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Effect of pH, Carbohydrates, and NaCl on Functional Properties of Whey Proteins

M. A. Ghanimah* and Eman M. Ibrahim
Department of Dairy Science, Faculty of Agriculture, Kafrelsheikh University,Egypt

THE effect of pH (7,5 and 3), and the addition of glucose, sucrose and Litesse II, as well as sodium chloride (1,2 and 3%) on the functional properties of whey protein isolate and whey protein concentrate was investigated. Foaming capacity and emulsifying activity index were significantly affected by the change of the pH in the prepared solutions. The addition of glucose to protein solutions had a slight impact (P>0.05) on the functional properties, while the foaming capacity of whey protein isolate solution significantly increased with the presence of sucrose. Litesse II significantly increased the foaming capacity of whey protein isolate and whey protein concentrate solutions, whereas the emulsifying activity index of whey protein isolate solution significantly increased with adding 1 and 2 % Litesse II. The addition of NaCl significantly increased the foaming properties of whey protein isolate and whey protein concentrate solutions and increased the emulsifying activity index of whey protein concentrate solution.

Keywords: Carbohydrates, Emulsifying, Foaming, pH, NaCl, WPI, WPC.

Introduction

Whey proteins are well known for their high nutritional value and health benefits (Krissansen,2007). Whey protein isolates (WPI, comprising ≥ 90% protein) and whey protein concentrates (WPC, comprising 34-80% protein) are among the available commercial forms of whey products, which are widely used in the food industry and dairy sector due to their unique functional characteristics, like foaming and emulsifying properties (Foegeding et al. ,2002).

Foams and emulsions are dispersed air/water and oil/water systems, respectively (Kilara and Vaghela et al., 2018). Protein function in foam properties as a surfactant by adsorbing at the freshly created air/water interface during bubble formation, and consequently lowers the interfacial tension and promotes bubble formation (Davis and Foegeding, 2007). Foaming performance of proteins is assessed by foaming capacity (FC) and foaming stability (FS). Higher FC implies greater incorporation of air bubbles, while FS describes the ability of proteins to form a strong and cohesive film around air bubbles (Patel and Kilara, 1990). Foams function as structuring materials in many food products and represent in the form of bread, ice cream and various baked goods (Ercelebi and Ibanoglu, 2009). Emulsifying properties are usually described as emulsification capacity, which reflects the ability of the protein to rapidly adsorbs at the water/oil interface during the formation of the emulsion and preventing flocculation and coalescence (Webb et al., 2002). Emulsions can improve the texture, appearance and organoleptic attributes of many food products in general (Fachin and Viotto, 2005).

Most of the food formulations contain a variety of ingredients such as carbohydrates, especially glucose and sucrose, which may interact with whey proteins and may affect their functionality (Kulmyrzaev et al., 2000). It was established that several factors such as the changes in the pH and ionic strength (salt type and concentration) have a major impact on the functional properties of whey proteins (Kulmyrzaev et al., 2000).

Litesse II® is a white - to- cream colored powder, which is described as the third generation of the polydextrose. Litesse II is specially developed for using in light foods provides a higher level of sugar and fat replacement, and it has prebiotic properties (Mitchell, 1996). The objective of the present research was to investigate the effect of the pH, and the addition of glucose, sucrose, Litesse II and NaCl on foaming and emulsifying properties of WPI and WPC.

* Corresponding author: Email: m.ghanimah@yahoo.com
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Materials and Methods

Instantized whey protein isolate (WPI) and whey protein concentrate (WPC) were kindly provided by Hilmar Ingredients, USA. The chemical composition of these products is shown in Table 1. Glucose, sucrose, and NaCl were obtained from El- Gomhouria Chemical Company, Egypt. Litesse II® was supplied by Danisco Inc., USA.

