

Functional Properties of Proteins Isolated From Industrially Produced Sunflower Meal

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Abstract

Protein isolate 1 (PI1) and protein isolate 2 (PI2) were prepared from industrially produced sunflower meal by using isoelectric and ethanol precipitation respectively. The water absorption capacity of PI1 was 6 times higher than that of PI2 and was significantly reduced by the presence of 0.03 M and 0.25 M NaCl. Oil absorption capacity of both protein isolates was not influenced by NaCl supplementation. Foam capacity of PI1 and PI2 was pH-dependent. While the foam capacity of both isolates was improved by either 0.03 M or 0.25 M NaCl, the foam stability was negatively influenced by the addition of NaCl at all pH values with except for pH 4. Emulsifying activity of PI1 and PI2 was lowest at pH 4. The emulsions exhibited relatively high stability (> 90%) under all studied conditions. Knowledge of the influence of pH and boundary concentrations of NaCl on the functionality of sunflower meal protein isolates could be beneficial for their future potential application in food industry.

Keywords: Sunflower protein isolates; Functionality; Sodium chloride; pH

1 Introduction

Plant proteins are good sources of essential amino acids for vegetarians and an alternative food for humans where animal protein is either expensive or scarce because of unfavorable natural factors. Sunflower meal is a by-product of oil production with high protein content (30%-50%) (Bau, Mejean, Debry, & Mohtadina, 1983; Damodaran, 1997). However, its common utilization as a feed supplement in animal nutrition is limited due to relatively high fibre content (Senkoylu & Dale, 2006; Raza, Ashraf, Pasha, & Latif, 2009). The application of the sunflower meal as an unconventional protein source for human consumption is an alternative approach which could lead to more complete and efficient use of this by-product.

The utilization of plant protein isolates in the food industry depends on their functional properties since they affect the formulation, preparation and quality of food products (Mahajan & Dua, 2002). For example, protein isolates with high water- and oil absorption capacity are more desired for the preparation of meat derived products and bread, while protein isolates with good emulsifying and foaming properties are preferably applied in salad dressings, soups and frozen products (Ahmedna, Prinyawiwatkul, & Rao, 1999; Kanu et al., 2007).

Functional properties of proteins are determined by their size, shape, and structure of molecules, amino acid content and sequence (Damodaran, 1997). They also depend on extrinsic factors such as pH, salt concentrations, temperature, chemical reagents and interactions with other food

components (Kinsella, Damodaran, & German, 1985). Among them, the impact of salts on protein functionality is of great interest since the system protein-water-salt is a major determinant of the quality and the consumer acceptance of food products (Aremu, Olaofe, Akintayo, & Adeyeye, 2008; Andualem & Gessesse, 2013). The pH of the food system affects hydrophobicity, net charge and electrostatic repulsive forces of proteins thus modulating their interactions and functionality.

The choice of a method for preparation may also result in protein isolates with different functionality. Organic solvents are commonly used to prepare high purity protein samples (Martínez-Maqueda, Hernández-Ledesma, Amigo, Miralles, & Gómez-Ruiz, 2013). However, they are not appropriate for food applications. The extraction/precipitation of proteins with alcoholic or aqueous solutions is less toxic and more advantageous when used in the food industry but may also lead to altered functionality of the protein isolates (Moure, Sineiro, Dominguez, & Parajo, 2006).

Most published studies on sunflower meal proteins have been performed under mild laboratory conditions where no change of the native structure and functions of the proteins occurred (Pawar, Patil, Sakhale, & Agarkar, 2001; González-Pérez et al., 2004; Pickardt et al., 2009). However, sunflower proteins obtained from industrially produced meal may have different functional characteristics due to the impact of the technological parameters of oil production and sunflower seed pre-treatment. The protein isolates from industrially obtained sunflower meal, however, are of a higher practical interest since they lead to a better and more efficient use of this by-product. Knowledge of the influence of pH and boundary concentrations of NaCl on the functionality of sunflower meal protein isolates could facilitate their potential application in food industry. The purpose of this research was to explore water- and oil absorption capacity, foaming and emulsifying properties of proteins isolated from industrially produced sunflower meal by using isoelectric or ethanol precipitation. The functional properties were studied at different pH in the presence of two levels of NaCl (0.03 M and 0.25 M) and compared to those

when no salt was added to the system.

