Contents lists available at ScienceDirect



## **Applied Food Research**



journal homepage: www.elsevier.com/locate/afres

# Assessment of Jerusalem artichoke as a source for the production of gluten-free flour and fructan concentrate by ultrafiltration



### M.V. Ostermann-Porcel, A.N. Rinaldoni, M.E. Campderrós\*

Instituto de Investigaciones en Tecnología Química (INTEQUI-CONICET), Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, San Luis 5700, Argentina

#### ARTICLE INFO

Keywords: Jerusalem artichoke flour Membrane concentration Ultrafiltration Fructans' extraction

#### ABSTRACT

Jerusalem artichoke (JA) is a tuber considered a functional food. The tubers accumulate a high amount of fructans (inulin and fructo-oligosaccharides) although tubers also have a high content of minerals and proteins of high biological value. In this study Jerusalem artichoke flour (JAF) was obtained by means of two drying methodologies: convective air oven drying and freeze-drying. Physico-chemical characteristics were analyzed in both samples, as well as a colorimetric analysis and microstructure. As a by-product of the production of JAF, a residual liquid is obtained from which fructans can be extracted, this process included separation steps in aqueous medium without the addition of organic solvents. The optimal extraction conditions were: solid/solvent ratio of 1:2 (Kg JA / L distilled water) during 40 min at 90  $\pm$  5 °C. The flours obtained presented a high protein and fiber content, 9.73-9.94% and 1.21-1.47% respectively, while the morphology of the matrix of protein and inulin could be observed by microscopy. These results allow them to be considered as an alternative raw material for the production of gluten-free products in order to overcome the nutritional deficiency that gluten-free products generally have. The fructans extraction yield was affected by the temperature and storage time of the tubers. The concentration of fructans was performed by ultrafiltration, the process was stopped when the desired volume concentration ratio was achieved (VCR = 3.24). The concentrate stream was lyophilized. The results showed the following centesimal compositions: 6.65% proteins; 0.22% fat; 7.50% ash; 6.60% moisture and 4% inulin. The use of JA tubers allowed to obtain a gluten-free alternative flour with good nutritional characteristics. Therefore, JA tubers present a high potential for the production of inulin through chemical-free methods, from the by-product of the JAF process.

#### 1. Introduction

Jerusalem artichoke (JA), *Helianthus tuberosus L.*, is an herbaceous crop that belongs to the Asteraceae family. Its reproduction is agamic, producing tubers for its propagation like a seed (Ibarguren & Rebora, 2013). It is a plant with an annual cycle that produces fibrous roots with short rhizomes that end in underground caulinar tubers. The particularity of this crop is that JA tubers accumulate high levels of inulin and fructo-oligosaccharides instead of starch (Yovchev et al., 2021). Inulin is a water-soluble fiber that consists of a mixture of oligo- and/or polysaccharides of  $\beta$  (2  $\rightarrow$  1) linked D-fructose units with a terminal glucose residue which are classified as fructans (Rubel et al., 2018). Inulin is used in food to support the growth of beneficial bacteria in the colon. Among the nutritional benefits of this fructan, can de indicated that they supply of dietary fiber, low caloric value, have proteins of high biological value a better bioavailability of calcium and magnesium absorption, reduction of the risk of suffering cancer and the reinforcement

of the immune response (Wang, 2009; Neyrinck et al., 2015; Díaz et al., 2019; Tarifa et al., 2021; Rubel et al., 2021). JA tubers contain a large amount of inulin, 85% of the dry weight corresponds to inulin-type fructans (Moon et al., 2019).

The JA tubers can be harvested in the fall or left underground for storage until spring harvest. If they are stored in chambers, it should be at 0 °C and between 90 and 95% relative humidity. Tubers that are saved for seed should not be frozen during storage (Rebora et al., 2011). Nowadays, a fast and inexpensive method has not been developed to process a large quantity of tubers, for this reason their accumulation after harvest causes quality deterioration. The main causes of the loss of quality are dehydration, spoilage and sprouts appearance, as well as the degradation of reserve carbohydrates (Ritsema & Smeekens, 2003). These carbohydrates are located in the cellular vacuoles of fresh plant tissues as solute in a colloidal state. Some authors suggest that the freezing temperature is optimal to avoid significant variations in the fructans composition (Abeynayake et al., 2015).

\* Corresponding author.

E-mail address: mcampd@gmail.com (M.E. Campderrós).

https://doi.org/10.1016/j.afres.2022.100201

Received 8 March 2022; Received in revised form 20 July 2022; Accepted 22 August 2022 Available online 23 August 2022

2772-5022/© 2022 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/)



Fig. 1. Flow chart of the process of obtaining Jerusalem artichoke flour (JAF).

Currently, there is great interest in the food industry for the design, development and marketing of special foods for different consumer demands. JA is a tuber considered a functional food; it is classified as a natural prebiotic. The main nutrient in Jerusalem artichoke tubers is soluble fiber (mainly fructans of the inulin type), however JA is not the industrial source commonly used to obtain derivatives with potential applications in food industry. Among the numerous alternatives of raw materials to use in this challenge, the possibility of applying JA tubers to produce flour arises.

