Green technologies for the extraction of proteins from jackfruit leaves (*Artocarpus heterophyllus* Lam)

Laura Cristina Moreno-Nájera¹ · Juan Arturo Ragazzo-Sánchez¹ ·
Cristina Regla Gastón-Peña² · Montserrat Calderón-Santoyo¹

Received: 6 February 2020 / Revised: 1 September 2020 / Accepted: 9 September 2020 / Published online: 6 October 2020
© The Korean Society of Food Science and Technology 2020

Abstract The application of emerging technologies such as ultrasound, microwaves and high hydrostatic pressure, allows the extraction of compounds in a sustainable manner from a vegetable matrix with a high value such as jackfruit leaf proteins (*Artocarpus heterophyllus* Lam). Currently, the main method of protein extraction is based on the precipitation with the use of an aggressive solvent, therefore it is necessary to optimize extraction methods with a minimum waste production. In the protein extraction of jackfruit leaves, we obtained a content of 84.1 mg/g using solvents. On the other hand, emergent extractions such as ultrasound, microwaves, and high hydrostatic pressure showed concentrations of 96.3, 95.6 and 147.3 mg/g, respectively. In addition, we found that the best extraction agent was 0.5 M NaCl, offering a range of possibilities that support green technologies as an imperative change in the food industry.

Keywords Ultrasound · Microwave · High hydrostatic pressure · Jackfruit leaf · Protein

Introduction

The jackfruit (*Artocarpus heterophyllus* Lam) tree has leaves which are bright dark green in color, oval in shape and alternate in the arrangement in adult branches and lobed in young branches (Piña-Dumoulin et al. 2010). The trees should be pruned to the first lateral branch, which causes vertical growth to slow down and increases the branching of the canopies before the rainy season (Larrea-Wachtendorff et al. 2015). Likewise, the elimination of the old branches at the end of the season increases the penetration of light in the cups, enhancing the production of fruits. The production of jackfruit leaves varies depending on multiple factors such as the density of sowing, the application of fertilizers, the weather or the pruning technique. For *Morus* spp in India, it has been reported sub-products of the pruning up to 40 ton/ha of fresh leaves per year (approximately 10 tons of dry leaf). The jackfruit leaves have been used in low concentrations due to their properties as palliative methods to treat anemia, diarrhea, and cough. Besides, young jackfruit leaves have also been utilized in minimum quantities as forage material, the rest of the leaves are discarded contributing to the formation of organic solid waste (Jaiswal 2012). In Chile, the use of leaves *Morus* spp as a food source for livestock has been encouraged. This action is because of its palatability and protein content in leaves and tender stems that varies between 13 and 28% dry basis according to the variety (Reddy and Elanchezhian 2019). Besides this, it has an excellent profile of essential amino acids. Likewise, trees with a warm sub-humid climate, such as orange and mandarin, have an estimated protein content of 4.6, and 6.2% wet base, respectively (Reyes-Santamaría et al. 2000). Therefore, the extraction of jackfruit leaf proteins is a sustainable alternative to give added value to pruning

✉ Montserrat Calderón-Santoyo
montserratcalder@gmail.com; mcalderon@tecni.mx

Laura Cristina Moreno-Nájera
themis_laura@hotmail.com

Juan Arturo Ragazzo-Sánchez
arturoragazzo@hotmail.com; jragazzo@tecni.mx

Cristina Regla Gastón-Peña
cristina.gaston51@gmail.com;
cristina.gaston@icidca.azcuba.cu

¹ National Technologic of Mexico/Technologic Institute of Tepic, 2595 Tecnológico Av., 63175 Tepic, Nayarit, Mexico

