EFFECT OF ULTRASOUND TREATMENT ON PHYSICOCHEMICAL, FUNCTIONAL AND NUTRITIONAL PROPERTIES OF A SAFFLOWER (*CARTHAMUS TINCTORIUS* L.) PROTEIN ISOLATE

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ABSTRACT

The effect of ultrasound exposure time (15 and 30 min) at 130 W and 40 kHz on the physicochemical, functional, and nutritional properties of a safflower protein isolate (SPI) was evaluated. The moisture content, bulk density, and a_w of the SPI were significantly ($P < 0.05$) decreased by ultrasound compared to the untreated control. In contrast to the control, the SPI exposed to ultrasound for 30 min had increased protein solubility (by 9.7% and 3.7% at pH 6 and 7, respectively), least gelation concentration (by 2.0% at pH 6), and oil absorption capacity (by 3.0%). No significant differences ($P < 0.05$) were observed in amino acid composition, chemical score, or predicted protein efficiency ratio of the control and the SPI exposed to ultrasound. Ultrasound treatment would benefit the application of SPI in the food industry for ground meat formulations, meat substitutes and extenders, doughnuts, baked goods, and soups.

Keywords: ultrasound treatment, safflower protein isolate, physicochemical properties, functional properties, nutritional properties

1. INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is one of the oldest cultivated crops and usually is grown for flowers that are used for coloring, flavoring foods, dyes, medicinal properties, and livestock feed (PEIRETTI, 2017). Safflower is gaining attention due to its oil yield potential and the ability to grow under high temperatures, drought, and high salinity (HUSSAIN *et al*., 2016). Safflower is produced in over 20 countries, but in 2016, the Russian Federation, Kazakhstan, Mexico, and the USA were the main producers, producing about 71% of the 948,516 t of safflower produced worldwide (FAO, 2018).

Oil and meal are the two main products that come from safflower production. Oil is the primary product and has both food and industrial uses. The seed oil content of safflower ranges from 30 to 45% (LIU *et al*., 2016). Safflower meal, a by-product of the safflower-oil industry, contains approximately 20-25% protein, and is currently marketed as animal feed (TIRIL and KERIM, 2015). However, according to some studies, a protein isolate from safflower meal could represent an opportunity to recover proteins for human consumption (ULLOA *et al*., 2011; PAREDES-LÓPEZ and ORDORICA-FALOMIR, 1986).

On the other hand, the application of ultrasonic technology in the food industry is currently attracting much attention (HU *et al*., 2013). Ultrasound is an acoustic wave with a frequency >20 kHz (O'SULLIVAN *et al*., 2016a). High intensity ultrasound (HIUS, 20-100kHz) might have a wide variety of applications in the food industry (ZHANG *et al*., 2014) because it can alter the physicochemical properties and/or structure and functional properties due to cavitation effects on vegetable proteins such as soy protein isolate (JAMBRAK *et al*., 2009), black bean protein isolate (JIANG *et al*., 2014), and jackfruit seed protein isolate (RESENDIZ-VAZQUEZ *et al*., 2017).

A recent study by XIONG *et al.* (2018) showed that ultrasonic treatment caused partial unfolding of the proteins of pea protein isolate, which improved foam ability and stability. According to MALIK *et al*. (2017), solubility, emulsifying capacity, emulsion stability, foaming capacity, foam stability, and oil binding capacity of a sunflower protein isolate were improved significantly after application of HIUS. ZHANG *et al*. (2018) found that the HIUS treatment modified the protein structure and significantly enhanced the solubility of rice proteins, as well as emulsifying properties and foaming properties. However, the results of the application of ultrasound treatment on protein properties depend on some conditions such as frequency, amplitude, time, temperature, ionic strength, pH, and concentration, as well as intrinsic characteristics of the protein source (HIGUERA-BARRAZA *et al*., 2016). To our knowledge, no studies have focused on the potential effects of HIUS application on safflower protein isolate (SPI). Therefore, the objective of this study was to evaluate the effect of HIUS on the physicochemical, functional, and nutritive properties of a protein isolate obtained from safflower meal.