The aim of this study is to produce and characterize an alternative gluten-free flour, as an alternative source for formulating food. The production of JAF generates a liquid waste that could be reused. In contrast to a linear economy of production, consumption and waste, this work promoting and implementing a circular economy, in which fructans could be extracted from this liquid waste by employing membrane technology. The latter encourages a constant flow and an environmental solution, in which waste or industrial by-products can be used as resources to reenter the production system as new raw materials. Furthermore, as for the health beneficial potential of inulin, its extraction from natural source has become a subject of interest in many food research programs (Ahmed & Rashid, 2019)

#### 2. Materials and methods

#### 2.1. Raw materials

For the experiments, a white skin variety of JA tubers were used. The samples were harvested 10 months after sowing in the province of San Luis (Argentina). 10 kg were received and processed as detailed in the Fig. 1 in order to obtain JAF. The tubers were stored at a temperature of  $4 \pm 1$  °C to maintain the hygienic, sanitary and nutritional quality of tubers.

#### 2.2. Jerusalem artichoke flour processing

The elaboration of the Jerusalem artichoke flour (JAF) was carried out as shown in the following flow chart (Fig. 1). The tubers were washed manually using a brush to remove soil residues and reduce the microorganism load on the surface, then separated into two batches. Samples from each batch were sliced into 3-4 mm thick, 12 by 12 mm slices, without damaging the structure, using a domestic food processor. After that, samples were cooked at  $85 \pm 5$  °C for 30 min, the aim of this stage is to subject the raw material to temperatures that modify the chemical structure of the components to generate nutrients more easily digestible by human digestive enzymes and inactivate the endogenous enzymes. Jerusalem Artichoke tubers deteriorate rapidly due to their high moisture content. To address these limitations, it must be dried as soon as possible under appropriate conditions to maintain product integrity and allow its use as a human food supplement. Two drying methods were evaluated, oven convective drying and freeze-drying, to optimize the production of Jerusalem artichoke flour and to evaluate whether the methodologies used influence the physicochemical composition and/or techno-functional properties of the flour. For lyophilization, a batch of the Jerusalem Artichoke slices were arranged in stainless steel trays, frozen at -40 °C and dried using a lyophilizer (Rificor S.A., Argentina) for 48 h at 25  $\pm$  5 °C and 50  $\mu mHg$  presure until constant weight. The second batch of Jerusalem Artichoke slices was oven-dried (San Jor, SE 70 SD, Argentina) with air current at 70  $\pm$  5 °C until constant weight. The flours obtained were labeled as: JAF (l) and JAF (o) for freeze-dried and ovendried flour, respectively. Finally, flour samples obtained were placed in hermetically sealed plastic bags using a vacuum sealing machine (Oster, V2240, USA), duly labeled and stored until the time of analysis and/or use to avoid oxidation. Flour yield after drying was obtained from the following equation:

Flouryield = [flourobtained(g)/tubersused(g)]x100((1))

The dehydrated samples were subjected to a grinding process (200  $\mu$ m), using an ultracentrifugal mill (Retsch ZM 200, Germany) at 21,732 g force in order to obtain a powder of homogeneous granulometry.

As a by-product of this process, a residual liquid is obtained from which fructans can be extracted and concentrated. The extraction of fructans includes steps in an aqueous medium, without the addition of organic solvents, which is of interest both for use in the food industry as soon as for the care of the environment. For the extraction at laboratory scale, the following operations were carried out: Solid-liquid extraction, filtration and membrane concentration and finally, freezedrying. Physicochemical characterization of the powder obtained, and determination of the inulin content were carried out. The different experimental stages are described below.

#### 2.3. Extraction and concentration of fructans from the waste water

#### 2.3.1. Solid-liquid extraction

The solid-liquid extraction is the first step to isolate and characterize compounds of interest, in this operation the compound's integrity must be maintained while the co-extraction of other undesirable compounds must be avoided. An aqueous extraction of fructans was carried out through fresh tubers of JA. Water as a solvent meets the conditions of selectivity and low viscosity to be able to easily penetrate through the pores of the solid. In the extraction process, the smaller the particle size, the greater the interfacial area and, therefore, the greater the mass transfer. But, if the particles are very small, they can compact and make extraction difficult. In order to determine the efficiency of the extraction process, the soluble solids extracted from the samples of Jerusalem artichoke were quantified. A solid solvent ratio (Kg of JA)/ (L of distilled water) was evaluated: [1: 2]. Soluble solids were measured using a refractometer (ZGRB-32ATC, Hand Held, China). For extraction processes, the temperature is a fundamental variable. Increasing temperature increases solubility and the mass transfer coefficient in the material and therefore, the extraction. The maximum working temperature was set at 90  $\pm$  5 °C in consideration of the evaporation of the solvent (distilled water), although it is possible to carry out experiments at higher temperatures, preventing the water from evaporating. According to previous studies by Apolinário et al. (2014) during extraction at high temperatures, inulin can degrade when the pH is below 5, reason why the pH was measured throughout the process. Three replications were made for each extraction.

#### 2.3.2. Filtration and membrane concentration

The membrane filtration process represents a very efficient and economical way of separating suspended or dissolved components in a liquid while concentrating compounds of interest, depending on the pore size of the membrane used.