² AZCUBA Sugar Group, 171 23th Av., Havana, Cuba

waste, through the use of emerging technologies such as ultrasound, microwaves and high hydrostatic pressure. Precipitation with solvents is the most used technique for the extraction of proteins. Nowadays, the trend is the use of natural products due to the interest to minimize risks in the production process and decrease the use of chemical substances. Consequently, the implementation of green technologies (eco-friendly) in the business industry is an indicator of good environmental practices and a high purchasing value of products. In this regard, ultrasound through acoustic cavitation can be used as an alternative extraction method. In this method, the sound is transported as a wave through a liquid, with alternating cycles of compression and expansion that generate negative pressures for the formation of microbubbles or cavities, until they collapse violently during a cycle of low pressure, reaching values of temperature, pressure and shear force up to 5000 °C, 1000 atm and 200 m/s, respectively (Ochoa-Rivas et al. 2017). These microflows are able to damage the plant cell membrane, enabling the extraction of proteins by the increase in the contact surface with the solvent. It has been found that the extraction of proteins by ultrasound or ultrasound-assisted techniques, reaches more efficient results than some traditional methods such as thermal extraction. The technologies previously mentioned are more economical and simple than unconventional methods such as extraction by supercritical fluids, thus it can be used in the industry (Saini and Keum 2018). Ochoa-Rivas et al. (2017) evaluated the effects of the ultrasound-assisted microwave for the alkaline extraction of peanut proteins. The results of that investigation showed 77% more protein compared to conventional methods. In the same study, an increase in the purity of the proteins extracted, water absorption, foaming, and gelling activities were recorded, since it contributed to the extraction of peanut protein for the production of high purity isolates. Similarly, Djilani et al. (2006) mentioned that microwave extraction is more efficient than conventional methods such as solvent extraction at room temperature and thermal reflux because microwaves generate heat within the material, which leads to faster heating speeds in less time as compared to the conventional heating methods (Li et al., 2010; Wannberg et al. 2006). This effect is attributed to microwave irradiation, which causes excitation of molecules by ion migration and dipole rotation, contributing to a rapid transfer of energy to solvent (Djilani et al. 2006). This, in turn, helps to destabilize the aqueous envelope of the proteins by thermal partial denaturation and induces their precipitation. Choi et al. (2006) carried out two types of methods to extract soy protein by using precipitation with a water bath through temperature variations and microwaves. These authors found a higher yield in the use of microwaves compared to the conventional method. This effect was

attributed to the microwave radiation that dramatically affected the microstructure of the soy cells, which allowed an increase in its extraction. On the other hand, the high hydrostatic pressure as an extraction method has been recognized as an environmentally friendly technology by the FDA that is already applied in the industry (Yang et al. 2011). In the high hydrostatic pressure, the material is compressed by the transmitting medium around the food, which causes a decrease in the volume that varies according to the pressure and temperature applied (Barbosa-Cánovas et al. 1999). This, in turn, favors the dissociation of the oligomeric proteins, causing the unfolding of their chains, which alters their quaternary and tertiary structure (Tabilo-Munizaga et al. 2014). It has been demonstrated that pressures above 200 MPa induce the denaturation of proteins, altering the balance of non-covalent interactions that stabilize the native conformation of many proteins (Chao et al. 2018). Nevertheless, most of the studies carried out related to protein interaction with high pressure have been focused on enzyme deactivation. In this respect, the use of this technology as a green extraction method is not well documented, hence, it is a field of innovation in a growing market where the commitment to the environment represents a responsibility. Therefore, in this work, we seek to optimize the extraction conditions of jackfruit leaf proteins with green technologies such as ultrasound, microwaves and high hydrostatic pressure using “eco-friendly” solvents.

Materials and methods

Biological material

The leaves were collected in June 2017 from “Las Varas”, Compostela, Nayarit (latitude 21.178333 and longitude – 105.136944) at 20 msnm. Pruning leaves free of damage and impurities were selected, washed with water and then dried in CA550 thermal chamber (NOVATECH, Guadalajara, Mexico) at 40 °C for 3 h. After, the enervation and petiole were removed from the leaves, ground and homogenized in Pro 900 (NUTRIBULLET, Los Angeles USA) until a fine powder was obtained. Finally, the powder was sieved in a 20 mesh (0.841 mm) and kept at – 80 °C until further use.

Proximal chemical analysis of the jackfruit leaf

Moisture, crude protein, lipids, and ash contents in the jackfruit leaves were determined according to the procedures established by the AOAC official methods (AOAC 2000). Gravimetric method AOAC 931.04 for moisture content, Kjeldahl method AOAC 955.04 for total protein

content, Soxhlet method AOAC 920.39 for lipids content, and AOAC 940.26 for ash content. The nitrogen-free matter was calculated based on the difference of the sum of the previous determinations and the total mass of samples.

Denaturation temperature

A non-denaturing extraction by maceration was carried out. 3 g of lyophilized proteins and 10 mL of deionized water were placed in a cold crucible and then placed in an ice bath 1243 (BOEKEL SCIENTIFIC, Feasterville, USA) with constant stirring. After, 20 mL of 70% $(\text{NH}_4)_2\text{SO}_4$ was added, macerated under stirring and then left overnight. Subsequently, the sample was placed in tubes and centrifuged at 11,000 rpm for 10 min at 4 °C in a centrifuge Z306 (HERMLE, Wehingen, Germany). The sediment was discarded and the supernatant was placed under stirring in an ice bath. 90% $(\text{NH}_4)_2\text{SO}_4$ was added in a 1:1 v/v ratio and the resulting mixture was centrifuged under the conditions previously described. The precipitate was filtered through a 100 μm Millipore filter to eliminate excess salts. The calorimetric analysis was carried out in DSC-250 equipment (TA instruments, Delaware, USA) using a heating ramp of 20–150 °C at a heating rate of 5 °C/min and inert atmosphere of nitrogen (Paulsson and Dejmek 1990). The data was analyzed in TRIOS 4 software.