2 Materials and Methods

2.1 Preparation of protein isolates

Two protein isolates were prepared from sunflower meal provided by a local oil factory, as described by Ivanova, Koleva, Tziporkov, and Pishtiyski (2011) and Ivanova, Chalova, Koleva, Pishtiyski, and Perifanova-Nemska (2012). Briefly, 100 g sunflower meal (average particle size 1.02 mm) were suspended in 300 ml 10% NaCl (pH 6) and the volume was subsequently adjusted to 1000 ml with 10% NaCl (pH 6). The suspension was incubated at 40°C for 60 min to extract proteins. Solid phase was separated by filtration. Protein isolate 1 (PI1) was prepared by isoelectric precipitation of the extracted proteins. After adjusting the pH to 2.5 with 6 N HCl, the protein precipitate was collected by centrifugation at 5000 x g for 15 min (MPW-251, Med. Instruments, Poland), washed three times with 100 ml HCL (pH 2.5) and dried by lyophilization for 24 h (Lyovac GT2, Leybold-Heraeus, Germany). Protein isolate 2 (PI2) was obtained by addition of 1250 ml 96% ethanol to 500 ml protein extract. Protein precipitate was collected by centrifugation at 5000 x g for 15 min, washed three times with 100 ml 96% ethanol and dried overnight at 40°C in an oven (SLW 53, Pol-Eko Aparatura, Poland).

2.2 Determination of water- and oil absorption capacity

Water absorption capacity (WAC) was determined as described by Rodríguez-Ambriz, Martínez-Ayala, Millán, and Dávila-Ortiz (2005). Each protein sample (100 mg) was mixed with 1 ml distilled water and vortexed (Advanced Vortex Mixer – ZX3, VELP Scientifica, Usmate (MB), Italy) for 30 s. The resulted suspension was incubated at room temperature for 30 min, centrifuged for 20 min at 1800 x g (22°C) and the supernatant was decanted for 10 min at 45° angle. WAC was calculated by dividing the weight of the absorbed water (g) by the weight of the protein sample (g).

Oil absorption capacity (OAC) was determined by the method of Lin and Zayas (1987). Each protein sample (100 mg) was mixed with 1 ml sunflower oil and vortexed (Advanced Vortex Mixer – ZX3, VELP Scientifica, Usmate (MB), Italy) for 30 s. The emulsion was incubated at room temperature (22°C) for 30 min and subsequently centrifuged at 13600 x g for 10 min. The supernatant was decanted and drained for 20 min at 45° angle. OAC was calculated by dividing the weight of the absorbed oil (g) by the weight of the protein sample (g).

2.3 Foam capacity and stability

Foam capacity and stability were determined as described by Sze-Tao and Sathe (2000) with some modification. An aliquot of 20 ml protein solution (0.5 mg/ml) was whipped for 70 s in a graduated cylinder by hand. The foam stability was defined as the volume of the foam that remained after 60 min at room temperature (22°C) and was expressed as a percentage of the initial foam volume. Foam capacity was determined by volume increase (%) immediately after whipping and was calculated by the formula $(V_2 - V_1) / V_1 \times 100$ where V_2 is the volume of protein solution after whipping and V_1 is the volume of solution before whipping.