2. MATERIAL AND METHODS

2.1. Materials and chemicals

The safflower meal (23% protein, moisture 8%, 5.4% ash, 1.4% fat, and total carbohydrates 62.2%) used in this study was purchased from Aceitera La Junta, S.A. de C.V. (Limited Company of Variable Capital) (Guadalajara, Jalisco, Mexico). All chemical reagents were J.T. Baker analytical grade and purchased from Diseño Tecnológico en Laboratorios, S. A. de C.V. (Guadalajara, Jalisco, México).

2.2. Preparation of protein isolate

The SPI was prepared according to the method reported by ULLOA *et al*. (2011). Briefly, the protein was recovered in batches of safflower meal suspension (120 L) by adding one part safflower meal to 30 parts filtered tap water, which was then mixed for 45 min at room temperature (25°C). The pH of the suspension was adjusted to 9.0 with diluted NaOH during mixing. The insoluble residue of the protein suspension was separated by continuous centrifugation. The protein extract was concentrated using a pilot-scale ultrafiltration unit (Osmonic Inc., Minnetonka, USA) equipped with a polysulfonate membrane cartridge with a molecular weight cut-off of 100 kDa. Extracts were concentrated to one-fifth of their original volume, then diluted with filtered tap water and concentrated by diafiltration for further purification of protein retentate. Dilution consisted of adding an amount of water equal to four times the retentate volume after the first concentration. Three cycles of diafiltration were required to produce a protein isolate with a protein content ≥90.0% dry weight. Finally, the diafiltrated protein extract was spray dried in a Model Tower No. 1 drier (Niro Atomizer, SA, Monterrey, Nuevo León, Mexico) to obtain the powder of protein isolate.

2.3. Ultrasound treatment

Aqueous suspensions of SPI containing 10% of protein (w/v) were prepared in 100-mL beakers by magnetic stirring for 15 min. Ultrasound treatment was performed in an ultrasound bath Branson Model MTH-3510 (130 W and 40 kHz; a tank capacity of 5 L; internal dimensions of 290 × 150 × 150 mm; an acoustic energy density of 0.026 W cm^{-3}). Beakers containing SPI suspension were placed directly into the ultrasound bath to receive the treatment for 15 or 30 min, while the control SPI suspension was placed into a water bath at 25°C; the temperature during ultrasound treatment increased by <2°C. The ultrasonic intensity introduced in the system measured by calorimetry according to JAMBRAK *et al*. (2014) was 1 Wcm^{-2}. Afterward the suspensions of SPI exposed to ultrasound and the control were lyophilized in a FreeZone Freeze-Dry System (Labconco, Kansas City, MO, USA) and stored at room temperature in sealed containers for further analysis.

2.4. Physicochemical and microstructure characteristics

Moisture, protein (N × 6.25), and ash contents were determined according to AOAC methods (1995). Water activity (a_w) was measured at 25°C using an AquaLab 4TEV (Decagon Devices Inc., Pullman, Washington, USA), on coarse powder samples (3 g). Prior to testing samples, the water activity meter was turned on and allowed to warm up for 30 min and calibrated by filling a plastic disposable cup half filled with a saturated sodium chloride solution. The accuracy of water activity values was ±0.003. The color was determined with a Minolta CR-400 color meter (Konica Minolta Sensing Ltd, Inc., Tokyo, Japan). The measured values were expressed according to the CIELAB color scale L^* (lightness), a^* (redness-greenness), and b^* (yellowness–blueness). The L_s^*, a_s^*, and b_s^* values of the white standard tile used as reference were 94.44, -0.23 and 3.89, respectively. Total color difference (ΔE) was calculated as:

$$\Delta E^* = [(L_s^* - L^*)^2 + (a_s^* - a^*)^2 + (b_s^* - b^*)^2]^{1/2} \quad (1)$$

The bulk density was determined in triplicate using the method described by PIORNOS *et al*. (2015) and expressed as g mL^{-1}.