Molecular mass of jackfruit leaf proteins

SDS-PAGE was performed to determine the molecular mass profile of the leaves proteins (Kobayashi et al. 2016). 20 μL of the protein samples and standards were mixed with 5 μL of loading buffer 5 $\times$ and heated at 100 °C for 5 min. 20 μL of the protein samples and the standard were loaded into the gel. The proteins were separated by SDS-PAGE on a 12% polyacrylamide gel at 220 V for 90 min and stained with Coomassie blue.

Protein extraction

5 g of leaf powder was mixed with 30 mL of 1 M NaOH in distilled water and stirred for 30 min. 1 M NaOH was added until pH of 12.5 and then centrifuged at 5000 rpm for 20 min in Z306 centrifuge (HERMLE, Waseerburg, Germany). The liquid was adjusted to pH 2 with 1 M HCl until precipitated. The sample was filtered and the retentate was dried for 6 h at 30 °C in the NOVATECH chamber (Boye et al. 2010).

Ultrasound-assisted extraction

The sample was suspended in NaCl (0.5, 1 or 2 M) in a ratio of 1:5 m/v, stirred 15 min at 25 °C and then sonicated for 10, 15 or 20 min at 25 °C in the sonicator JP-4820 (KENDAL, Florida, USA) at 42 kHz. The NaCl residues were removed with water by centrifugation at 5000 rpm for 15 min. The pellet was suspended in 70% ethanol and stirred for 15 min at 25 °C. Ultrasound was applied again (10, 15 or 20 min) at 42 kHz and centrifuged at 5000 rpm for 15 min. The supernatant was removed and the pellet was resuspended with water. The sediment was sonicated again (10, 15 or 20 min), with ethanol (1:3 v/v) at 70% added with 1% sodium acetate (in relation to the volume of ethanol added 0, 1 or 2%) keeping the agitation conditions. The sample was centrifuged without washing. 70% ethanol (1: 3 w/v) added with sodium acetate as the reducing agent (0, 1 or 2% in relation to the ethanol volume added) was incorporated to the sediment under stirring. The sonication conditions were the same as the previously mentioned (10, 15 or 20 min). The residues were centrifuged with ethanol and sodium acetate, removing with successive washes of 1:1 water–ethanol solution at room temperature. Finally, it was filtered and dried at 30 °C for 6 h in the thermal chamber CA550 (NOVATECH, Guadalajara, Mexico) (Ochoa-Rivas et al. 2017).

Microwave-assisted extraction

10 g of sample was placed in a ratio of 1:5 respect to the extraction solvent (0.5 M NaCl, 96% ethanol or absolute methanol) and CH_3COONa addition (1, 2 or 3%). The microwave extraction (Whirlpool WMC30516HZ, USA) was carried out for 2, 3 or 4 min (applying pulses of 30 s) at 1200 W. The precipitate was separated by filtration with a 0.2 μm filter. The sample was dried at 30 °C for 2 h in the CA550 thermal chamber.

High hydrostatic pressure-assisted extraction

The 1:5 sample ratio with respect to the extraction solvent (0.5 M NaCl, 96% ethanol or absolute methanol) was vacuum packed with high-density polyethylene bags using Multivac equipment. High pressure was applied to CIP42260 equipment (Erie PA USA) at 100, 200 or 300 MPa, for periods of 10, 15 and 20 min. The precipitate was separated by filtration with a 0.2 μm filter. It was dried at 30 °C for 2 h in CA550 thermal chamber.

Determination of jackfruit leaf proteins

The extracts obtained by ultrasound, microwave, and high hydrostatic pressure were analyzed by the Bradford method

(Bradford 1976). 2.5 mg of the samples were diluted in 1 mL of distilled water. In a 96-well microplate, 20 μ L of distilled water, 20 μ L of the sample and 190 μ L of the 75% Bradford reagent were added. The absorbance was determined at 595 nm on SYNERGY HTX microplate reader (BIOTEK, Vermont, USA) by readings each 20 s. A calibration curve was made with bovine serum albumin according to the conditions specified in the reagent.