2.4 Emulsifying properties

Emulsifying activity and emulsion stability were determined as described by Neto, Narain, Silva, and Bora (2001). Five milliliters protein solution (0.5 mg/ml) was homogenized with 5 ml sunflower oil for 60 s at 1000 rpm by using an homogenizer (Ultra Turrax IKA T18 Basic, Germany). The emulsion was centrifuged at 1100 x g for 5 min and the height of the emulsified layer was recorded. The emulsifying activity was calculated as a ratio of the height of the emulsified layer and the height of the total content of the tube and multiplied by 100 to express in percent. Emulsion stability was established after heating. Therefore, hereafter emulsion stability should be considered as emulsion heat stability. The protein emulsion was heated up to 80°C in a water-bath (WNB 29, Memmert GmbH + Co.KG, Ger-

many) and kept under the same conditions for 30 min. It was subsequently cooled down to room temperature (22°C) and centrifuged at 1100 x g for 5 min. Emulsion stability was calculated by the height of emulsified layer after heating divided by the height of the emulsified layer before heating and multiplied by 100 to express in percent. NaCl was added to a test system to reach a final concentration of either 0.03 M or 0.25 M as appropriate. The influence of pH on foaming and emulsifying properties was tested by varying pH from 2 to 10 with an increment of 2 using NaOH or HCl.

2.5 Statistical analysis

The data are presented as means of results obtained from two independent experiments \pm standard deviations. They were analyzed by one-way analysis of variance (ANOVA) using Statgraphics Centurion (version XVI, 2009) statistical program (Stat Point Technologies, Ins., Warrenton, VA, USA). Mean differences were established by Fisher's least significant difference test for paired comparison with a significance level $\alpha = 0.05$.

3 Results and Discussion

3.1 WAC and OAC of protein isolates

Vegetable protein for food applications is an alternative to animal protein and represents a significant contribution to human dietary protein intake (Moure et al., 2006). The sunflower meal protein isolates, analyzed in the current study, are rich in sulfur-containing amino acids and arginine and exhibit sufficient water solubility over a wide pH range as previously established in our laboratory (Ivanova, Chalova, Koleva, Pishtiyski, et al., 2013).

Water/oil absorption capacity is the ability of proteins to absorb and retain liquid which influences texture and mouth feel characteristics of food products (Okezie & Bello, 1988). In our study, the WAC of PI1 (8.57 ± 0.40 g/g) was approximately 6 fold higher than the WAC of PI2 (1.42 ± 0.04 g/g) (Table 1). It was

Table 1: Water and oil absorption capacity of protein isolate 1 and protein isolate 2 at different concentrations of NaCl

	Water absorption capacity, g H ₂ O/g protein			Oil absorption capacity, g oil/g protein		
	NaCl concentrations, M			NaCl concentrations, M		
	0.00	0.03	0.25	0.00	0.03	0.25
Protein isolate 1	8.57 ± 0.40 ^a	5.81 ± 0.17 ^b	2.48 ± 0.04 ^c	1.41 ± 0.04 ^a	1.78 ± 0.03 ^a	1.49 ± 0.24 ^a
Protein isolate 2	1.42 ± 0.04 ^a	1.45 ± 0.11 ^a	1.62 ± 0.16 ^a	1.12 ± 0.01 ^a	1.15 ± 0.33 ^a	1.37 ± 0.17 ^a

^{a-c} Means in a row for a particular functional property with common superscripts do not differ significantly ($p \geq 0.05$)

comparable to the WAC of commercial soybean protein isolates such as Purina Protein 500E (7.9 ml/g) and 760 (8.8 ml/g), and Protein-max 90HE (8.4 ml/g) (Zayas, 1997). The WAC of PI2 was close to the WAC of a meal prepared from mixed sunflower cultivars (Rahaman, Ahmed, Babiker, & Mahgoub, 2006) and some plant proteins such as cashew nut protein concentrate (1.74 ml/g) (Ogunwolu, Henshaw, Mock, Santos, & Awonorin, 2009) and jojoba protein concentrate (Wiseman & Price, 1987).

Statistical differences in the WAC of PI1 and PI2 were also observed when studied in the presence of NaCl. NaCl is a common ingredient used in food industry to enhance flavour, preserve food, and improve processing but is a strong modulator of protein functionality (Aremu et al., 2008; Andualem & Gessesse, 2013). In our study, the functional properties of the protein isolates were studied at two levels of NaCl, namely 0.03 M and 0.25 M, which corresponded to the most common boundary concentrations of NaCl used in the preparation of commercial food products. According to (Dragoev, Vulkova-Yorgova, & Balev, 2008), NaCl content of different type unprocessed meat may vary from 0.16% to 0.25%. (Antova & Georgieva, 2008) reported that commercial butter contained 0.1% to 0.2% NaCl. Higher NaCl concentrations are allowed for application in bread (1.2%) and processed meat products (2.0%) (BDS 3412, 1979; BDS 7168, 1993).