The microstructure of the freeze-dried SPI samples was observed with a scanning electron microscope (SEM; SEC, Mini-SEM SNE-3200M, South Korea) at an accelerating voltage of 20 kV. Before using the SEM, the samples were coated with gold using an ion sputter coater (MCM-100, SEC).

2.5. Functional properties

Protein solubility profile of the SPI as a function of pH was determined according to the method reported by PIORNOS *et al*. (2015). The water absorption capacity (WAC) and oil absorption capacity (OAC) were measured according to the method described by ULLOA *et al*. (2011) and expressed as g water or oil absorbed per g protein. Soy oil (Fábrica de Aceites la Central, S. A. de C.V., Guadalajara, Jalisco, México) was used in the determination of OAC. The emulsifying activity (EA) and emulsion stability (ES) were determined according to the method reported by DENG *et al*. (2011) using soy oil. The least gelation concentration (LGC) at pH 2, 4, 6, 8, and 10 was determined according to the method reported by BENELHADJ *et al*. (2016). Foaming capacity (FC) and foam stability (FS) were measured using the method described by STONE *et al*. (2015).

2.6. Amino acid composition and protein nutritive quality

Hydrolysis and quantification of amino acids were performed according to the methods described by VÁZQUEZ-ORTÍZ *et al*. (1995) using a Pro Star-210 Varian high-performance liquid chromatographic system (Varian Associates, Inc. USA). Amino acids were expressed on a protein basis, equivalent to g per 16 g of protein. The tryptophan content was not determined.
The nutritive quality of proteins was estimated by determination of chemical score (CS) and predicted protein efficiency ratio (PER) according to the procedure described previously by ULLOA *et al*. (2015).

2.7. Statistical analysis

Analyses of samples were done in triplicate and data were analyzed using one-way ANOVA. Significant differences ($P < 0.05$) between samples were determined from a Tukey's test using SPSS Statistics Version 20 (IBM Corporation, New York, USA).

3. RESULTS AND DISCUSSION

3.1. Physicochemical and microstructure characteristics

The effect of ultrasound exposure time on the physicochemical characteristics of SPI is shown in Table 1. The protein and ash contents of the SPI exposed to ultrasound for 15 and 30 min were not significantly ($P > 0.05$) different from the control (Table 1). However, the moisture content of SPI was significantly ($P < 0.05$) reduced from 5.08% (control) to 4.01% and 4.53% when samples were exposed to ultrasound for 15 and 30 min, respectively. The lower moisture content of SPI treated with ultrasound was due to a higher effect of compressions and expansions induced by the sound waves passing through the food medium, which make moisture removal easier (AWAD *et al*., 2012).
Bulk density is a property used to characterize powder products. It is of great importance for economical and functional reasons, for example, for reducing packaging costs, which depends on the combined effects of interrelated factors, like particle size, number of

contact points, and intensity of attractive inter-particle forces (PIORNOS *et al*., 2015). As shown in Table 1, the exposure to ultrasound of SPI significantly decreased the bulk density 4.9–6.5% as compared to the control. Such a decrease in the bulk density was due to the samples being treated with ultrasound and freeze drying, as these samples had larger and more heterogeneous structures in protein isolates in comparison with the control (HU *et al*., 2013; RESENDIZ-VAZQUEZ *et al*., 2017).

The a_w of a food system is a thermodynamic property, which is defined as the ratio of water vapor pressure of food to the saturated water vapor pressure at a given temperature. It is considered to be a good quality indicator for the safety and stability of foods with respect to microbial growth and biochemical reactions (TADAPANENI *et al*., 2017). The a_w of SPI exposed for 15 and 30 min to ultrasound was lower than that of the control (Table 1). As discussed previously, sound waves passing through the food medium make moisture removal easier, which also influenced the reduction in a_w of SPI as was observed in this study. However, both the control and the SPI exposed to ultrasound had values of a_w below a limiting level to ensure microbial stability, because it is generally accepted that no microbial growth will occur at $a_w < 0.66$ (ULLOA *et al*., 2015).