Optimization

In order to optimize the extraction parameters of the three techniques, response surface methodology based on a CCD (central composite design) model with a factorial design 3^3 and maximization of the response variable (protein extracted concentration) were used. Validation of the model of response surface methodology was performed by comparison of the estimated extracted proteins obtained by the model and experimental values. Least significant differences (LSD) test was performed to find statistically significant differences among techniques and to determine the best extraction method. All statistical analysis were conducted using the STATISTICA software version 10.

Results and discussion

Proximal chemical analysis of the jackfruit leaf

The proximal chemical analysis indicated that the humidity percentage was lower than leaves of sub-humid warm climate, such as the soursop, which is 63% (Table 1). The low percentage of humidity of the jackfruit leaves ($55.87 \pm 3.4\%$) was attributed to the fact that the leaves analyzed in this study were a product of pruning before the rainy season in the months of May and June, in which the average relative humidity is from 47 to 62% (INEGI, 2017). This helps the evaporation of water from the leaf to achieve a water balance with the environment and therefore a reduced humidity. The protein content (19.33% dry base) was consistent with the 13–20% of mulberry leaf proteins reported by Reddy and Elanchezhian (2008) which is indicative of a good source of nitrogen available for use as

forage and livestock feed. Moreover, it provides an excellent source for the extraction of isolates, hydrolysates, and protein concentrates. Consistent percentages with the ash content (16.1%) in mulberry trees were found. In the mulberry trees, the ash content is up to 17% which represents a high ash content, therefore, the jackfruit leaf is an excellent source of minerals.

Determination of denaturation temperature

The enthalpy of denaturation can be defined as the heat absorbed during the unfolding of the proteins (Porrás-Godínez et al., 2015). In the jackfruit leaf proteins, we observed an enthalpy relaxation value of 3.7154 J/g. In the thermogram, an endothermic peak was recorded at 111.96 °C (Fig. 1A), attributed to the denaturation temperature. Hydrogen bonds and the hydrophobic effect are the dominant non-covalent interactions in the stability of proteins. The endothermic transitions observed in the thermogram corresponds to the opening temperature of the globular structure accompanied by rupture of non-covalent bonds. The enthalpy change is the result of the combination of the endothermic reactions produced by the breakdown of the intermolecular bonds of the native protein and the new bonds that formed in the aggregates (Porrás-Godínez et al., 2015). When losing the compact structure, the nonpolar interior of the protein is exposed to the solvent and thus the water molecules are structured in such a way that they solvate these groups, so that the highest specific heat (C_p) in the denatured protein reflects the extra energy that is required to break the structure of the water around the non-polar groups. The importance of non-polar amino acids in stability is based on the abundance of non-polar groups in the interior of the native protein (Porrás-Godínez et al., 2015). The variation of enthalpy due to the unfolding of the protein is a thermodynamic parameter, which is associated with reversible processes, that uses very slow heating rates and very low concentrations of proteins to avoid thermal aggregation (Liu et al., 2000).

Determination of the molecular mass of extracted proteins

Electrophoresis results exhibited problems to obtain the electrophoretic profile because at the time of extraction no protective agent is added, nor does it perform any chemical treatment that eliminates compounds of a non-protein nature. Hence, for this analysis, the jackfruit protein was extracted with TCA-Acetone (Pineda-Guerra et al., 2016) and an extraction with loading buffer (ISTA) for the analysis of plant proteins rich in secondary metabolites that interfere with the separation of proteins and affect the quality of electrophoresis (ISTA, 2018). The SDS-PAGE

Table 1 Chemical composition (%) of the jackfruit leaf

Humidity	55.87 ± 3.4
Protein	8.69 ± 0.5
Fat	1.83 ± 0.2
Nitrogen free extract	27.74 ± 0.1
Ashes	7.24 ± 0.1

Fig. 1 Characterization of extracted protein from jackfruit leaf. (A) Thermogram of the temperature denaturing for proteins. (B) Electrophoregram of molecular mass distribution of the proteins

gel (Fig. 1B) shows the distribution of the molecular mass of the jackfruit leaf proteins. The extraction with TCA-Acetone showed diffuse and fine bands from 116 to 14 kDa. In an analogous way, the extraction by the loading buffer showed a well-defined proteins bands from 70 to 14 kDa because the extraction with the loading buffer does not contain any lytic agent, showing only the proteins soluble in these compounds. The SDS (a component of the β -Mercaptoethanol buffer), does not show any specificity for hydrophilic or hydrophobic compounds. However, due to the extraction of the proteins contained in the cell or the vacuoles was not carried out, only the low molecular weight proteins exposed to the medium can be extracted (Preece et al., 2017).