Both levels of NaCl (0.03 M and 0.25 M) influenced the WAC of PI1 as the addition of 0.25 M NaCl led to more than 3 fold decrease of the WAC when compared to the WAC of the isolate in water. Although to a smaller extent,

increases of NaCl concentrations diminished the WAC of groundnut protein (Aremu et al., 2008). In contrast, no statistical differences in WAC of PI2 were established after supplementation with NaCl (Table 1). The differences in the WAC of PI1 and PI2 may be due to the purity and protein content of the two protein isolates. PI1 contained 94.25% protein and 1.34% ash, while PI2 was characterized with lower protein (75.34%) and higher ash content (13.26%). The amount of total carbohydrates of PI2 (4.56%) exceeded the amount of total carbohydrates established in PI1 (1.74%). In contrast, a higher content of total lipids (2.60%) was found in PI1 when compared to PI2 (1.50%) (Ivanova et al., 2011). According to Kinsella et al. (1985), the presence of non-protein components may impair protein unfolding and the exposure of additional binding sites to water molecules thus diminishing their WAC.

In contrast to WAC, OAC of PI1 (1.41 ± 0.04 g/g) and PI2 (1.12 ± 0.01 g/g) were found to be similar (Table 1). They were close to the OAC of a soy protein isolate (1.3 ± 0.02 g/g) established by Alkahtani and Abouarab (1993) but lower than the data reported by L'Hocine, Boye, and Arcand (2006). According to the latter, the OAC of soy isolates may reach 9 g/g depending on pre-treatment of the seeds and the methods used for the preparation of the protein isolates. NaCl did not affect the OAC of either PI1 or PI2 ($p \geq 0.05$).

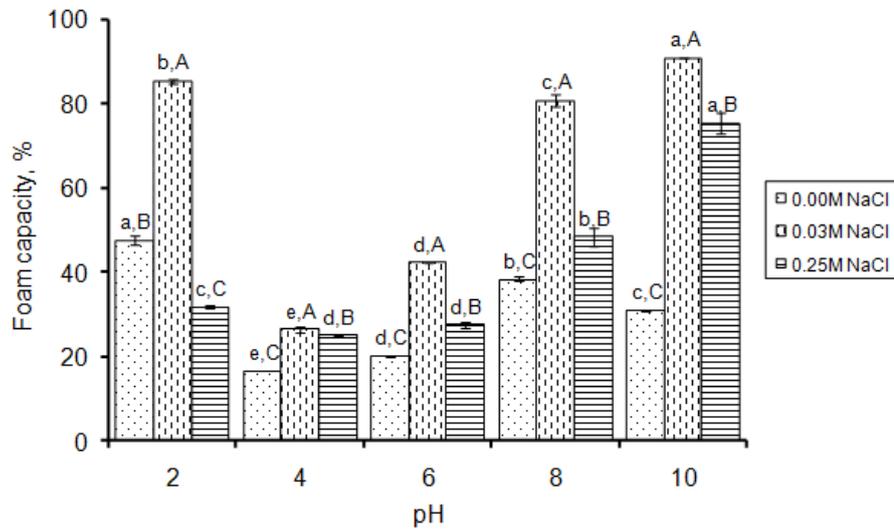


Figure 1: Foam capacity of protein isolate 1 at different pH and NaCl concentrations.
a-e Means with same lowercase letter for a particular NaCl concentration do not differ significantly ($p \geq 0.05$).
A-C Means with same capital letter for a particular pH value do not differ significantly ($p \geq 0.05$).