The aqueous extract obtained in the previous stage, was filtered in successive stages using a canvas filter, to separate the insoluble particles (remains of JA tubers) from the aqueous solution. Once clarified, the liquid fraction was the feed stream for the microfiltration (MF) process. The solution was thermostatted through a hot-cold bath with recirculation (MGW LAUDA K4R, Germany) through a coil that was placed inside it to control the stability of the selected temperature. This feed solution is driven by means of a variable speed pump (General Electric, USA), through a 5–10  $\mu$ m polyethylene microfiltration front filter (Pall Corporation, USA). This operation reduces the amount of bacteria and spores, acting as a cold pasteurization of the feed, furthermore it protects the UF membrane from fouling (Gonzalez et al., 2016).

The micro-filtered solution constitutes the feed stream in the next ultrafiltration (UF) step. The ultrafiltration module consists of a Millipore PELLICON system (Millipore PLGC-type, USA) composed of layers of polysulfone membranes, presented in the form of sheets stacked with a polypropylene separating screen. The molecular weight cutoff is 10 kD, the membrane effective surface area is 0.47 m2, having the advantage of large transfer area in small volume. The equipment consists of two pressure sensors, to control the feed (inlet) and concentrate (outlet) pressures. The concentration of fructans by UF was carried out by continuously removing the permeate stream until the desired concentration, was achieved. The operational conditions used were: Sample Temperature: 19.5 ± 0.5 °C; Speed: 12 Hz and Transmembrane Pressure: 3.5 bar, defined as the pressure gradient of the membrane. The streams obtained in this stage were labeled as: feed (the micro-filtered solution) (F), and the currents after passing the UF membrane: permeate (P) and concentrate (C). The cleaning of the fouled membrane was performed by applying a "Cleaning in Place" (CIP) after each filtration the membrane is cleaned online according to the instructions provided by the manufacturer. The protocol used was distilled water - NaOH (pH: 11.5  $\pm$  0.5) - distilled water, each step is carried out at 40  $\pm$  2 °C for 30 min, a final cleaning stage was carried out with NaClO 300 ppm at the same temperature and pressure to ensure disinfection and a final water rinse. The hydraulic permeability of the membrane was always recovered, with which it was verified that the cleaning procedure was carried out correctly.

#### 2.3.3. Freeze-drying

Freeze drying (FD) was performed using a freeze-dryer (Rificor S.A., Argentina). In the FD process, the streams (F, P and C) were spread uniformly on a stainless-steel tray. The samples (400 g) were frozen at -40 °C and then were lyophilized under the following operational conditions:  $25 \pm 5$  °C;  $50 \ \mu$ mHg; 72 h. The resulting powder from each stream was labeled (Ext-F, Ext-P, and Ext-C, respectively) and stored in hermetic flask, in a cool place (15–25 °C) until its use.

#### 2.4. Physicochemical analysis

Physicochemical analyses of samples, JAF and fructans extracts, were determined in triplicate according to standard replication (AOAC, 2016). Moisture content was determined by gravimetric method (AOAC 925.10), dry matter by weight difference (AOAC 925.23), ash by incineration (AOAC 945.46), protein content by determination of total nitrogen by the Kjeldahl method (Selecta, Spain) (AOAC 991.22), fat content by Soxhlet extraction (Shanghai QianJian Instrument Co. Ltd, China) (AOAC 945.39), and carbohydrate by difference. For the determination of water activity, an important property from a food safety point of view, an AquaLab PRE water activity meter was used.

#### 2.4.1. Determination of the inulin content

The quantification of inulin in the freeze-dried material was assessed by high performance liquid chromatography (HPLC), AOAC 985.29 143 method, using an ion exchange column with a refractive index detector (Zuleta & Sambucetti, 2001). The chromatograph uses a Aminex HPX-87C (Bio-Rad, USA) column and a refractive index detector. Deionized water was used at 85° C as mobile phase and a flow rate of 0.6 ml /min.

#### 2.5. Color analysis

A Mini Scan EZ digital spectrophotometer (Hunterlab, USA) was used for color analysis. The colorimeter was calibrated with a white/black color standard. The results reported are the average of three measurements in each sample in the coordinates of the CIELAB color space, which consists of a Cartesian system defined by three colorimetric coordinates: L\*, a\* and b\*, which allow describing the color of any object. L\* indicates the brightness; a\* indicates the saturation or deviation of the achromatic point of L\* towards red (a\* > 0) or towards green (a\* < 0); and b\* is the pitch angle that defines the deviation of L\* on the yellow (b\* > 0) or blue (b\* < 0) axis.

#### 2.6. Microstructure study

The microstructure of flour was evaluated across analysis of image by scanning electron microscopy (SEM, LEO1450VP, Zeiss, Germany). The samples were mounted on double-sided adhesive carbon on aluminum sample holders. The micrographics were determined under VP mode (variable pressure), using 300x magnification. The low vacuum mode of SEM is a special type, where the chamber can be maintained at low vacuum at 70 Pa, while the column remains under vacuum. In this way, it is possible to observe biologically sensitive samples without dehydrating or metalizing with gold (Ostermann Porcel et al., 2017).

