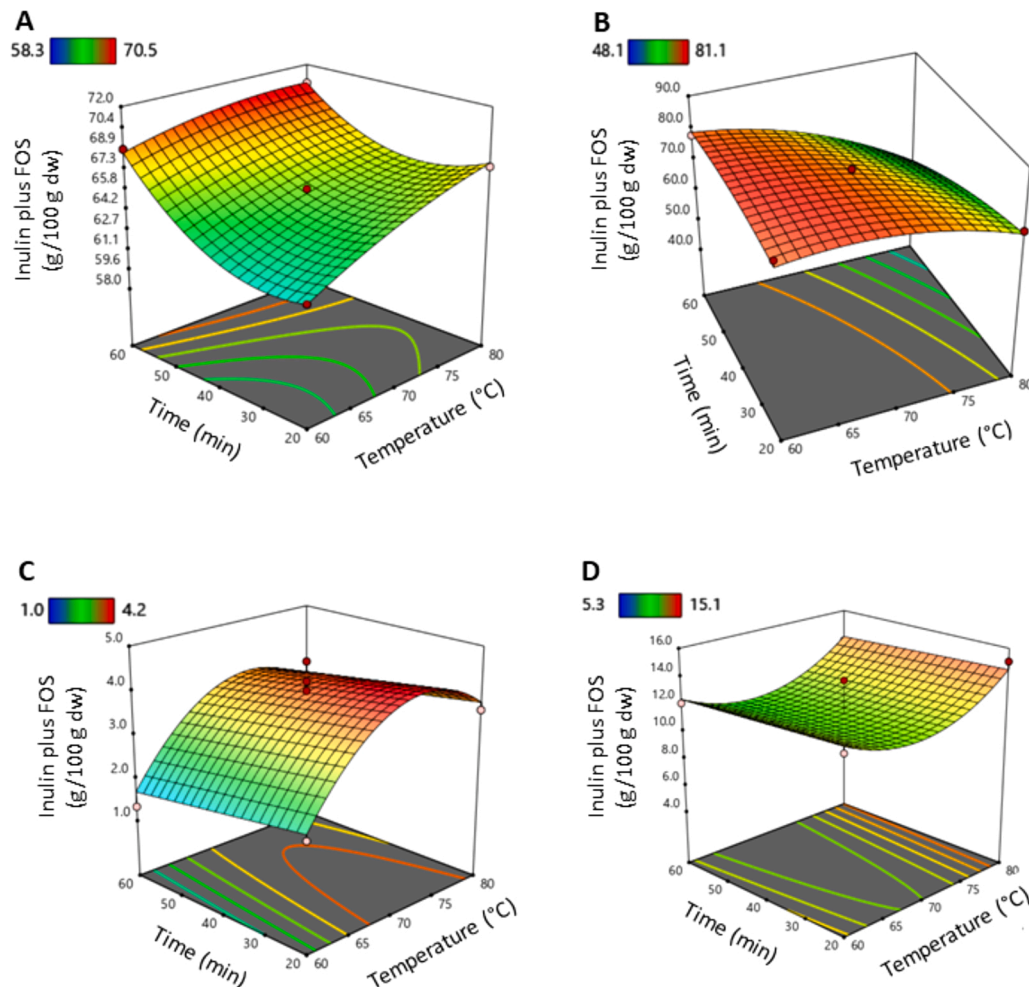


Fig. 1. Response surface and contour plots showing the effect of temperature and time on inulin plus FOS (fructo-oligosaccharides) extraction from samples of: A) Chicory root, B) Jerusalem artichoke tuber, C) Globe artichoke by-product, and D) Globe artichoke core. For each surface and contour plot, the level of solvent to solid ratio was held at its central value 25 mL/g.

samples under study (Table 2, Supplementary materials 1 and 2), but its low cost and immediate availability would support its potential to be used by the food industry as an alternative source of inulin with prebiotic potential.