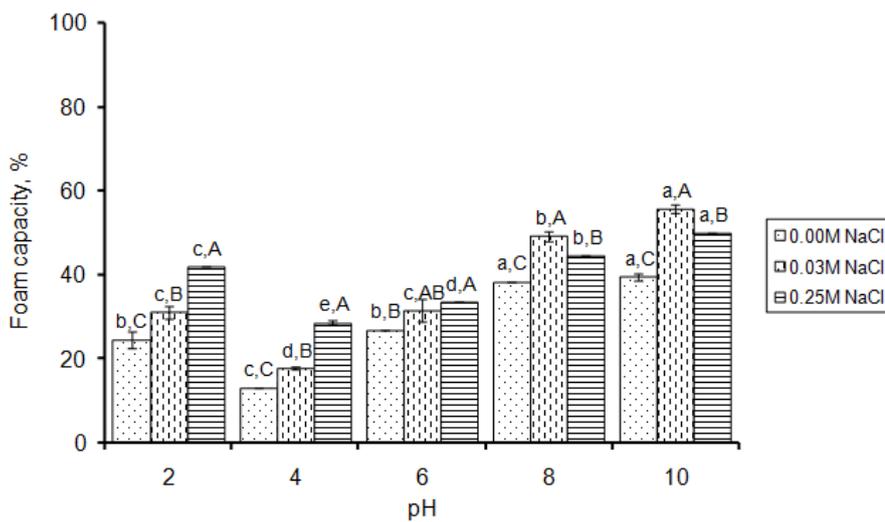


Figure 2: Foam capacity of protein isolate 2 at different pH and NaCl concentrations.
a-c Means with same lowercase letter for a particular NaCl concentration do not differ significantly ($p \geq 0.05$).
A-C Means with same capital letter for a particular pH value do not differ significantly ($p \geq 0.05$).

3.2 Foaming properties of PI1 and PI2 at different pH

Variations in pH influenced the foam capacity of both protein isolates either in the presence of NaCl or without salt (Fig. 1 and Fig. 2). In general, the trend followed pH-dependent solubility of the protein isolates previously established in our laboratory (Ivanova et al., 2013). A similar observation was reported by Khalil, Ragab, and Hassanien (1985) who studied the functional properties of proteins isolated from oilseeds.

The foam capacity of PI1 was the lowest at pH 4 under all tested conditions (Fig. 1). Without addition of NaCl, the alteration was more prominent at pH 2 where approximately 3 fold increase of the foam capacity was observed. According to González-Pérez et al. (2004), the smaller foam volume of sunflower proteins in neutral or alkaline media is due to the relatively large molecules and stable conformation of helianthinin which impair their absorption at system interfaces. In acidic medium ($\text{pH} < 3$), globulins dissociate to monomers which increases the volume of the foam.

Foam capacity of PI2 was also pH dependent (Fig. 2). However, in contrast to PI1, it reached maximal values at pH 8 and 10 which corresponded to the solubility profile of the isolate under the conditions studied (Ivanova et al., 2013). The presence of NaCl improved the foam capacity of both protein isolates (Fig. 1 and 2). However, the influence of the lower concentration of NaCl (0.03 M) on the foam capacity of PI1 was stronger than that of the higher NaCl concentration (0.25 M) (Fig. 1). In general, the influence of NaCl on foam capacity of PI1 and PI2 followed the solubility pattern of the protein isolates at different pH values (Ivanova et al., 2013). At the pH corresponding to high solubility of protein isolates, the influence of NaCl on the foam capacity was stronger than that at the pH providing conditions for low solubility of the isolates. The foam stability of PI1 and PI2 is presented in Table 2. The minimum foam stability of both isolates in water without addition of NaCl was observed at pH 4. Both levels of NaCl improved the foam stability of PI1 and PI2 at pH 4 only. For all other pH values, the addition of either 0.03