#### 2.7. Statistical analysis

One-way analysis of variance was used to study differences between the batters and cakes measurements. Fisher's least significant differences (LSD) test were used to establish the significance of differences among mean values with significance level of 95% (p < 0.05). The statistical analyses were performed using Statgraphics Centurion XVIII software (StatPoint Technologies Inc, Warrenton, USA).

Table 1

Physicochemical composition of Jerusalem artichoke flour compared with rice flour.

Sample	Proteins % (w/w)	Fiber % (w/w)	Fat % (w/w)	Ash % (w/w)	Moisture % (w/w)	Carbohydrates % (w/w)
JAF (o) JAF (l) Rice Flour (*)	$9.94 \pm 0.07^{a}$ $9.73 \pm 0.22^{a}$ 7.96-12.89	$\begin{array}{l} 1.47 \pm 0.08 \ ^{a} \\ 1.21 \pm 0.34^{a} \\ 0.42 0.53 \end{array}$	$\begin{array}{c} 1.78 \pm 0.07^a \\ 1.86 \pm 0.03^a \\ 0.26 1.49 \end{array}$	$\begin{array}{l} 6.57 \pm 0.11^a \\ 6.29 \pm 0.05^a \\ 0.08  0.82 \end{array}$	$5.17 \pm 0.18$ $5.35 \pm 0.07$ 10.45	75.07ª 73.04 ª 79.49–89.69

Within each column averages followed by the same letter are not significant different (p < 0.05). Results are expressed as mean  $\pm$  SD. (\*) (Asmeda et al., 2015).

#### 3. Results and discussion

#### 3.1. Jerusalem artichoke flour yield

The flour yield after drying was 25% for JAF (l) and 16% for JAF (o). The low yield demonstrates the efficiency of drying, since the JA tuber has a high initial moisture content (80%). By freeze-drying, a higher yield in dry flour is obtained, however this technology has been associated with a relatively high energetic cost; consequently, its utility would depend on the development of special applications of the flour.

# 3.2. Physicochemical and morphologic characterization of Jerusalem artichoke flours

The results of chemical composition of the flours are presented in Table 1. When comparing the protein and fiber content of the processed Jerusalem artichoke flours, no statistically significant differences were observed. However, when compared JAF with commercial gluten-free flours such as rice flour it is observed that JAF have an improved nutritional value, constituting a nutritional alternative for people with celiac disease. In addition, it is considered that the nutritional value of Jerusalem artichoke tuber proteins is due not only to the fact that they contain the essential amino acids, but also have a good amino acid balance that determines their high nutritional and biological value (Rakhimov et al., 2003). The fat content did not show statistically significant differences between the two samples analyzed. However, the results obtained were low, since these are flours obtained from roots and tubers, which by their nature show a lower amount of fat than flours from cereals such as rye (5.11%) and wheat (1.76%) (Drakos et al., 2017; Kowalski et al., 2022). The average ash content of the flours obtained was 6.5% w/w. It should be noted that, according to the literature, the main minerals found in Jerusalem Artichoke tubers are potassium, calcium and magnesium (Kang et al., 2007). The high Ca content ensures bone development and stability, while potassium and magnesium are important in the cation balance of the human organism, muscle contraction and nerve transmission (Kang et al., 2007). The elimination of water provides excellent protection against the main causes of food spoilage, such as the proliferation of microorganisms, which cannot develop in an environment without water, so that the scarce availability of water prevents or reduces food spoilage. In addition, under these conditions, enzymatic activity is not possible either, and most of the chemical reactions are paralyzed or slowed down, giving the food a longer shelf life (Vega-Mercado et al., 2001). It is observed that the flours dried by oven (JAF (o)) and freeze-drying (JAF (l)) have a moisture content below 15% w/w, complying with the value stablished by the Argentine Food Code for commercial flours. As previously mentioned, Jerusalem Artichoke can be considered a functional food due to its high content of inulin, 16 to 20 % of the fresh weight of the tuber. The Jerusalem artichoke flours presented a value of 75% w/w of carbohydrates, formed mostly by fructans, reducing sugars and sucrose. The inulin content in the flour was determined by HPLC, and the content was 3% w/w. The potential of this crop as a source for obtaining this carbohydrate is important, as it is one of the plant species with the highest proportion of this carbohydrate, also associated with the high yield per unit area (Ibarguren et al., 2013).

 Table 2

 Colorimetric analysis of Jerusalem artichoke flour and commercial flours

Sample	L*	a*	<b>b</b> *	
JAF (0) JAF (1)	$\begin{array}{c} 62.70 \pm 1.30^a \\ 81.02 \pm 0.83^b \end{array}$	$\begin{array}{c} 5.23 \pm 0.55^{b} \\ 2.47 \pm 0.13^{a} \end{array}$	$\begin{array}{l} 9.60 \pm 0.90^{b} \\ 5.31 \pm 0.17^{a} \end{array}$	
Cassava flour (*) Wheat flour (*) Corn flour (*)	$86.2 \pm 0.85$ $91.8 \pm 0.04$ $82.3 \pm 0.88$	$\begin{array}{c} 11.71 \pm 0.04 \\ 9.3 \pm 0.17 \\ 28.5 \pm 1.22 \end{array}$	$\begin{array}{c} 11.7 \pm 0.04 \\ 9.28 \pm 0.17 \\ 28.2 \pm 1.16 \end{array}$	

Within each column averages followed by the same letter are not significant different (p < 0.05). Results are expressed as mean  $\pm$  SD. (\*, Von Atzingen et al., 2005).