Table 1. Effect of ultrasound exposure time on the physicochemical properties of safflower protein isolate**.**

Property	Ultrasound exposure time (min)		
	0 (control)	15	30
Protein content (%)	87.54±0.52	88.17±0.13	87.03±1.01
Ash content (%)	6.92±0.11	6.88±0.12	6.81±0.13
Moisture content (%)	5.08^{a}±0.13	4.01^{c}±0.12	4.53^{b}±0.11
Bulk density (g mL^{-1})	0.61^{a}±0.1	0.57^{b}±0.1	0.58^{b}±0.1
Water activity	0.341^{a}±0.002	0.286^{c}±0.007	0.300^{b}±0.004
Color			
*L** (lightness)	60.62±0.11	60.35±0.19	60.19±0.21
*a** (redness-greenness)	4.21±0.06	4.23±0.03	4.18±0.08
*b** (yellowness-blueness)	17.45±0.29	17.41±0.17	17.77±0.25
Δ*E* (color difference)	36.70±0.17	36.94±0.21	37.22±0.27

Values are mean±standard deviation of three determinations. Values followed by different superscript letters in the same line are significantly different ($P < 0.05$).

The color characteristics of SPI exposed to ultrasound for 15 and 30 min in comparison with the control treatment are shown in Table 1. There were not significant ($P > 0.05$) differences in L^*, a^*, b^*, and ΔE values among samples of control SPI and those exposed to ultrasound. The effect of ultrasound on color in food depends on intrinsic characteristics of food and ultrasound conditions (BI *et al*., 2015). ADEKUNTE *et al*. (2010) reported a decrease in L^*, a^* and b^* values of tomato juice after sonication due to the degradation of lycopene. CHENG *et al*. (2007) found that guava juice treated by ultrasound for 30 min showed a significant change in the ΔE value due to a decrease in L^* value and an increase in a^* and b^* values. VALERO *et al*. (2007) found that ultrasound treatments had no significant effect on color scoring in orange juice. The degradation of color might be due to the effect of cavitation of ultrasound that could induce both chemical (e.g., by generation of radicals) and mechanical degradation of biomolecules (ADEKUNTE *et al*., 2010); however, such a phenomenon was not observed in the SPI exposed to ultrasound in this study. In general, a similar behavior on the effect of ultrasound exposure time on the

physicochemical properties of safflower protein isolate of this study was reported by FLORES-JIMÉNEZ *et al*. (2019) for canola protein isolate**.**
Fig. 1 shows a set of SEM images of control SPI (A) and SPI exposed to ultrasound for 15 (B) and 30 min (C). It was observed that samples B and C obtained after ultrasonic treatments and freeze-drying had larger and more heterogeneous structures than sample A (untreated SPI). These results might have been caused by ultrasound treatment inducing the unfolding of proteins and increasing the surface hydrophobic groups of the SPI molecules (HU *et al*., 2013; JIANG *et al*., 2014).

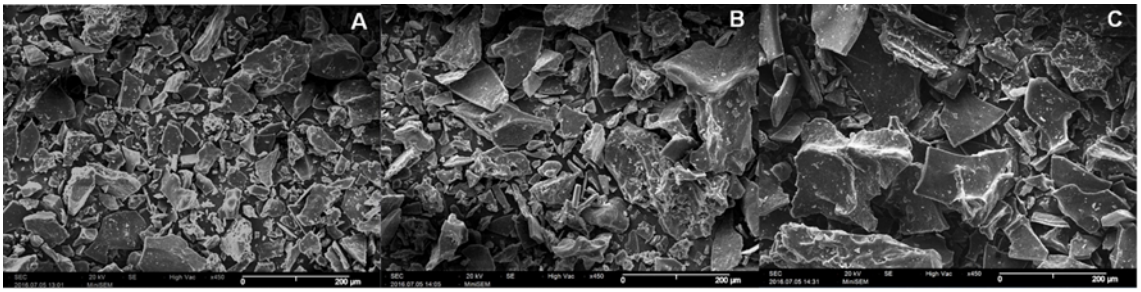

Figure 1. Effect of ultrasound exposure time (control = A, 15 min = B and 30 min = C) on the microstructure of safflower protein isolate.