M or 0.25 M NaCl diminished the foam stability with the exception of pH 2 where 0.25 M NaCl improved the foam stability of PI1 to $91.51 \pm 3.70\%$. These results are probably a consequence of the influence of pH and NaCl on protein molecular charge and interactions. At the isoelectric point of the sunflower proteins (pH 4), the total charge of protein molecules is close to zero which leads to their aggregation and coagulation. The higher molecular weight complexes impair the formation of viscoelastic protein film at the boundary of the two phases which is mandatory for stabilization of the foam. At pH values differing from the pH of the isoelectric point, the protein molecules are either positively or negatively charged which determines their electrostatic repulsion. NaCl exhibits a similar influence on protein molecules. Chloride anions neutralize the positive charge of protein molecules thus moving isoelectric point to lower pH values. As a result, the addition of NaCl leads to a balance between protein-protein and protein-water interactions and the formation of a net-like structure at the interface which improves the foam stability at pH 4. In alkaline media, where protein molecules are negatively charged, the addition of NaCl increases electrostatic repulsion of proteins which forms an unstable boundary protein layer (Schnepf, 1992; Damodaran, 1997).

3.3 Emulsifying properties of protein isolates

The ability to form and stabilize emulsions is an important functional characteristic of sunflower protein isolates when used as food ingredients (Pawar et al., 2001). The emulsifying activity of PI1 and PI2 as a function of pH at two different levels of NaCl (0.03 M and 0.25 M) is presented in Table 3. Both protein isolates expressed minimum emulsifying activity at pH 4. Lowest emulsifying activity of protein isolates in the pH area of the isoelectric point was reported by Mao and Hua (2012) and Ogunwolu et al. (2009) who studied the functional properties of walnut and cashew nut proteins respectively. According to Damodaran (1997), most plant proteins are poor emulsifiers at the pH around their isoelectric points due to the low level of hydra-

Table 2: Influence of pH on foam stability of protein isolate 1 and protein isolate 2

	NaCl, M	Foam stability, %				
		pH				
		2	4	6	8	10
Protein isolate 1	0.00	82.18±0.25 ^{bc,B}	50.00±0.00 ^{d,B}	98.13±0.88 ^{a,A}	84.22±2.11 ^{b,A}	81.54±0.40 ^{c,A}
	0.03	50.84±1.18 ^{b,C}	89.29±5.06 ^{a,A}	80.74±4.91 ^{a,B}	56.79±4.55 ^{b,C}	50.00±0.00 ^{b,C}
	0.25	91.51±3.70 ^{a,A}	58.57±2.02 ^{d,B}	84.93±0.44 ^{b,B}	68.93±1.51 ^{c,B}	57.78±3.14 ^{d,B}
Protein isolate 2	0.00	82.38±1.34 ^{c,A}	66.67±0.00 ^{d,B}	85.71±0.00 ^{b,A}	91.49±0.26 ^{a,A}	84.26±1.32 ^{bc,A}
	0.03	74.17±1.18 ^{a,B}	77.50±3.54 ^{a,A}	76.39±1.97 ^{a,B}	74.78±4.74 ^{a,B}	73.61±1.97 ^{a,B}
	0.25	72.73±0.00 ^{cd,B}	79.52±2.45 ^{a,A}	77.78±0.00 ^{ab,B}	75.00±0.00 ^{bc,B}	70.00±0.00 ^{d,B}

^{a-d} Means in a row with same lowercase letter do not differ significantly (p≥0.05).

^{A-C} Means in a column for a particular protein isolate with same capital letter do not differ significantly (p≥0.05).

Table 3: Influence of pH on emulsifying activity of protein isolate 1 and protein isolate 2

	NaCl, M	Emulsifying activity, %				
		pH				
		2	4	6	8	10
Protein isolate 1	0.00	52.90±2.55 ^{a,A}	44.75±1.77 ^{b,A}	53.66±3.67 ^{a,A}	54.53±3.44 ^{a,A}	55.75±3.47 ^{a,A}
	0.03	57.14±0.00 ^{a,A}	45.45±2.40 ^{c,A}	51.40±2.86 ^{b,A}	54.13±2.02 ^{ab,A}	58.77±0.62 ^{a,A}
	0.25	53.70±1.19 ^{b,A}	2.90±0.06 ^{c,B}	55.20±1.62 ^{b,A}	53.69±0.51 ^{b,A}	59.17±1.18 ^{a,A}
Protein isolate 2	0.00	49.00±1.41 ^{ab,B}	45.85±1.20 ^{b,B}	52.70±0.28 ^{a,B}	51.30±1.84 ^{a,A}	51.55±3.18 ^{a,B}
	0.03	58.22±0.35 ^{a,A}	47.62±1.35 ^{c,B}	53.10±0.34 ^{b,B}	53.33±0.00 ^{b,A}	59.57±1.75 ^{a,A}
	0.25	60.88±1.25 ^{a,A}	53.00±1.34 ^{c,A}	55.63±0.52 ^{bc,A}	53.59±0.36 ^{c,A}	57.17±1.65 ^{b,AB}