Isoelectric precipitation

The content of proteins found in the jackfruit leaf by isoelectric precipitation was 84.1 mg/g in a dry base. Shen et al. (2008) mentioned that proteins in plant cells are poorly soluble in water due to their hydrophobic nature and disulfide binding between protein molecules. In this method, the pH of the proteins represents its alkaline point (pH at 12) which is recommended not to reach, due to a severe alkaline extraction leads to the racemization of amino acids, reduction of protein digestibility and the damage of some amino acids (lysine and cysteine) that diminish the nutritive value of proteins (Schwass and Finley, 1984).

Ultrasound-assisted extraction

The ultrasound-assisted extraction revealed a direct dependence of the processing time ($p = 0.00002$),

obtaining concentrations in a range from 3.72 to 9.74% on a dry basis (Fig. 2). The mathematical model obtained in this investigation showed a significant effect on the prediction of protein extraction by ultrasound with a value of $p = 0.0009$, and $R^2 = 0.72$. The second order mathematical model obtained for this design (Eq. 1) is a relationship between the observed ranges and the empirical prediction of ultrasound-assisted protein extraction.

$$\begin{aligned} \% \text{ protein} = & 10.40 - 0.95x + 0.03x^2 - 2.10y + 0.20y^2 \\ & + 0.14xy - 0.10(2)x - 0.24(2.06) + 4.13 \end{aligned} \quad (1)$$

The optimal value for proteins extraction was 96.3 mg/g, exceeding the concentration by isoelectric precipitation with the characteristic of represents a green technology since it was used as extraction solvent NaCl 1 M. Experimental validation using the optimal parameters gave 92 ± 1.2 mg/g. Because the experimental value was inferior than the estimated value, the model is considered as a reserved model (Table 2). Kim et al. (2018) extracted proteins from porcine myocardium using NaCl and other salts. The authors attribute a change in the ionic strength of the solution, therefore a reduction of the electrostatic charge between the nearby groups, producing the solvation of ions (Salting-in). On the other hand, we determined that the best time of sonication was at 20 min. These results can be due to the phenomenon of cavitation. The mechanism of extraction by ultrasound involves two variables, the first is diffusion through cell walls, and the second the extraction of the cellular content of the solvent when the cells are already fragmented. According to Veggi et al. (2013), this is a consequence of the ultrasonic waves that modify the physical and chemical properties of the vegetable matrix, reflecting in a high percentage of extracted proteins.

Fig. 2 Model optimization using response surface of ultrasound-assisted extraction of proteins, (desired level = 1). The graph shows the effect of the Molarity of NaCl extraction solvent (0.5, 1 and 2 M), time of sonication (10, 20, and 30 min) and the level of sodium acetate (0, 1 and 2%). Analysis by DCC (n = 3)

Table 2 Validation for optimization models

Method	Theoretical (mg/g)	Experimental (mg/g)
Isoelectric point	–	84.1 a
Ultrasound	96.3 b	94.9 ± 1.2 b
Microwave	95.6 b	87.6 ± 0.8 b
High hydrostatic pressure	147.3 c	135.9 ± 1.5 c

The results indicate the value ± standard deviation. Different letters indicate significant differences. LSD Fisher stockings ($\alpha = 0.05$)

In addition, we found a sodium acetate content of 1% as the optimum parameter for extraction. This can be explained to the fact that the salt added causes an opposite effect to solubilization by salting, competing for solvation with the water molecules with the proteins. This, in consequence, causes a decrease in the amount of soluble protein available for extraction (Fig. 2). Validation for these optimal values is shown in Table 2.

Microwave-assisted extraction

The results for the extraction of proteins in jackfruit leaf by microwave showed a variation of 3.6–10.4% in function of

time and time-solvent interaction ($p = 0.00008$ and 0.0003 , respectively) with an $R^2 = 0.75$. The extraction with 0.5 M NaCl showed the best results followed by ethanol and methanol, as well as 4 min processing and no addition of CH_3COONa . The methanol showed an extraction estimated value of 95.6 mg/g, which represents the lowest percentage of protein obtained by the three emerging extraction methods (Fig. 3). The mathematical model for this extraction method is shown in Eq. 2. Experimental validation using the optimal parameters gave 87.6 ± 0.8 (Table 2). Then, the model is considered reserved.