3.2.3. Prediction and validation of optimal conditions for inulin extraction

Optimal conditions to maximize inulin extraction from selected plant material predicted by the response surface methodology are summarized in Table 4. Optimal conditions for inulin extraction were found to be dependent on vegetable source. Thus, for all samples the best results were achieved from 62 to 80 °C of temperature, with a variable time of 22–60 min and a solvent volume to solid ratio of 28–37 mL/g. The following optimal conditions were found for: a) chicory root, a maximum I + FOS of 70.8 g/100 g dw was achieved at temperature = 77.4 °C, time = 59.4 min and solvent to solid ratio = 27.8; b) Jerusalem artichoke tuber, a maximum I + FOS of 81.4 g/100 g dw was achieved at temperature = 62.4 °C, time = 21.7 min and solvent to solid ratio = 32.3; c) globe artichoke by-product, a maximum I + FOS of 4.3 g/100 g dw was achieved at temperature = 69.3 °C, time = 22.6 min and solvent to solid ratio = 37.4 and d) globe artichoke core, a maximum I + FOS of 15.5 g/100 g dw was achieved at temperature = 79.9 °C, time = 21.9 min and solvent to solid ratio = 34.2. Optimal conditions reported (Lingyun et al., 2007) for maximizing inulin extraction (83.6 %) from Jerusalem artichoke differed from ours. Thus, these authors used higher temperature (76.65 °C), lower solvent to solid ratio (10.56 mL/g) and similar extraction time (20 min). Finally, I + FOS content at different

Table 4

Optimal conditions for the extraction of inulin plus FOS from plant materials.

Sample	Independent variables			Inulin plus FOS (g/100 g dry weight)	
	Temperature (°C)	Time (min)	Solvent to solid ratio v:w (mL/g)	Predicted	Experimental
Chicory root	77.4	59.4	27.8	70.8	70.50 ± 0.29
Jerusalem artichoke tuber	62.4	21.7	32.3	81.4	81.12 ± 1.51
Globe artichoke by-product	69.3	22.6	37.4	4.3	4.22 ± 0.01
Globe artichoke core	79.9	21.9	34.2	15.5	15.07 ± 0.61

FOS = fructo-oligosaccharides.

time and temperature for a fixed solvent to solid ratio = 25 mL/g, including optimal regions, are shown in Fig. 2.

Predicted values of I + FOS for each selected plant material at optimal conditions were experimentally validated. The confidence