3.2. Functional properties

Solubility is one of the most important functional attributes of proteins because it affects the texture, color, and the sensory properties of products containing them. It is closely associated with water retention capacity and other physicochemical and functional properties. Solubility of proteins depends on the composition, molecular weight, and surface characteristics of constituent amino acids and environmental factors such as pH, temperature, and ionic strength, and it can be affected by some chemical and physical treatments (TIMILSENA *et al*., 2016).
Table 2 shows the effect ultrasound exposure time on the protein solubility of SPI at different pH values. Only at pH 6 and pH 7, did the SPI exposed to ultrasound for 30 min have a beneficial effect on protein solubility, increasing 9.7% and 3.7%, respectively, in comparison to the control. This increase in protein solubility may be due to the conformational change during ultrasonic treatment and formation of soluble protein aggregates from insoluble protein (HU *et al*., 2013). However, such an increase in protein solubility depends on ultrasonic conditions (JIANG *et al*., 2014), as well as the pH at which the proteins are solubilized, as was observed in this study. Besides, for safflower proteins, the solubility values were minimum at the pH value of 5.0, which is the isoelectric point of safflower proteins, for ultrasound treatments and control treatment.
The results of WAC and OAC by effect of ultrasound exposure time of SPI are shown in Table 3. The WAC decreased from an initial value of 2.14 g H_2O g^{-1} protein to 1.94 g H_2O g^{-1} protein after ultrasound treatment for 30 min. According to RESENDIZ-VAZQUEZ *et al*. (2017), the ultrasound treatment might denature the molecular structure of proteins and cause an increase in the hydrophobic surface, which can lead to low values of WAC, as was observed in proteins from jackfruit seeds. In contrast, the OAC of samples of SPI exposed to ultrasound for 15 and 30 min were significantly ($P < 0.05$) higher compared to the control SPI. The OAC increased from an initial value of 0.99 g oil g^{-1} protein to 1.19 g oil g^{-1} protein and 1.30 g oil g^{-1} after exposure to ultrasound for 15 and 30 min, respectively. The

increase of OAC in proteins might be attributed to the exposure of hydrophobic groups after ultrasound treatment (HIGUERA-BARRAZA *et al*., 2016; ZHOU *et al*., 2016), which allowed the physical entrapment of oil (MEINLSCHMIDT *et al*., 2016).

Table 2. Effect of ultrasound exposure time at different pH on protein solubility (%) of safflower protein isolate.

pH	Ultrasound exposure time (min)		
	0 (control)	15	30
2	90.95±0.68	87.22±1.43	88.10±0.06
3	81.06^{a}±0.68	65.32^{b}±0.68	67.16^{b}±0.69
4	17.98^{a}±0.68	12.59^{b}±0.01	13.73^{b}±0.01
5	14.57^{a}±0.01	12.11^{b}±0.66	13.20^{ab}±0.69
6	49.61^{b}±1.37	50.32^{b}±0.01	54.41^{a}±0.61
7	61.16^{b}±0.01	57.94^{c}±1.28	63.44^{a}±0.04
8	89.75^{a}±0.74	71.88^{b}±0.68	73.49^{b}±1.43
9	92.32^{a}±0.12	86.34^{b}±1.31	88.32^{ab}±1.38
10	95.72^{a}±0.68	90.26^{b}±1.37	93.00^{ab}±0.06

Values are mean±standard deviation of three determinations. Values followed by different superscript letters in the same line are significantly different ($P < 0.05$).

Table 3. Effect of ultrasound exposure time on water and oil absorption capacity of safflower protein isolate.