TABLE 1. Chemical composition (%) of WPI and WPC (as provided by the manufacturer)

	WPI	WPC
Moisture%	5.5	5.6
Protein%	92.6	81.4
Fat%	0.8	4.0
Ash%	2.5	2.8

Foaming properties

Solutions of WPI or WPC (5% w/v) were prepared and kept overnight in a refrigerator. The pH was adjusted to 3.0, 5.0 and 7.0 pH using 1 *N* HCl or 0.1 *N* NaOH.

Glucose, sucrose, Litesse II, and NaCl were separately added to the prepared WPI and WPC solutions in ratios of 0 (control), 1, 2 and 3% (w/v) with continuous stirring.

One hundred milliliter of each solution was whipped for 5 min using an electric blender. The foaming capacity%(FC) and foaming stability%(FS) were computed according to Patel et al. (1988).

Emulsification properties

Solutions of WPI or WPC (2% w/v) were prepared and kept overnight in a refrigerator. The pH was adjusted to 3.0, 5.0 and 7.0 pH using 1 *N* HCl or 0.1 *N* NaOH.

Glucose, sucrose, Litesse II, and NaCl were separately added to WPI and WPC solutions in ratios of 0 (control), 1, 2 and 3% (w/v) with continuous stirring.

Volumes of 75 ml of WPI or WPC solutions and 25 ml of sunflower oil were mixed together in a blender for 3 min at room temperature. The emulsification capacity (EC) was determined by the turbidimetric method described by Pearce and Kinsella (1978). The EC expressed as emulsifying activity index (EAI, m^2/g) was computed using the equation given by Herceg et al. (2007).

Statistical analysis

Analysis of variance and Duncan's test (at $P<0.05$) were carried out and the average and standard error were computed using the SPSS program (version 16), SPSS Inc., Chicago, IL, USA.

Results and Discussion

The effect of pH on foaming capacity (FC) and foaming stability (FS) of WPI and WPC solutions is illustrated in Fig 1. The data show that the FC significantly ($P < 0.05$) increased with the decrease of the pH. The highest FC values were recorded at pH 3.0 (124.5% and 109.5% for WPI and WPC solutions, respectively), while the lowest values ($P<0.05$) were observed at pH 7.0 (92% and 82% for WPI and WPC solutions, respectively). The FS values insignificantly increased ($P>0.05$) with the decrease of the pH of WPI solution, whereas the lowest ($P<0.05$) FS value was recorded at pH 7.0 – WPC solution, while at pH 5.0-WPC solution possessed the highest value.

Figure 2 indicates that emulsifying activity index (EAI) was significantly affected by changing the pH of the solutions. The highest value ($P<0.05$) was recorded at pH 7.0 in case of WPI solution (206.3 m^2/g), while at pH 5.0 – WPC solution showed the highest ($P<0.05$) EAI value (186.73 m^2/g) in comparison with the other WPC solutions. These results agree with those of Lam and Nickerson (2015), who found that the EAI of WPI solution was greater at both pH 7.0 and 3.0. They attributed that to WPI aggregates were smaller to allow faster migration and integration into the interface. The changes in pH affect the conformation and the net charge of the adsorbed protein layers at the interface (Phillips et al., 1994). Moreover, the pH of protein solution may affect the solubility of proteins by changing the repulse among molecules (Peligrine and Gasparetto, 2005). At pH 5.0, the net charge is minimized, and the aggregation of whey proteins was the highest due to a reduction in electrostatic repulsion between proteins and rigid protein films (Zayas, 1997 and Lam & Nickerson ,2015). The increment of pH from 5 to 7 pH increases the stability of whey protein emulsions, probably due to an increase in repulsion by the electrostatic charge of the protein (Yamauchi et al., 1980 and Leman et al., 1988).