Scanning electron microscopy allows the observation and surface characterization of inorganic and organic materials, thus providing information about the surface morphology of the analyzed material. The images obtained for the freeze dried JAF are presented in Fig. 2. The globular proteins present in the sample can be observed; these are composed of one or several polypeptide chains coiled on themselves, forming a rounded three-dimensional structure, amorphous and of uniform size. The particles with concavities represent the inulin.

#### 3.3. Colorimetric analysis of Jerusalem artichoke flours

The results of the colorimetric analysis of JAF (1) and JAF (0) flours are presented in Table 2. In addition, this table compares the data obtained with data from flours available on the market (Von Atzingen et al., 2005). Statistically significant differences were observed in the colorimetric parameters evaluated: L\*, a\* and b\* for the Jerusalem artichoke flours produced. Thus, it is corroborated that the drying technique used influences the coloration of the flour obtained. Both samples, JAF (l) and JAF (o), presented a brightness higher than 50, which reflects the clarity of the processed Jerusalem artichoke flours. The freeze-dried sample presented an L\* value of 81.02 while in the JAF (o) sample a value for L\* of 62.70 was determined; this lower value is a consequence of the nonenzymatic browning reactions generated in the process (70  $\pm$  5 °C). In both samples the value of a\* was inclined towards red color, while the value of b\* towards yellow color. The flour brightness parameter is one of the main aspects linked to consumer preference. The average value of L\* obtained for JAF (1) is very close to other commercial flours with and without gluten, while the value obtained for JAF (o) is significantly lower. As for the values of parameters a\* and b\*, the same positive deviation is observed in all cases, with more intense shades for corn and cassava flours, and the values found for JAF are closer to those of wheat flour.

#### 3.4. Extraction of fructans from Jerusalem artichoke tubers

It has been described that the main factors that influence the yield of fructans extraction from JA tubers employing hot water as solvent include temperature, extraction time and solid to solvent ratio.

#### 3.4.1. Solid-liquid extraction

Throughout the extraction process, measurements of soluble solids were taken until its value remained constant. As it was mentioned, temperature has an important influence on the extraction performance of

ultrafiltration process.



Fig. 2. Scanning electron microscopy of freeze-dried Jerusalem artichoke flour (200 µm) at different magnitudes: (A) 200x; (B) 1000x and (C) 2000x.



**Fig. 3.** Soluble solids (°Brix) as a function of temperature (°C) for the extraction process of fructans, using water as solvent in a ratio [1:2].



Fig. 4. Soluble solids (°Brix) vs. time (min).

fructans when using water as a solvent. Fig. 3 shows the soluble solids (°Brix) as a function of temperature (°C). As expected, it is observed that increasing the temperature favors the extraction. The solubility of fructans decreases with decreasing temperature (Moerman et al., 2004) and their solubility in water decreases with the increasing degree of polymerization, being the molecules with a high degree of polymerization those with the lowest solubility. Lingyun et al. (2007) determined that the pH variable does not significantly influence the inulin extraction performance, therefore, in this study the pH was likewise measured throughout the process obtaining values in a range of 5.8-6.5. The influence of the storage of the Jerusalem artichoke tubers on the extraction of fructans was evaluated. Samples were stored frozen for 4 months and were compared with freshly harvested samples. When comparing a batch of tubers stored during 4 months at  $-2^{\circ} \pm 1^{\circ}$ C with a batch of freshly harvested tubers (without storage), the same fructan concentration is obtained but in a statistically significant shorter extraction time for the frozen sample (Fig. 4). Samples stored for 4 months reached 6 °Brix at 110 min, while samples without storage reached 6 °Brix at 180 min into the process. This would indicate that storing the tubers allows for shorter extraction times, which also translates into lower energy de-

Table 3
Results of experimental parameter determined during the

Parameter	Feed (F)	Concentrate (C)	Permeate (P)
pH °Brix Density (g/cm³)	$\begin{array}{l} 5.40 \pm 0.40^{a} \\ 6.20 \pm 0.50^{a} \\ 1.032 \pm 0.003^{a} \end{array}$	$\begin{array}{l} 5.75 \pm 0.57^a \\ 8.20 \pm 0.20^b \\ 1.034 \pm 0.001^a \end{array}$	$\begin{array}{l} 5.72 \pm 0.66^a \\ 6.80 \pm 0.50^a \\ 1.030 \pm 0.001^a \end{array}$

Within each column averages followed by the same letter are not significant different (p < 0.05). Results are expressed as mean  $\pm$  SD.

mand. Although the concentration of soluble solids in both conditions (frozen and freshly harvested tubers) reach a maximum of 6 °Brix, it can be observed (Fig. 4) that samples frozen for 4 months present a higher concentration of soluble solids at the beginning of the extraction. Water is contained in the cell structure, so the formation of large crystals can break the cell walls and produce losses of the product structure that will not be recovered when thawed. For this reason, the fructans are more exposed for extraction. Furthermore, by storing the tubers at less than 4°C it avoids the degradation of inulin to sucrose (Saengthongpinit & Sajjaanantakul, 2005; Rubel et al., 2018).