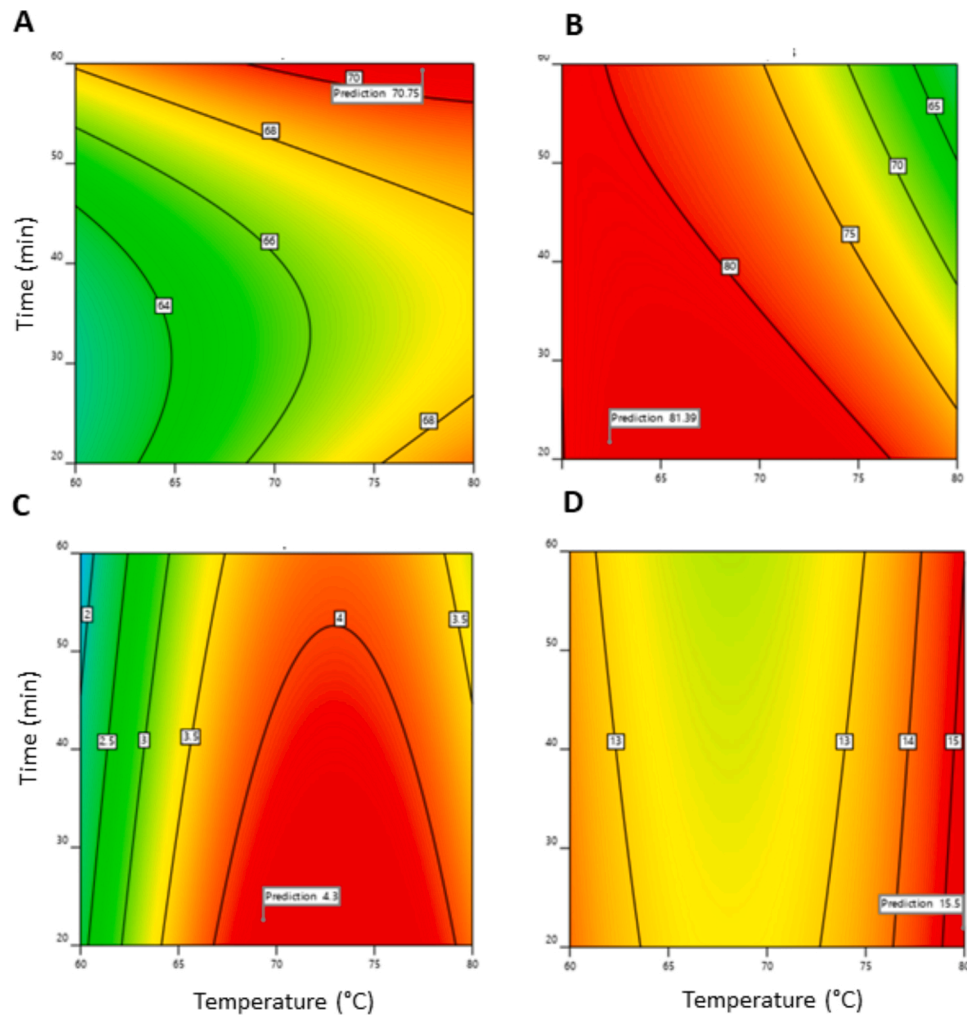


Fig. 2. Amount of inulin plus FOS (fructo-oligosaccharides) extracted and predicted at optimal conditions for: A) Chicory root, B) Jerusalem artichoke tuber, C) Globe artichoke by-product, and D) Globe artichoke core. For each surface and contour plot, the level of solvent to solid ratio was held at its central value 25 mL/g.

intervals for chicory root (95 % PI low = 69.49 and 95 % PI high = 72.27), Jerusalem artichoke tuber (95 % PI low = 72.23 and 95 % PI high = 90.03), globe artichoke by-product (95 % PI low = 3.2 and 95 % PI high = 5.5) and globe artichoke core (95 % PI low = 12.1 and 95 % PI high = 18.3) confirmed that models established are suitable and effective (Table 4).

The differences found in globe artichoke samples with inulin values reported in the literature (Ruiz-Cano et al., 2014; Shoaib et al., 2016; Singh et al., 2019) could be possibly attributed to the intrinsic characteristics of this raw material during extraction, such as its solubility and high degree of polymerization, as well as to another factors, all of which could affect the variability of temperature, time, and global recovery process.

Finally, I + FOS (g/100 g dw) contents (Table 4), in descending order are as follows: Jerusalem artichoke (81.12 ± 1.51), chicory (70.50 ± 0.29), artichoke core (15.07 ± 0.61) and artichoke by-product (4.22 ± 0.01). All values were slightly lower than corresponding raw materials except for Jerusalem artichoke sample. This fact could be attributed to some degree of hydrolyses of I + FOS during extraction, which could release reducing sugars. The most important difference between the two globe artichoke samples could be attributed to their different composition. Globe artichoke by-product is composed by the outer bracts and stalks of the inflorescence while the edible core is formed by the inner and tender bracts. In this regard, if only the most external bracts of the inflorescence from globe artichoke were considered as the by-product, without considering the stalks, then its inulin content would be

considerably higher.

3.3. Characterization of inulin extracted from plant material

3.3.1. Fourier transformed infrared spectra

In the present study, Fourier Transformed Infrared from Attenuated Total Reflectance -FTIR-ATR spectroscopy- was used to characterize the recovered inulin-rich extracts (Gómez-Ordóñez and Rupérez, 2011).

Common to all samples, two bands appeared in the $4000\text{--}2000\text{ cm}^{-1}$ region of the FTIR spectra (data not shown): a broad band centred at 3260 cm^{-1} assigned to hydrogen bonded O-H stretching vibrations and a weak signal at 2926 cm^{-1} due to C-H stretching vibrations. In addition, the medium to strong IR absorption bands at $1200\text{--}970\text{ cm}^{-1}$ were mainly due to C-C and C-O stretching in pyranoid ring and to C-O-C stretching of glycosidic bonds. An intense absorption in this spectral region is common for all polysaccharides (Gómez-Ordóñez et al., 2011).

FTIR spectra in the range $2000\text{--}650\text{ cm}^{-1}$ of raw materials (chicory root, Jerusalem artichoke, core and by-product of globe artichoke) compared to optimized inulin extracts and inulin standards are presented in Fig. 3. These infrared spectra demonstrated that the sample extracts show as the typical band of inulin-type fructan (Fig. 3 A-b, B-b, and C-c). In particular, the band at 935 cm^{-1} has been reported as typical from inulin and it is attributed to the (2→1)-glycosidic bond (Barclay et al., 2016; Romano et al., 2018). In addition, in all inulin-rich extracts and standards, characteristic peaks at $1075\text{--}1100\text{ cm}^{-1}$ indicated C-O and C-C stretching vibrations of pyranose ring from inulin.