Fig. 3 Model optimization using response surface of the microwave-assisted extraction of proteins, (desired level = 1). The graph shows the effect of sodium acetate (0%, 1% and 2%), type of extraction solvent (NaCl 0.5 M = 1, ethanol 96% = 2 and absolute methanol = 3) and exposure time (2, 3, and 4 min). Analysis by DCC (n = 3)

$$\begin{aligned} \% \text{ protein} = & 5.67 - 3.74x + 1.27x^2 - 0.622y + 0.22y^2 \\ & + 0.15xy - 0.2(3.0)x - 0.28(3.0)y + 4.4 \end{aligned} \quad (2)$$

The results previously mentioned might be explained with the salting-in effect, which causes the surface charges of the protein to neutralize hydration and solubility decrease, which then leads to precipitation, known as the salting-out effect (Xu et al., 2015). The addition of organic solvents such as ethanol and methanol reduces the dielectric constant of the medium that decreases the electrostatic forces present in protein molecules, contributing to their aggregation and precipitation (Cheftel et al., 1989). Ochoa-Rivas et al. (2017) varied the parameters of power and time in the extraction of peanut proteins using the microwave. The authors found the best yields at the longest time (4 min) and power evaluated. These same authors reported extraction times greater than 8–10 min for a yield of 55%. On the other hand, Liu et al. (2011) mentioned that the temperature reached during exposure to microwaves must be proportional to the denaturation temperature. Therefore, a positive relationship exists between the microwave

temperature and extraction of soy proteins, reaching the optimum at 60.1 °C (Denaturing temperature of soybeans 60 °C).

High hydrostatic pressure-assisted extraction

The results for the extraction of proteins in the leaf of jackfruit by high hydrostatic pressure showed a variation from 2.4 to 17.4%. The predictive mathematical model for this extraction method is described in Eq. 3. The synergistic interactions of the evaluated variables were both statistically significant, for the pressure ($p = 0.0003$) and for the type of solvent ($p = 0.0005$). The mathematical model evaluation was significant for the extraction of proteins by high hydrostatic pressure $p = 0.00006$ with an $R^2 = 0.856$. The effects of the pressure in relation to the solvent showed a synergic effect in the maximization of the extraction percentages (Fig. 4). The optimal parameters were 300 MPa of pressure with NaCl 0.5 M as the solvent and 20 min of time process to obtain 147.3 mg/g of protein. The validation for these optimal values by obtaining

Fig. 4 Model optimization using response surface of high hydrostatic pressure-assisted extraction of proteins, (desired level = 1). The graph shows the effect of pressure (100, 200 and 300 MPa), the type of extraction solvent (NaCl 0.5 M = 1, ethanol 96% = 2 and absolute methanol = 3) and exposure (10–15–20 min) Analysis by DCC (n = 3)

the experimental value gave 135.9 ± 1.5 of protein (Table 2). Then, again, a reserved model was obtained.

$$\begin{aligned} \% \text{ protein} = & 15.39 - 0.11x + 0.65x^2 - 0.08y + 0.0001y^2 \\ & - 0.00xy - 0.2(15)x + 0.003(15)y - 1 \end{aligned} \quad (3)$$

During exposure to the high hydrostatic pressure, proteins undergo conformational changes in their structure due to the destabilization of weak interactions, which contribute to their tertiary and quaternary structure (Chao et al., 2018). In this regard, in the protein of the jackfruit leaf, a decrease in the concentration of extracted proteins at 200 MPa was observed. This behavior could be due to the formation of disulfide bonds, which are favored by the increase of the pressure and the formation of ionic bonds with the salts and solvents of the medium. This increases its solubility and therefore, decreases its extraction. This solubility effect is diminished at 300 MPa in which the solubility of the protein decreases and therefore, an increase in the concentration of extracted proteins is observed. Again, the best green extraction solvent was 0.5 M NaCl. The effect of salty solubility is caused by changes in the ionization tendency of the $-R$ dissociable

groups of the protein (Wood, 2000). In the same way, we observed that the hydrostatic pressure has a significant effect on the extraction of proteins due to the concentration was increased at a higher pressure. One possible explanation is to the fact that at the moment in which the pressure is exerted, the extraction matrix (jackfruit leaf) suffer an increase in the porosity of the membranes when the osmotic and hydrostatic pressure are equal (Cheftel et al., 1989) following the principle of Le Chatelier. Consequently, it causes greater contact between the extraction solvent and the compound of interest, increasing the diffusion of the solvent in the plant cells, which in turn facilitates the extraction of proteins. The most outstanding method was the high hydrostatic pressures using 0.5 M NaCl as an extraction solvent because it showed a greater concentration of extracted proteins compared with the other techniques and solvents tested in this investigation.