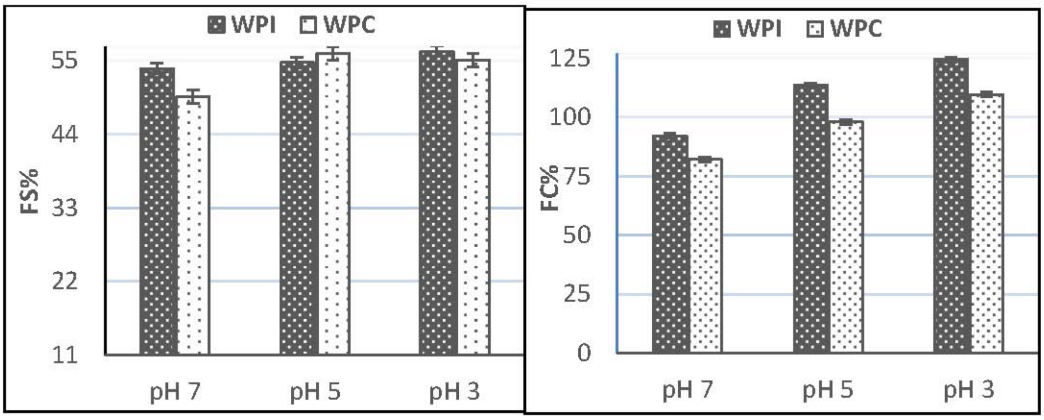

Fig. 1. Effect of pH on the FC and FS of WPI and WPC solutions

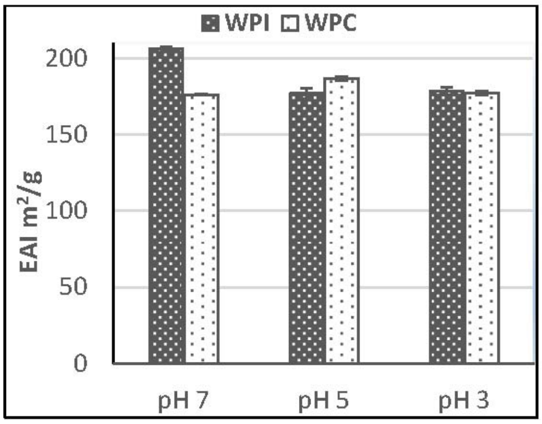

Fig. 2. Effect of pH on EAI of WPI and WPC solutions

The effect of the addition of some carbohydrates on FC, FS, and EAI of WPI and WPC solutions is presented in Table 2. In all cases, the WPI solutions possessed better functional properties than those of WPC solutions. This could be attributed to the high protein and low fat and ash content in WPI (Table 1). It has been reported that the functional properties of whey products are related to the protein content and the composition of the product (Heino et al., 2007 and Ghanimah, 2018).

Clearly, the addition of glucose slightly increased (*P*>0.05) the FC of WPI solutions, and the EAI of WPI and WPC solutions. The addition of sucrose significantly(*P*<0.05) increased the FC and slightly increased (*P*>0.05) the EAI of the WPI solutions. The WPC solutions with sucrose had slightly higher FC and lower (*P*>0.05) FS and EAI values than the control.

A similar trend of results was observed by Farrag (2008), who found that adding sucrose increased the foaming performance of whey protein concentrate. The possible explanation such results may be the addition of sucrose increase the bulk viscosity and influence the surface properties of the protein films (Zayas ,1997). Proteins adsorb faster to the air/water interface in the presence of sucrose (Zayas, 1997).

An opposite trend of results was observed by Yang and Foegeding (2010), who found that increasing sucrose concentration (0-63.3 g/100 mL) gradually decreased foam overrun of WPI solution and increased foam stability. It is clear from Table 2 that the addition of Litesse II significantly (*P*<0.05) increased the FC of WPI and WPC solutions. The FS values of the control and Litesse- treated solutions were not statistically different. The EAI of WPI solution significantly increased by adding 1 and 2% Litesse II and decreased (*P*<0.05) with 3% Litesse II. Litesse II addition had insignificant effect (*P*>0.05) on the EAI of WPC solution. These results may be attributed to sugars and polysaccharides encourage protein-protein interactions and lead to the development of multilayer cohesive protein film at the interface, which prevents foam collapse and enables the formation of more stable foam (Adebowale and Lawal, 2003). It is well known that sugars and polysaccharides have no affinity for the air-water interface (Bos and van Vliet, 2001). Farrag (2008) found that WPC with starch and inulin showed an improvement in the foaming and emulsifying properties. Herceg et al. (2007) mentioned that some literatures have shown that specific interactions between proteins and polysaccharides may result in the formation of a complex with substantially improved emulsifying, foaming and rheological properties.