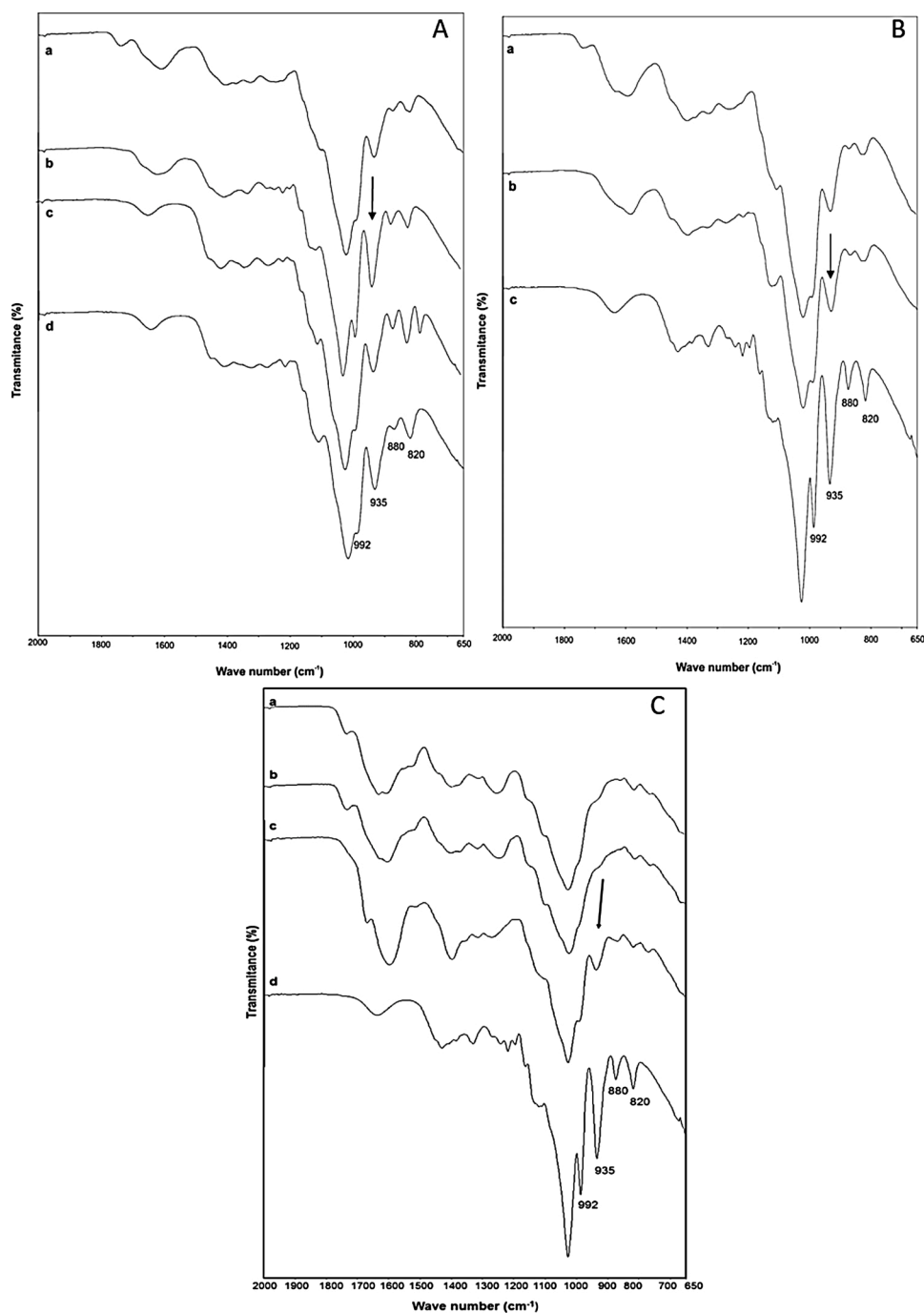


Fig. 3. Fourier transformed infrared spectra of different raw samples, optimal extracts and inulin standards. A: (a) Chicory root; (b) Inulin extract from chicory root; (c) Oligofructose (Raftilose® P95 from ORAFTI); (d) Inulin (Raftiline®bHP-Gel from ORAFTI). B: (a) Jerusalem artichoke tuber; (b) Inulin extract from Jerusalem artichoke tuber; (c) Inulin standard from chicory (Sigma). C: (a) Globe artichoke core; (b) Globe artichoke by-product; (c) Inulin extract from globe artichoke by-product; (d) Inulin standard (Sigma).