The solubility of proteins is determined by three main factors such as the degree of hydration, the density, and distribution of charges along the chain, as well as the presence of non-protein compounds such as phosphates, carbohydrates or lipids that may have a stabilizing effect (Badui et al., 2006). In addition, the solubility of proteins

depends fundamentally on pH, ionic strength, type of solvent and temperature (Cheftel et al., 1989). Therefore, the highest protein extraction yield can be attributed to the ionic strength in combination with the contact surface at high hydrostatic pressure. It is important to mention that macromolecules such as proteins can undergo a reversible denaturation during the application of high hydrostatic pressure. This effect is reinforced by Le Chatelier's Principle which together with the Pascal Principle, governs the physical process of APH. According to this principle, the pressurization accelerates the reactions that involve a volume change, favoring them in the direction of its decrease (Larrea-Wachtendorff et al., 2015)

It was demonstrated that the emerging methods are effective and sustainable processes, comparable with the traditional procedures for the extraction of jackfruit leaf proteins. The method of high hydrostatic pressures using 0.5 M NaCl as an eco-friendly solvent produced high yields of protein. To our knowledge, this is the first report that uses this green technology in the extraction of proteins from vegetable sources.

Acknowledgments This study was carried out with the support of "National Technologic of Mexico" Project No. 6811.18-P and is part of the activities of the CYTED network No. 319RT0576. The authors thank CONACYT (Mexico) for the scholarship No. 466318.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- AOAC International. Official method 931.04, Official method 940.26, Official method 955.04, Official method 920.39. Association of Official Analytical Chemists, Washington, DC, USA (2000)
- Badui S, Guerrero I, López E, Armenta R. Química de los alimentos. 4a ed. Ed. Pearson, México, 717 p (2006)
- Barbosa-Cánovas GV, Palou E, Pothakamury UR, Swanson BG. Conservación no térmica de alimentos. Ed. Acribia. S.A. Department of Biological Systems Engineering, Washington State University, USA. (1999)
- Boye JI, Aksay S, Roufik S, Ribéreau S, Mondor M, Farnworth E, Rajamohamed SH. Comparison of the functional properties of pea, chickpea and lentil protein concentrates processed using ultrafiltration and isoelectric precipitation techniques. *Food Res. Int.* 43: 537-546 (2010)
- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248-254 (1976)
- Chao D, Jung S, Aluko RE. Physicochemical and functional properties of high pressure-treated isolated pea protein. *Innov. Food Sci. Emerg. Technol.* 45: 179-185 (2018)
- Cheftel J, Cup J, Lorient D. Proteínas alimentarias., Ed. Acribia. Zaragoza, España. 346 p (1989)
- Choi IL, Choi SJ, Chun JK, Moon TW. Extraction yield of soluble protein and microstructure of soybean affected by microwave heating. *J. Food Process. Preserv.* 30: 407-419 (2006)
- Djilani A, Legseir B, Soulimani R, Dicko A, Younos C. New extraction technique for alkaloids. *J. Braz Chem Soc.* 17: 518-520 (2006)
- INEGI. Banco de información INEGI. Retrieved December 14, 2018 from <http://www.inegi.org.mx/biinegi/> (2017)
- ISTA International Seed Testing Association. In: International rules for seed testing. Zurich, Switzerland. p 345 (2018)
- Jaiswal BS. *Artocarpus*: A review of its traditional uses, Phytochemistry and Pharmacology. *Int. J. Pharma. Bio. Sci.* 3(4): 104-111 (2012)
- Kim HK, Kim YH, Kim YE, Jung SK, Lee NH, Song KM. Effects of salts on ultrasonic extraction of protein from porcine myocardium. *Food Bioprod. Process.* 108: 12-17 (2018)
- Kobayashi Y, Yang T, Yu CT, Ume C, Kubota H, Shimakura K, Shiomi K, Hamada-Sato N. Quantification of major allergen parvalbumin in 22 species of fish by SDS-PAGE. *Food Chem.* 194: 345-353 (2016)
- Larrea-Wachtendorff D, Tabilo-Munizaga G, Moreno-Osorio L, Villalobos-Carvajal R, Pérez-Won M. Protein Changes Caused by High Hydrostatic Pressure (HHP): A Study Using Differential Scanning Calorimetry (DSC) and Fourier Transform Infrared (FTIR) Spectroscopy. *Food. Eng. Rev.* 7: 222-230 (2015)
- Li JF, Wei F, Dong XY, Guo LL, Yuan GY, Huang FH, Jiang ML, Zhao YD, Li GM, Chen H. Microwave-assisted approach for the rapid enzymatic digestion of rapeseed meal. *Food Sci. Biotechnol.* 19: 463-469 (2010)
- Liu L, Yang C, Guo QX. A study on the enthalpy-entropy compensation in protein unfolding. *Biophys. Chem.* 84: 239-251 (2000)
- Liu Y, Zhao G, Ren J, Zhao M, Yang B. Effect of denaturation during extraction on the conformational and functional properties of peanut protein isolate. *Innov. Food Sci. Emerg. Technol.* 12: 375-380 (2011).
- Ochoa-Rivas A, Nava-Valdez Y, Serna-Saldívar SO, Chuck-Hernández C. Microwave and Ultrasound to Enhance Protein Extraction from Peanut Flour under Alkaline Conditions: Effects in Yield and Functional Properties of Protein Isolates. *Food. Bioprocess. Technol.* 10: 543-555 (2017)
- Paulsson M, Dejmeek P. Thermal Denaturation of Whey Proteins in Mixtures with Caseins Studied by Differential Scanning Calorimetry. *J. Dairy Sci.* 73: 590-600 (1990)
- Pineda-Guerra Y, Betancur-Echeverri J, Pedroza-Díaz J, Delgado-Trejos E, Rothlisberger S. Análisis proteómico del veneno de la abeja africanizada: comparación de métodos de extracción. *Acta Biol. Colomb.* 21: 619-626 (2016)
- Piña-Dumoulin G, Quiroz J, Ochoa A, Magaña-Lemus S. Caracterización físico-química de frutas frescas de cultivos no tradicionales en Venezuela: la yaca. Physical-chemistry characterization of fresh fruits of no traditional cultivations in Venezuela: the yaca. *Agron. Trop.* 60: 35-42 (2010)
- Porras-Godínez MR, Pedroza-Islas R, Franco-Rodríguez G, San Martín-Martínez E. Thermal treatment effect on wettability of edible films from whey protein isolated and mesquite (*Prosopis* spp.) gum. *Agrociencia* 49: 1-13 (2015)
- Preece KE, Hooshyar N, Krijgsman A, Fryer PJ, Zuidam NJ. Intensified soy protein extraction by ultrasound. *Chem. Eng. Process. Process. Intensif.* 113: 94-101 (2017).
- Reddy DV, Elanchezian N. Evaluation of tropical tree leaves as ruminant feedstuff based on cell contents, cell wall fractions and polyphenolic compounds. *Livest. Res. Rural Dev.* 20: 77 Retrieved March 26, 2019, from <http://www.lrrd.org/lrrd20/5/redd20077.htm> (2008)