Property	Ultrasound exposure time (min)		
	0 (control)	15	30
Water absorption capacity (g H_2O g^{-1} protein)	2.14^{a}±0.03	2.15^{a}±0.03	1.94^{b}±0.02
Oil absorption capacity (g oil g^{-1} protein)	0.99^{c}±0.01	1.19^{b}±0.02	1.30^{a}±0.01

Values are mean±standard deviation of three determinations. Values followed by different superscript letters in the same line are significantly different ($P < 0.05$).

Proteins are of particular interest as emulsifying agents in food formulations such as frozen desserts, salad dressings, comminuted meats, mayonnaise, cake batters, milks, and coffee whiteners, due to their ability to adsorb and form viscoelastic films at oil-water interfaces (O'SULLIVAN *et al*., 2016b). According to the results presented in Fig. 2, ultrasound treatment had no effect on EA and ES of SPI. YANJUN *et al*. (2014) reported that ultrasound pretreatment increased the emulsifying activity index (EAI) and ES index of milk proteins. ZHOU *et al*. (2016) found that the ultrasound treatment increased or decreased EAI of soybean glycinin depending on the ionic strength. In another study, the EA of defatted rice bran protein concentrate was higher ($P < 0.05$) than that of the ultrasound treatment, but the ES was not significantly different ($P > 0.05$) from the control (CHITTAPALO and NOOMHORM, 2009). The emulsifying properties of food proteins depend upon the solubility, molecular flexibility, surface hydrophobicity, and stability of the protein structure (ZHOU *et al*., 2016), which can be modified or not when are exposed to different ultrasound conditions as was observed in this study.

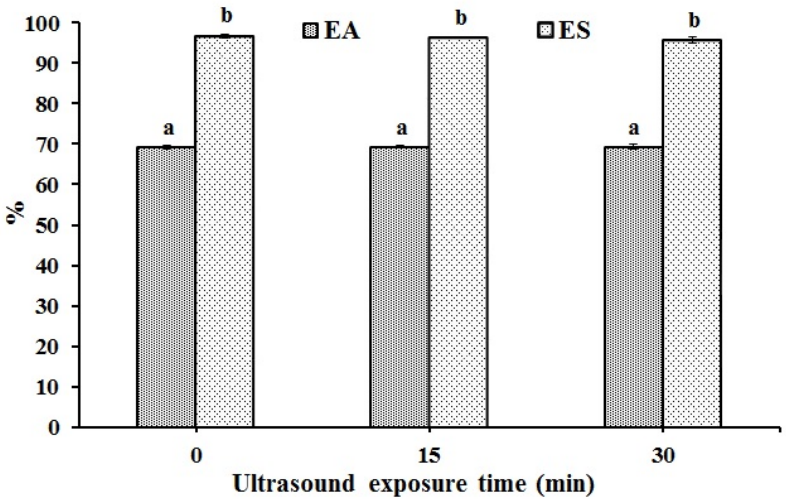

Figure 2. Effect of ultrasound exposure time on the emulsifying activity (EA) and stability (ES) of safflower protein isolate.

Gel properties are important functional characteristics of protein isolates, which are widely used as gelling agents to improve the texture and water holding capacity of meat products. Gelation is often an aggregation of denatured proteins, which involves the formation of a network, retaining significant amounts of water and transforming liquid sample to solid, which exhibits a certain degree of order where both covalent and non-covalent interactions are involved (CHEN *et al*., 2016). LGC indicates the gelling capacity of protein. Fig. 3 shows that the SPI exposed to ultrasound for 15 and 30 min at pH 2-10 had no effect on LGC, except for the ultrasound treatment of 30 min at pH 6, where the LGC increased from 6.0% (w/w) to 8.0% (w/w), which implies a reduction of the gelling capacity. The behavior of the LGC of SPI by effect of pH in this study was similar to that showed for lupin (PIORNOS *et al*., 2015), jackfruit (RESENDIZ-VAZQUEZ *et al*., 2017), and cashew nut shell (YULIANA *et al*., 2014) protein isolates.