These and other bands occurring at 820 cm^{-1} , 880 cm^{-1} , 992 cm^{-1} , are present in inulin standards (Fig. 3) and have been reported in inulin from chicory, Jerusalem artichoke, globe artichoke and garlic by other authors (Mudannayake et al., 2015; Shao et al., 2012).

According to the extraction results, Jerusalem artichoke had the highest amount of inulin + FOS, followed by chicory root (Table 4). This can be also observed in the FTIR-spectra, with an important peak in the characteristic inulin band at 935 cm^{-1} , in both samples (Fig. 3A-b, B-b). Likewise, globe artichoke with little amount of inulin in the extracts, also showed a smaller inulin band at 935 cm^{-1} .

3.3.2. Profile of inulin and low molecular weight carbohydrates by HPLC

The extracts obtained from each of the samples at optimal conditions were analysed by HPLC (Table 5) and FTIR-spectroscopy (Fig. 3). All

sample extracts had a similar profile of low molecular weight carbohydrates - I + FOS - but differed in their concentration.

Chicory and Jerusalem artichoke gave the highest amount of inulin ($\approx 54\text{ g}/100\text{ g dw}$), while artichoke by-product ($0.70\text{ g}/100\text{ g dw}$) and core ($1.71\text{ g}/100\text{ g dw}$) gave the lowest inulin content. Three LMWCs, which retention times matched the available galacto-oligosaccharide standards, are most probably fructo-oligosaccharides with polymerization degrees from DP4 to DP2, such as: inulotetraose (Fru4), inulotriose (Fru3), and inulobiose (Fru2). Alternatively, they could be oligosaccharides of fructose with a terminal glucose unit, such as: nystose or kestotetraose (Glu-Fru3) and kestose (Glu-Fru2).

Likewise, chicory and Jerusalem artichoke samples also showed higher FOS content ($5.4\text{--}14.3\text{ g}/100\text{ g dw}$) than globe artichoke samples ($1.3\text{--}1.6\text{ g}/100\text{ g dw}$). As previously reported, low molecular weight

Table 5

Profile of inulin and low molecular weight carbohydrates determined by HPLC in samples extracted at optimized conditions (g/100 g dry weight).

Sample	Inulin	FOS (sucrose included)				Reducing sugars		Total LMWC	Inulin plus FOS
		DP4	DP3	DP2	Sucrose	Glucose	Fructose		
Chicory root	54.16 ± 0.10 ^b	2.55 ± 0.01 ^b	2.11 ± 0.02 ^b	0.76 ± 0.03 ^a	4.25 ± 0.03 ^b	0.33 ± 0.01 ^c	1.23 ± 0.01 ^c	65.39 ± 0.12 ^b	63.83 ± 0.17 ^b
Jerusalem artichoke tuber	54.57 ± 0.06 ^a	6.16 ± 0.02 ^a	7.33 ± 0.03 ^a	0.81 ± 0.01 ^a	7.18 ± 0.01 ^a	0.36 ± 0.03 ^c	1.77 ± 0.04 ^a	78.15 ± 0.09 ^a	76.02 ± 0.06 ^a
Globe artichoke by-product	0.70 ± 0.05 ^d	0.29 ± 0.02 ^d	0.33 ± 0.03 ^d	0.73 ± 0.02 ^b	1.70 ± 0.03 ^d	3.83 ± 0.04 ^b	1.43 ± 0.02 ^b	9.01 ± 0.14 ^d	3.75 ± 0.09 ^d
Globe artichoke core	1.71 ± 0.04 ^c	0.46 ± 0.03 ^c	0.49 ± 0.06 ^c	0.69 ± 0.03 ^b	3.32 ± 0.08 ^c	4.50 ± 0.08 ^a	1.38 ± 0.07 ^b	12.54 ± 0.34 ^c	6.67 ± 0.09 ^c

FOS = fructo-oligosaccharides; DP4= polymerization degree of 4 monomers; DP3= polymerization degree of 3 monomers; DP2= polymerization degree of 2 monomers; LMWC = low molecular weight carbohydrates.

carbohydrates from Jerusalem artichoke are inulin-type polyfructans (Li et al., 2015). Chicory root and Jerusalem artichoke tuber contain natural FOS with a degree of polymerization up to 7 and inulin up to 60 DP (Li et al., 2015; Meyer and Blaauw, 2009).