#### 3.4.2. Microfiltration-ultrafiltration membrane process

A characteristic decay of the permeate flow until reaching almost stationary values was observed. The rapid fall of the flow in the first moments is attributed to the phenomenon of concentration polarization. This is produced by a concentration of solutes retained near the membrane, due to a semi-permeable nature, which causes a higher concentration of solutes on the surface of the membrane than within the solution with which some of these solutes return to the membrane. The rejected solutes are deposited on its surface forming a viscous and gelatinous layer, which is usually responsible for the decrease in the filtration flow. The flow can continue to decrease slowly as the filtration progresses, the final decrease in the flow may be a consequence of the concentration of the solute layer having reached the point of super-saturation, which produces fouling itself, or clogging of the pores of the membrane (Marshall et al., 1993; Palatnik et al., 2015). Concentration of the raw materials by ultrafiltration was carried out by continuously removing the permeate flow, and the process was stopped when the desired concentration was achieved. This was determined through the concentration factor (VCR), defined by Cheryan (1986) as the ratio between the initial volume of feed and the volume of the concentrate. For the calculation of the VCR, the volumes of the feed and the concentrate were taken into account. The VCR obtained for the ultrafiltration process was 3.24. pH and °Brix were determined to the streams obtained in the ultrafiltration process, the results are presented in Table 3, where it can be observed the major concentration of soluble solid in the C stream. Analyzing the results of Table 3, it is observed that there is no statistically significant difference between the samples with regard to the density and pH parameters. However, the concentrate presents a statistically significant difference in soluble solids (°Brix).

Table 4

Physicochemical composition and colorimetric analysis of lyophilized extracts.

Sample	Proteins % (w/w)	Fat %(w/w)	Ash %(w/w)	Moisture % (w/w)	Inulin %	a <sub>w</sub>	L*	a*	b*
Ext-F	$5.51\pm0.07^{b}$	$0.23\pm0.02^{a}$	$6.16\pm0.05^{\rm b}$	$6.18\pm0.03^a$	3.2	$0.372\pm0.01^a$	$57.92 \pm 3.71^{a}$	$\textbf{-0.45} \pm 0.04^{b}$	$13.35 \pm 0.14^{a}$
Ext-P	$4.81 \pm 0.05^{a}$	$0.29 \pm 0.03^{a}$	$5.47 \pm 0.05^{a}$	$5.67 \pm 0.17^{a}$	2.4	$0.378 \pm 0.01^{a}$	$80.03 \pm 2.72^{\circ}$	$-1.91 \pm 0.09^{a}$	$15.12 \pm 0.97^{\circ}$
Ext-C	$6.65 \pm 0.05^{\circ}$	$0.22 \pm 0.02^{a}$	$7.50 \pm 0.05^{\circ}$	$6.60 \pm 0.02^{a}$	4.0	$0.398 \pm 0.01^{a}$	$71.81 \pm 1.71^{b}$	$-0.43 \pm 0.03^{b}$	$12.43 \pm 0.31^{a}$

Within each column averages followed by the same letter are not significant different (p < 0.05). Results are expressed as mean  $\pm$  SD.

#### 3.4.3. Physicochemical composition of lyophilized extracts

When carrying out the extraction of fructans from the tubers, other compounds that are not carbohydrates were jointly extracted, so a physicochemical characterization was necessary. Physicochemical parameters evaluated are shown in Table 4. The result demonstrated that the protein concentration by the applied ultrafiltration process was effective. Indeed, the Ext-C that represents the concentrated from the feed, presented the following composition: 6.65% (w/w) proteins; 0.22% (w/w) fat; 7.50% (w/w) ash and 6.60% (w/w) moisture. As already mentioned, in JA tubers 85% of the dry weight corresponds to inulintype fructans. For this reason, the inulin content was determined in the lyophilized extracts obtaining 4% (w/w). Inulin polymers of plant origin are a subject of interest in many food research programs, for their low food caloric value and their dietary fiber effects (Zeaiter et al., 2019). The ash concentration was also observed, because the minerals present could interact with the proteins and thus bind to them. The reported results show that the concentrate has indeed, a higher inulin content. The water activity (a<sub>w</sub>) did not present a statistically significant difference between the three lyophilized extracts. The low values of a<sub>w</sub> allow to achieve product stability from a microbiological point of view. This study presents a simple method for the production of a lyophilized extracts containing fructans from the by-product generated in the production of JAF. These extracts could have potential applications as a functional additive in the formulation of food products.

#### 3.4.4. Colorimetric analysis of lyophilized extracts

The values obtained for the colorimetric parameters  $L^* a^*$  and  $b^*$  are presented in Table 4. The extraction of fructans from JA tubers also entails the extraction of other soluble compounds that can be found in the extract, such as polysaccharides, reducing sugars and sucrose, as well as phenolic compounds and enzymes which give haze and color to the extract. For this reason, Ext-F (Feed flow) has a lower brightness value (L\*), while the permeate flow is the one with the highest luminosity due to the fact that it contains fewer solids, which are responsible of the turbidity. When analyzing a\* and b\* parameters, a slight trend towards green and yellow respectively was observed in all samples.