The data presented in Table 3 show the effect of adding NaCl on FC, FS and EAI of WPI and WPC solutions. Adding sodium chloride showed significant impact on the functional properties. The addition of sodium chloride significantly increased the FC and FS of WPI and WPC solutions. The EAI was significantly affected by the addition of NaCl. The NaCl-treated WPI solutions had significant lower EAI values than the control. The addition of sodium chloride to WPC solutions showed an opposite trend, where the EAI values significantly increased in the presence of NaCl, and no differences among the treated solutions were recorded. The apparent discrepancy between the findings may be due to the emulsifying capacity depends on the properties of the protein and vary with the source and the concentration of the protein as well as the viscosity of the system (Zayas, 1997).

These results agree with those of El-Desoki (2009), who found that the foaming properties of whey protein prepared from unsalted whey increased by increasing salt concentration, while emulsifying capacity decreased. Ercelebi and Ibanoglu (2009) found that the foam volume and stability of WPI increased when NaCl concentration increased from 0 to 0.05 *M* and decreased with further increase of the concentration.

The addition of low concentration of NaCl increased the protein solubility; as result of the salting in; at the air/water interface during the formation of foam (Zayas, 1997). The addition of NaCl improved foaming performance by modifying protein net charge, increasing the

TABLE 2. Effect of some carbohydrates on FC, FS and EAI of the WPI and WPC solutions (Means ± SE of 3 replicates)

Carbohydrate concentration	WPI solutions			WPC solutions		
	FC%	FS%	EAI(m^2/g)	FC%	FS%	EAI(m^2/g)
Glucose (%)						
0	92.0^{a}±2.0	51.5^{a}±1.02	220.7^{a} ±2.0	88.0^{a} ±2.0	49.9^{a} ±0.5	183.8^{a} ±2.6
1	96.0^{a}±2.0	51.53^{a}±0.1	221.1^{a} ±2.0	86.0^{a} ±0.0	48.9^{a} ±0.5	187.2^{a} ±2.0
2	97.0^{a}±1.0	51.26^{a}±0.2	223.06^{a} ±2.0	89.0^{a} ±1.0	51.8^{a} ±1.7	185.9^{a} ±3.0
3	96.0^{a}±1.0	52.2^{a}±0.18	228.33^{a} ±2.0	88.0^{a} ±0.0	48.4^{a} ±0.5	187.5^{a} ±1.3
Sucrose (%)						
0	91.7^{c} ±0.8	51.58^{a} ±0.6	204.95^{a} ±2.9	79.3^{a} ±1.3	51.5^{a} ±2.4	178.9^{a} ±1.5
1	96.0^{c}±1.3	52.1^{a} ±0.1	211.2^{a} ±3.9	80.3^{a} ±0.3	48.5^{a} ±1.5	170.4^{a} ±1.7
2	98.6^{ab}±0.5	51.7^{a} ±0.12	209.9^{a} ±1.9	84.1^{a} ±2.1	49.9^{a} ±0.5	167.1^{a} ±1.3
3	103.1^{a}±1.6	51.64^{a} ±0.6	210.6^{a} ±2.6	85.1^{a} ±1.1	49.4^{a} ±0.1	170.4^{a} ±1.6
Litesse II (%)						
0	90.5^{b}±1.5	50.53^{a} ±0.1	199.7^{b}±3.7	81.0^{c}±0.5	50.4^{a} ±2.3	182.9^{a} ±1.6
1	95.5^{ab}±1.4	51.15^{a} ±0.6	206.3^{b}±0.4	83.5^{b}±0.5	49.7^{a} ±0.8	184.5^{a} ±1.8
2	98.0^{a}±2.0	51.80^{a} ±0.6	224.7^{a}±2.0	84.0^{b}±0.5	48.5^{a} ±0.9	186.2^{a} ±1.3
3	99.0^{a} ±1.0	53.2^{a} ±0.7	197.5^{b}±3.1	86.0^{a}±0.0	48.6^{a} ±0.3	184.2^{a} ±3.2