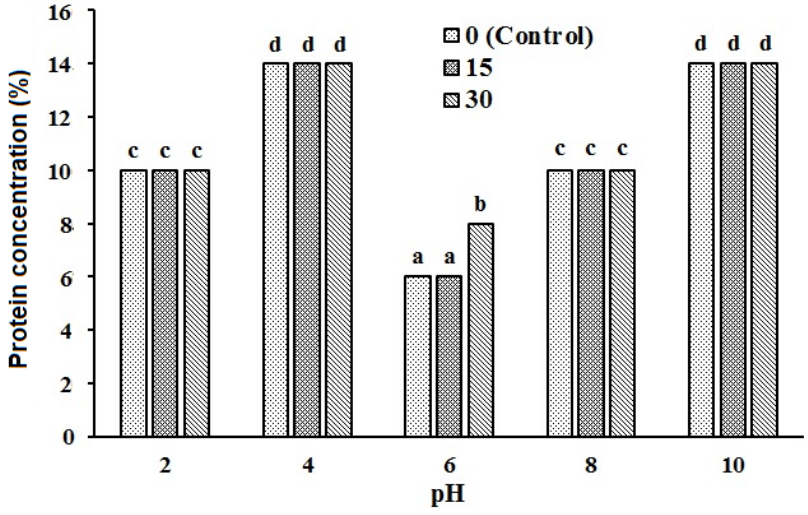

Figure 3. Effect of ultrasound exposure time on the last gelation concentration (LGC) of safflower protein isolate.

The FC and FS of protein isolates are functional properties that determine their application in food systems, where aeration and overrun is required (e.g. baked foods, whipped toppings and ice cream mixes) (SHEVKANI *et al.*, 2015). Because of surface-active properties, the proteins form foam when they are whipped (MALIK *et al.*, 2017). According to the results obtained in this study, the exposure to ultrasound for 15 and 30 min did not modify the FC and FS of SPI with respect to the control (Fig. 4).

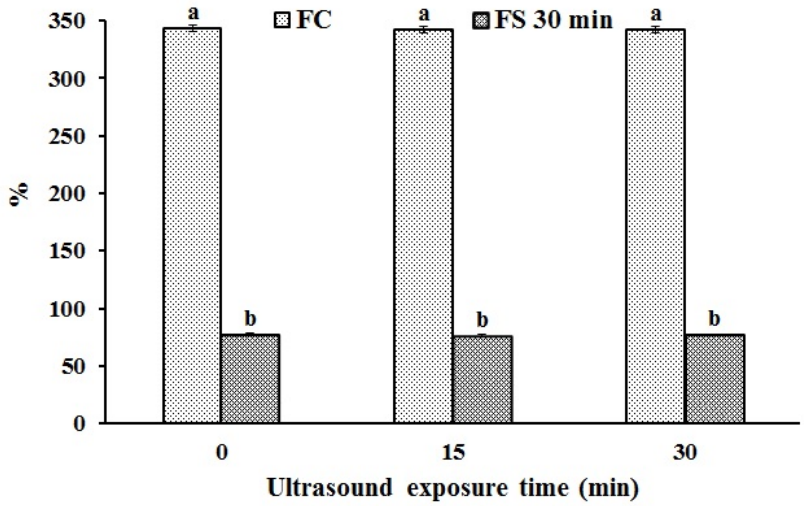

Figure 4. Effect of ultrasound exposure time on the foaming capacity (FC) and foam stability (FS) of safflower protein isolate.

Some reports showed that FC and FE of protein isolates from whey (JAMBRAK *et al.*, 2008) and soy (ZHANG *et al.*, 2014) were improved after ultrasound treatment for both 20 kHz and 40 kHz treatments, but no effect in foaming at 500 kHz treatment was observed, as occurred for SPI in this study. The improvement of the foaming properties in protein suspensions by ultrasound may be due to protein partial denaturation, which causes a higher air-water diffusion interface due to an increase in cohesiveness and flexibility of the foams (HIGUERA-BARRAZA *et al.*, 2016).