#### 4. Conclusion

Jerusalem artichoke is a sustainable and easily cultivated source for the production of flour and fructans. Two drying methodologies (convective air oven drying and freeze-drying) were evaluated for the production of Jerusalem artichoke flour (JAF). The drying processes evaluated did not significantly affect the chemical composition of the flours. However, statistically significant differences were observed in the colorimetric parameters evaluated: being the flour dried by lyophilization the one with the best characteristics presented and also similar to the rice flour. When comparing the protein and fiber content of JAF produced with commercial gluten-free flours such as rice flour, it was observed that JAF have a higher fiber and protein content so improved nutritional value. The matrix was adequately observed by electron microscopy. The optimal fructans extraction conditions found from the tubers were: solid/solvent ratio of 1:2 (Kg JA/L distilled water). The extraction was affected by the storage time and temperature of the JA tubers. The tubers storage at freezing temperatures presented better fructans extraction yields, since a higher concentration of soluble solids was obtained in a shorter time. The microfiltration and ultrafiltration process allows to concentrate fructans and the lyophilized concentrate (Ext-C) obtained were rich in proteins, ash and fructans (it presented 4% (w/w) of inulin). Therefore, they could have potential applications as a functional ingredient in the formulation of food products increasing added value in the production of tubers, with the consequent contribution to the development of the local agro-industry, enhancing JA cultivation.

The authors declare that the work has no impact on humans or animals, does not cause damage to the environment.

#### **Ethical Statement**

The manuscript is not submitted to another place for simultaneous consideration. The submitted work is original and is not have been published elsewhere in any form or language (partially or in full), unless the new work concerns an expansion of previous work. and that all listed authors have approved the manuscript before submission, including the names and order of authors;managing all communication between the Journal and all co-authors, before and after publication\*;providing transparency on re-use of material and mention any unpublished material included in the manuscript in a cover letter to the Editor;making sure disclosures, declarations and transparency on data statements from all authors are included in the manuscript as appropriate.

#### **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.

#### References

- Editor AOAC. (2016). In G. W. Latimer (Ed.), Official methods of analysis (20th ed.). USA: Association of Official Analytical Chemists. Editor.
- Abeynayake, S. W., Etzerodt, T. P., Jonaviclené, K., Byme, S. Asp. T., & Boelt, B. (2015). Fructan metabolism and changes in fructan composition during cold acclimation in perennial rygrass. *Frontiers Plant Science*. 10.3389/fpls.2015.00329.
- Ahmed, W., & Rashid, S. (2019). Functional and therapeutic potential of inulin: A comprehensive review. Critical Reviews in Food Science and Nutrition, 59(1), 1–13.
- Apolinário, A. C., Goulart de Lima, B. P., Esberard de Macedo, N., Pessoa, A., Converti, A., & da Silva, A (2014). Inulin-type fructans: A review on different aspects of biochemical and pharmaceutical technology. *Carbohydrate Polymers*, 101, 368–378.
- Asmeda, R., Noorlaila, A., & Norziah, M. H. (2015). Effects of different grinding methods on chemical and functional properties of MR 211 rice. *International Journal of Food Engineering*, 1(2), 111 -111. 10.18178/ijfe.1.2.111-114.
- Cheryan, M. (1986). Ultrafiltracion handbook. USA: Technomic Publishing Co., INC.
- Díaz, A., Bomben, R., Dini, C., Viña, S. Z., García, M. A, Ponzi, M., & Comelli, N. (2019). Jerusalem artichoke tuber flour as a wheat flour substitute for biscuit elaboration. *LWT - Food Science and Technology*, 108, 361–369.
- Drakos, A., Kyriakakis, G., Evageliou, V., Protonotariou, S., Mandala, I., & Ritzoulis, C. (2017). Influence of jet milling and particle size on the composition, physicochemical and mechanical properties of barley and rye flours. *Food Chemistry*, 215, 326–332.
- Gonzalez, U., Ferraris, M. P., Fernández, S., Campderros, M., & Menéndez, C. (2016). Nutritional value analysis of atriplex lampa protein concentrates obtained by thermocoagulation and ultrafiltration. *International Journal of Current Advanced Research*, 5-10, 1284–1289 ISSN: E: 2319-6475; ISSN: P: 2319-6505.
- Ibarguren, L., & Rebora, C. (2013). El cultivo de topinambur: generalidades sobre su ecofisiología y manejo. Horticultura Argentina, 32(77), 35–41.
- Kang, I., Yu Suk Kim, M. D., & Choongbai Kim, M. D. (2007). Mineral deficiency in patients who have undergone gastrectomy. *Nutrition*, 23(34), 318–322.
- Kowalski, S., Mikulec, A., Mickowska, B., Skotnicka, M., & Mazurek, A. (2022). Wheat bread supplementation with various edible insect flours. Influence of chemical composition on nutritional and technological aspects. *LWT*, 159, Article 113220.
- Lingyun, W., Jianhua, W., Xiaodong, Z., Da, T., Yalin, Y., Chenggang, C., Tianhuab, F., & Fan, Z. (2007). Studies on the extracting technical conditions of inulin from Jerusalem artichoke tubers. *Journal of Food Engineering*, 79(3), 1087–1093.