Means in the same column with different superscripts differed significantly ($P < 0.05$)

TABLE 3. Effect of NaCl (%) on FC, FS and EAI of WPI and WPC solutions

NaCl concentration%	WPI solutions			WPC solutions		
	FC%	FS%	EAI(m^2/g)	FC%	FS%	EAI(m^2/g)
0	95^{b}±2.0	51.3^{b}±0.7	190.0^{a}±1.3	80^{c}±0.5	47.95^{b}±.9	196.1^{b}±2.1
1	108^{a}±1.5	57.2^{a}±0.6	186.2^{ab}±1.25	86^{b}±2.0	51.2^{a}±.9	206.6^{a}±2.6
2	104^{a}±2.0	55.9^{a}±0.9	176.5^{bc}±2.9	89.5^{b}±0.5	51.8^{a}±.2	217.7^{a}±2.7
3	107^{a}±1.4	57.0^{a}±0.5	167.7^{c}±3.0	94^{a} ±2.0	53.4^{a}±.4	221.5^{a}±0.2

Means in the same column with different lowercase superscripts differed significantly ($P < 0.05$).

protein adsorption at the interface due to the decrease in surface repulsions between adsorbed and nonadsorbed proteins, and decreasing the protein denaturation during foam formation (Hailing, 1981). Moreover, the addition of NaCl can impair electrostatic interactions and affect protein diffusion and unfolding at the interface (Zayas, 1997). Nishanthi et al. (2018) reported that high number of hydrophobic segments was probably exposed on the protein surface in salty-WPC due to sodium-induced whey protein denaturation, thus increasing the number of proteins absorbed to emulsion interface, enhancing emulsion activity.

Conclussion

This study indicates that the functional properties of whey proteins were affected by the change in the pH of their solutions. The addition of Litesse II and NaCl enhanced the FC of WPI and WPC solutions, while sucrose and Litesse II increased the FC and EAI of WPI solution, respectively. The addition of NaCl caused a different impact on the EAI of WPI and WPC solutions.

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تأثير ال pH ، الكربوهيدرات وكلوريد الصوديوم على الخواص الوظيفية لبروتينات الشرش

محمد عابد غنيمة وايمان محمد إبراهيم
قسم الألبان، كليه الزراعة ، جامعة كفر الشيخ ، مصر

تم في هذه البحث دراسة تأثير قيمه ال pH (٧، ٥ و ٣) واضافه الجلوكوز، السكروز و Litesse II وكذلك اضافه كلوريد الصوديوم (بنسبه ١، ٢ و ٣٪) على الخواص الوظيفية لبروتين الشرش المعزول والمركز. وجد ان سعة تكون الرغوة والنشاط الاستحلابى لمحاليل بروتينات الشرش المحضرة قد تأثرا بصوره معنويه بالتغير في pHهذه المحاليل. لم يؤدى اضافه الجلوكوز لمحاليل بروتينات الشرش الا لتأثير طفيف غير معنوي على الخواص الوظيفية لمحاليل بروتينات الشرش بينما زادت سعة تكون الرغوة معنويا بإضافة السكروز لمحلول بروتين الشرش المعزول. وجد ان اضافهLitesse II أدى الى زيادة معنويه في سعة تكون الرغوة لمحاليل كلا من بروتين الشرش المعزول والمركز بينما زاد النشاط الاستحلابى لمحلول بروتين الشرش المعزول بإضافة ١ و ٢ ٪ من .Litesse II أدت اضافه كلوريد الصوديوم الى حدوث زيادة معنويه لخواص تكوين الرغوة لمحاليل بروتين الشرش المعزول أو المركز، وقد أدى كذلك الى زياده معنويه في النشاط الاستحلابى لمحلول بروتين الشرش المركز.