^{a-c} Means in a row with same lowercase letter do not differ significantly (p≥0.05).

^{A-B} Means in a column for a particular protein isolate with same capital letter do not differ significantly (p≥0.05).

Table 4: Influence of pH on emulsion stability of protein isolate 1 and protein isolate 2

	NaCl, M	Emulsion stability, %				
		pH				
		2	4	6	8	10
Protein isolate 1	0.00	93.20±2.69 ^{bc,B}	100.0±0.00 ^{a,A}	90.30±1.98 ^{c,B}	95.75±0.21 ^{b,A}	93.65±0.21 ^{bc,B}
	0.03	100.0±0.00 ^{a,A}	93.67±3.90 ^{a,A}	100.0±0.00 ^{a,A}	97.44±3.63 ^{a,A}	98.81±1.68 ^{a,A}
	0.25	100.0±0.00 ^{a,A}	100.0±0.00 ^{a,A}	92.50±0.00 ^{c,B}	96.25±1.77 ^{b,A}	96.43±1.68 ^{b,AB}
Protein isolate 2	0.00	100.0±0.00 ^{a,A}	98.55±2.05 ^{ab,A}	98.65±1.91 ^{ab,A}	100.0±0.00 ^{a,A}	96.25±1.34 ^{b,B}
	0.03	100.0±0.00 ^{a,A}	86.97±1.78 ^{b,B}	100.0±0.00 ^{a,A}	98.75±1.77 ^{a,A}	100.0±0.00 ^{a,A}
	0.25	97.62±3.37 ^{a,A}	98.78±1.72 ^{a,A}	97.62±3.37 ^{a,A}	98.81±1.68 ^{a,A}	95.24±0.00 ^{a,B}

^{a-c} Means in a row with same lowercase letter do not differ significantly (p≥0.05).

^{A-B} Means in a column for a particular protein isolate with same capital letter do not differ significantly (p≥0.05).

tion and lack of electrostatic repulsive forces. At pH values differing from 4, the emulsifying activity of PI1 and PI2 varied from 45% to 60%. NaCl did not influence the emulsifying activity of PI1 at all pH values with except at pH 4 where it was highly reduced to 2.90 ± 0.06 by the addition of 0.25 M NaCl. The emulsifying activity of PI2 was enhanced by the higher level NaCl. Improvement of the emulsifying activity of the isolate by 0.03 M NaCl was reached only at pH 2 and pH 10 when compared to water. In general, the protein isolates formed emulsions which remained stable (> 90%) under all studied conditions (Table 4). Mahajan and Dua (2002) also reported that salts, NaCl and NaHCO_3 , did not change the emulsifying properties of amaranth seed meal. Stable emulsion properties of sunflower protein isolate were reported by González-Pérez et al. (2005) as well.

4 Conclusions

As a whole, the pH and NaCl, at the concentrations studied, influenced foam properties and emulsifying activity of PI1 and PI2. They slightly altered the emulsion stability of the isolates which remained above 90% under all studied conditions. NaCl significantly influenced the WAC of PI1 but not that of PI2 or the OAC of both isolates. The significant differences between PI1 and PI2 observed for a particular functional property were probably due to physico-chemical characteristics of the isolates which originated in the specifics of the methods for their preparations. Knowledge of the influence of pH and boundary concentrations of NaCl on the functionality of sunflower meal protein isolates could be beneficial for their future potential application in food formulation.

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