3.3. Amino acid composition and protein nutritive quality

Amino acid composition and nutritive quality of proteins in terms of CS and PER of SPI exposed to the ultrasound treatments in comparison with the control (Table 4) were not significantly different ($P < 0.05$). Of all the amino acids present in SPI, half corresponded to essential amino acids. On the other hand, glutamic acid was the amino acid with higher concentration in the SPI, as well as in the hempseed, soy (WANG *et al.*, 2008), and pennycress protein isolates (HOJILLA-EVANGELISTA *et al.*, 2014). According to amino acid requirements for adults (FAO/WHO, 1991), the first limiting amino acid of SPI was lysine. Therefore lysine was considered for calculating CS values for the SPI exposed to ultrasound for 15 min, 30 min, and the control, which were 48.4, 48.2, and 48.9 respectively, and were not significantly different ($P > 0.05$) from one another (Table 4).

Table 4. Effect of ultrasound exposure time on the composition of amino acid and protein quality of safflower protein isolate.

Parameter	Amino acid composition (g/16 g N) Ultrasound exposure time (min)			Reference for adults (FAO/WHO, 1991)
	0 (Control)	15	30	
Essential amino acid				
Lysine	2.69±0.08	2.66±0.15	2.65±0.05	5.5
Threonine	5.08±0.25	4.78±0.21	4.31±0.57	4.0
Valine	2.93±0.09	3.01±0.44	2.70±0.14	5.0
Methionine+cysteine	2.13±0.01	1.93±0.37	2.21±0.14	3.5
Cysteine				
Isoleucine	2.25±0.46	2.36±0.10	2.20±0.20	4.0
Leucine	6.39±0.94	7.06±0.36	6.57±0.14	7.0
Phenylalanine+tyrosine	4.10±0.94	4.16±1.12	3.56±0.60	6.0
Tyrosine	2.58±0.10	2.97±0.73	2.40±0.20	
Tryptophan	ND	ND	ND	
Total essential amino acids	25.58±0.70	25.96±1.10	24.19±0.80	
No essential amino acid				
Histidine	3.84±0.05	3.42±0.32	4.09±0.46	
Arginine	18.65±1.08	18.57±0.74	19.08±0.56	
Aspartic acid	6.78±0.57	6.73±0.41	6.60±0.48	
Serine	8.25±0.58	7.20±0.51	7.99±0.60	
Glutamic acid	20.96±0.86	22.28±0.73	21.17±0.83	
Glycine	8.83±0.52	9.09±0.58	9.61±0.26	
Alanine	7.11±0.37	6.75±0.55	7.27±0.86	
Total non-essential amino acid	74.42±0.81	74.04±0.75	75.81±0.90	
Nutritive quality				
Chemical score	48.9±1.53	48.4±2.7	48.2±0.83	
PER	2.16±0.22	2.42±0.20	2.26±0.04	

Values are mean±standard deviation of three determinations. Values followed by different superscript letters in the same line are significantly different ($P < 0.05$). ND = Not determined. PER = Predicted protein efficiency ratio.

With respect to the PER, the values obtained for the SPI exposed to ultrasound for 15 min, 30 min, and the control were not significantly different ($P < 0.05$) from one another and had values of 2.42, 2.26, and 2.16, respectively, which were higher than the values 2.14 and 2.04 for desi chickpea and soy protein isolates, respectively (WANG *et al*., 2010).

4. CONCLUSIONS

Application of ultrasound at 130 W and 40 kHz for 30 min to SPI increased the protein solubility at pH range of 6-7 and the OAC. It was demonstrated that the ultrasound did not affect the color parameters of L^*, a^*, b^* and ΔE, or the amino acid composition and nutritional quality of proteins of SPI. The improvement of the OAC of SPI could benefit its

application in the food industry for ground meat formulations, meat substitutes and extenders, doughnuts, baked goods, and soups.

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