All samples contained sucrose, glucose, and fructose (5.7–9.3 g/100 g dw), but glucose was the main sugar in artichoke samples which are derived from inflorescences, while sucrose was the predominant sugar in samples from Jerusalem artichoke tuber and chicory root, both of which are storage organs. The samples also contained trace amounts of three unidentified compounds (U1-U3), most probably monosaccharides, which could be intermediate compounds in inulin synthesis.

Total LMWC range between 65.4 g/100 g dw in chicory and 78.2 g/100 g dw in Jerusalem artichoke Globe artichoke samples have much lower total LMWC content (9.0 and 12.5 g/100 g dw). Total LMWC minus reducing sugars would let to compare with estimated values by colorimetry (Tables 4 and 5).

Inulin + FOS content ranged from 64 to 76 % in chicory roots and Jerusalem artichoke tubers, a much higher value than in artichoke samples. DP4 to DP2 FOS amount from 5.4–14.3 g/100 g dw. Globe artichoke samples ranged from 3.7 to 6.7 g/100 g dw), for by-product and core, respectively. These differences were expected, since roots and tubers are storage organs of plants (Van Laere and Van Den Ende, 2002), while inflorescence carbohydrates are actively metabolized into simple sugars, like in globe artichoke (Table 5).

Absolute values of inulin and other low molecular weight carbohydrates were higher by colorimetric method than by HPLC analysis. This could be due to differences between the instrumental techniques used. HPLC specifically quantified inulin and other low molecular weight carbohydrates using inulin, several oligosaccharides and other LMWC as standards. In this work, fructose was used as standard for determination of total carbohydrates and reducing sugars by colorimetric methods. Moreover, when inulin plus FOS content is estimated colorimetrically by difference between total carbohydrates and reducing sugar, non-reducing sugars such as sucrose are also accounted for.

Inulin plus FOS content by HPLC (Table 5) was more abundant in Jerusalem artichoke, followed by chicory, core, and by-product of globe artichoke. In chicory roots (Apolinário et al., 2017) inulin content ranges from 42 to 76 g/100 g dw (Shoaib et al., 2016). Jerusalem artichoke tubers could also store high levels of inulin and fructo-oligosaccharides (FOS), amounting to 45–75 g/100 g dw (Singh et al., 2019; Wilkins, 2008). Our values are in the top range for chicory (63.83 g/100 g dw) and Jerusalem artichoke (76.02 g/100 g dw).

4. Conclusions

Inulin plus FOS content was the highest in raw material of chicory root and Jerusalem artichoke tubers and the lowest in globe artichoke inflorescence (core 17.9 and by-product 6.2 g/100 g dw). Optimate conditions for extraction, aided by RSM model, were solvent to solid ratio 28–37 mL/g, temperature from 62 to 80 °C, and time from 22–60

min. The higher the inulin plus FOS contents in the starting raw material, the higher their global recovery. Inulin plus FOS content by descending order was Jerusalem artichoke, chicory, globe artichoke core, and by-product. For each independent variable, temperature, time, and solvent to solid ratio model suggested that inulin plus FOS content was mainly affected by linear and quadratic coefficients in all samples, except for globe artichoke samples in which there was not a significant interaction among factors. Total carbohydrate and inulin plus FOS contents were lower in artichoke by-product than in the other samples studied. FTIR spectra confirmed that optimized sample extracts showed typical absorption bands of inulin-type fructans. In particular, the band at 935 cm⁻¹ is attributed to the (2→1) - glycosidic bond of inulin.

Conditions for inulin extraction should be optimized for every vegetable source under study. Therefore, RSM model approach could be very helpful for the screening of novel sources of inulin like globe artichoke by-product. Its ready availability and low cost would support its potential to be used as a source of functional ingredients and prebiotics, nevertheless, it could be desirable to increase its inulin content by selecting the richer parts in inulin from the residue. Novel inulin sources could be alternative and complementary to the most traditional plant sources of inulin for the food industry.

Author contributions

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Selene Elizabeth Herrera-Vázquez: Methodology; Formal analysis
Luis Condezo-Hoyos: Software; Data curation; Model validation; Formal analysis

Eva Gómez-Ordóñez: Methodology; Formal analysis; Data curation
Pilar Rupérez: Experimental design; Data curation; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Roles/Writing - original draft; Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.indcrop.2021.113726>.

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