- Reyes-Santamaría MI, Villegas-Monter A, Colinas-León MT, Calderón-Zavala G. Peso específico, contenido de proteína y de clorofila en hojas de naranjo y tangerino. *Agrociencia*, 34: 49-55 (2000)
- Saini RK, Keum YS. Carotenoid extraction methods: A review of recent developments. *Food Chem.* 240: 90–103 (2018).
- Schwass DE, Finley JW. Heat and alkaline damage to proteins: Racemization and lysinoalanine formation. *J Agric Food Chem.* 32:1377–1382 (1984)
- Shen L, Wang X, Wang Z, Wu Y, Chen J. Studies on tea protein extraction using alkaline and enzyme methods. *Food Chem.* 107: 929-938 (2008)
- Tabilo-Munizaga G, Gordon TA, Villalobos-Carvajal R, Moreno-Osorio L, Salazar F N, Pérez-Won M, Acuña S. Effects of high hydrostatic pressure (HHP) on the protein structure and thermal stability of Sauvignon blanc wine, *Food Chem.* 155: 214-220 (2014)
- Veggi PC, Martinez J, Meireles MAA. Fundamentals of microwave extraction. In *Microwave-assisted extraction for bioactive compounds* pp. 15-52. Ed. Springer US (2013)
- Wannberg J, Ersmark K, Larhed M. Microwave-accelerated synthesis of protease inhibitors. In: Larhed M., Olofsson K. (eds) *Microwave Methods in Organic Synthesis*. Top. Curr. Chem. Springer, Berlin, Heidelberg 266:167-198 (2006).
- Wood E. *Lehninger's principles of biochemistry* 3rd Edition DL Nelson, M. Cox. Worth, New York, 1152 p (2000)
- Xu HN, Liu Y, Zhang L. Salting-out and salting-in: Competitive effects of salt on the aggregation behavior of soy protein particles and their emulsifying properties. *Soft Matter* 11:5926–5932 (2015)
- Yang B, Jiang Y, Shi J, Chen F, Ashraf M. Extraction and pharmacological properties of bioactive compounds from longan (*Dimocarpus longan* Lour) fruit-A review. *Food Res. Int.* 44: 1837-1842 (2011)

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.