- Marshall, A. D., Munro, P. A., & Trägardh, G. (1993). The effect of protein fouling in microfiltration and ultrafiltration on permeate flux, protein retention and selectivity: A literature review. *Desalination*, 91, 65 and references therein.
- Moerman, F. T., Van Leeuwen, M. B., & Delcour, J. A. (2004). Enrichment of higher molecular weight fractions in inulin. *Journal of Agricultural and Food Chemistry*, 52(12), 3780–3783.
- Moon, KB., Ko, H., Park, JS., et al., (2019). Expression of Jerusalem artichoke (Helianthus tuberosus L.) fructosyltransferases, and high fructan accumulation in potato tubers. *Applied biological chemistry*, 62, 74. 10.1186/s13765-019-0481-x.
- Neyrinck, A., Theulier, P., Jouret, A., Taminiau, B., Daube, G., Frederick, R., Cani, P., & Delzenne, N. (2015). Impact de nutriments prébiotiques de type inuline sur la glycémie: miseenévidence de nouvellesciblesthérapeutiques. *Diabetes & Metabolism*, 41(1), 82–83.
- Ostermann Porcel, M. V., Campderrós, M. E., & Rinaldoni, A. N. (2017). Effect of Okara flour addition on the physical and sensory quality of wheat bread. *MOJ Food Processing* & *Technology*, 4(6), 1–7. 10.15406/mojfpt.2017.04.00111.
- Palatnik, D. R., Ostermann Porcel, M. V., González, U., Zaritzky, N., & Campderrós, M. E. (2015). Recovery of caprine whey protein and its application in a food protein formulation. *LWT- Food Science and Technology*, 63, 331–338.
- Rakhimov, D. A., Arifkhodzhaev, A. O., Mezhlumyan, L. G., Rozikova, U. A., Aikhodzhaeva, N., & Vakil, M. (2003). Carbohydrates and proteins from Helianthus tuberosus. *Chemistry of Natural Compounds*, 39(3), 312–313.
- Rebora, C., Lelio, H., Ibarguren, L. & Gómez, L. (2011), Efecto de la densidad de plantación sobre el rendimiento de topinambur (*Helianthus tuberosus L.*) regado con aguas residuales urbanas, en: www.bdigital.uncu.edu.ar
- Ritsema, T., & Smeekens, S. (2003). Fructans: Beneficial for plants and humans. Current Opinion in Plant Biology, 6(3), 223–230.
- Rubel, I. A, Iraporda, C., Novosad, R., Cabrera, F. A., Genovese, D. B., & Manrique, G. D. (2018). Inulin rich carbohydrates extraction from Jerusalem artichoke (Helianthus tuberosus L.) tubers and application of different drying methods. *Food Research International*, 103, 226–233.

- Rubel, I. A., Iraporda, C., Manrique, G. D., Genovese, D. B., & Abraham, A. G. (2021). Inulin from Jerusalem artichoke (*Helianthus tuberosus L.*): From its biosynthesis to its application as bioactive ingredient. *Bioactive Carbohydrates and Dietary Fibre*, 26, Article 100281.
- Saengthongpinit, W., & Sajjaanantakul, T. (2005). Influence of harvest time and storage temperature on characteristics of inulin from Jerusalem artichoke (*Helianthus tubero*sus L.) tubers. Postharvest Biology and Technology, 37(1), 93–100.
- Tarifa, M. C., Piqueras, C. M., Genovese, D. B., Rubel, I. A., Sica, M. G., & Brugnoni, L. I. (2021). Symbiotic pectin microparticles with native Jerusalem artichoke (*Helianthus tuberosus L.*) enhance Lactobacillus paracasei subsp. Tolerans survival. *Revista Argenta Microbiología*. 10.1016/j.ram.2021.03.001.
- Vega-Mercado, H., Góngora-Nieto, M. M., & Barbosa-Cánovas, G. V. (2001). Advances in dehydration of foods. *Journal of Food Engineering*, 49, 271–289.
- Von Atzingen, M. C., & Machado Pinto, M. E. (2005). Evaluación de la textura y color de almidones y harinas en preparaciones sin gluten. *Ciencia y Tecnología Alimentaria*, (4), 319–323.
- Wang, X. (2009). Prebiotics: Present and future in food science and technology. Food Research International Review, 42(1), 8–12.
- Yovchev, A., & Le-Bail, A. (2021). Effect of Jerusalem artichoke flour on wheat dough physical and mechanical properties. *Applied Food Research*, (1), Article 100026.
- Zeaiter, Z., Regonesi, M., Cavini, S., Labra, M., Sello, G., & Gennaro, P. (2019). Extraction and characterization of inulin-type fructans from artichoke wastes and their effect on the growth of intestinal bacteria associated with health. *BioMed Research International*, 1–8, 10.1155/2019/1083952.
- Zuleta, A., & Sambucetti, M. E. (2001). Inulin determination for food labeling. Journal of Agricultural and Food Chemistry, 49(10), 4570–4572. 10.1021/jf0105050.