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Effect of Heat Treatments of Whey Proteins Supplemented with Sodium Caseinate on the Emulsions Properties.

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ABSTRACT

Whey proteins are often used as food ingredients in many food products not only for their nutritional properties, but also for their interesting physic – chemical properties. Emulsions were prepared from pasteurized, boiled and sterilized 3% WPC solution containing 5, 10, 15, 20, 25 and 30% of sodium caseinate at pH 7.0. The emulsifying activity index (EAI), the emulsion stability index (ESI) and creaming stability were measured after holding the emulsions at 4 °C for 24, 48 and 72 h. The results indicated that sterilization improved significantly the emulsifying activity index (EAI) followed by boiling and pasteurization compared with unheated samples. Unheated WPC samples showed (EAI) of 45.14 m^2/g increased to 47.77 m^2/g with pasteurization and increased to 55.38 m^2/g with boiling and increased to 60.20 m^2/g with sterilization treatment. Either emulsifying activity (EAI) or emulsifying stability ESI were improved significantly with addition sodium caseinate to WPC compared to emulsion prepared with WPC only. Also, sterilization for WPC/SC solutions appeared more emulsions stability ESI after 72 hrs of storage compared to other heat treatments. Moreover, addition of sodium caseinate to WPC solution led to more emulsion stability ESI during storage for 72 hrs. Sterilization enhancement significantly creaming stability of formulated emulsions compared to boiling and pasteurization. Addition of sodium caseinate improved creaming stability of resultant emulsions after storage of 72 hrs.

Keywords: Whey proteins, sodium caseinate, Heat treatment, Emulsion properties, Creaming Stability.

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INTRODUCTION

Liquid whey production exceeded 39 million kg in 2004 (USDA). Liquid whey processed into whey powder, whey protein concentrate and whey protein isolate with protein content up to 90 % (Carunchia, 2005).

Due to their exceptional functional properties and their ability to contribute unique and essential functional characteristics to the final products whey proteins (WPs) are used in food emulsion systems (Dickinson, 1994 and Huffman, 1996).

The high susceptibility of WPs to heat treatments is one of the main problems associated with application of WPs induced to aggregation (Singh & Havea, 2003). Different ideas have been proposed to solution this problem, for example by changing the surface properties (Bouaouina, et al., 2006), inducing conformational and structural changes in WPs to increase dispersibility and solubility (Dissanayake, et al., 2012).

Because of their excellent surface active properties, milk proteins are often play important structural and stabilization roles in food formulations (Dickinson, 2009). In food emulsion systems, milk proteins absorb as an excess layer at the oil–water interface and lower the interfacial tension between the phases. To improve the emulsifying properties, many food emulsions undergo thermal treatments including heating, chilling and/or freezing (Mun, et al 2008).

Recently, caseins were play important roles as molecular chaperones that may control on the denaturation and aggregation of WPs (Kehoe & Foegeding, 2011).

Heat treatment is essential step in most dairy product processing, this treatment affects the rheological properties and structure of milk protein emulsions and determines to a large extent the consumer acceptability of such products (McSweeney, et al., 2004). Heating of milk is not only to ensure microbiological safety, in other cases heat treatment is employed to improve the organoleptic properties of such dairy formulations, where milk is used as a food ingredient in milk-based products (del Angel & Dalgleish, 2006).

However from the applied point of view, it would be important to examine behaviour of commercially available casein preparations and their role in influencing the denaturation and aggregation of commercially available WPs. The aim of this work was thus to assess the impact of the casein inclusion and heat treatment on the properties of WPs formulated emulsion.

MATERIALS AND METHODS

Materials

- Whey Protein Concentrate (LACTOMIN 80) 81% protein, 5% water, 7% fat and 4.0% ash. Sodium caseinate(LACTONAT EN) 88.5% protein, 6% water, 1.5 fat and 4.5% ash. Both type of protein were obtained from Lactoprot Deutschland GmbH, Germany.
- Corn oil was obtained from local market by ARAMA Oils CO., 10^{th} of Ramadan Egypt.

Methods

Preparation of the WPC - sodium caseinate mixtures

Whey Protein Concentrate (WPC) and Sodium Caseinate (SC) were individually dispersed at 3% (w/v) in deionized water and stirred for at least 2 h at ambient temperature, and then stored overnight at 4°C to ensure complete dissolution. . Sodium azide (0.02%, w/w) was added to dispersions to prevent microbial growth.

Different volumes of the both aqueous protein solutions were mixed together under moderate magnetic stirring at ambient temperature to obtain 3% protein solutions of different WPC/SC ratios as follows:-

Table (1): The ratios between WPC and sodium caseinate mixtures .

Samples	Whey proteins Dispersion (ml)	Sodium Caseinate Dispersion (ml)	Total Volume (ml)
WPC	100	0.0	100
WPC-SC 1	95	5	100
WPC-SC 2	90	10	100
WPC-SC 3	85	15	100
WPC-SC 4	80	20	100
WPC-SC 5	75	25	100
WPC-SC 6	70	30	100

Heat treatment on the prepared protein mixtures:

WPC-SC solutions were adjusted to pH 7.0, then subjected to heat treatment at pasteurization of 72° for 15 sec, boiling of 100°C and sterilization at 121°C at 15 min.

Preparation of emulsions

Emulsions were prepared using 10 ml corn oil and 90 ml of 3% (w/w) total solids of heated WPC-SC solutions. Emulsions were then mixed using a high-speed blender (Hamilton Beach Model 600 AL Almond USA) for 2 min and then passed through QP laboratory homogenizer at 500 psi at room temperature. The emulsifying activity index (EAI) was measured and the emulsions were held at 4 °C for 24, 48 and 72 h, and the emulsion stability index (ESI) were determined. The chemical composition of emulsion was determined, Total Solids (TS) according to AOAC (1990); total protein and pH according to ling (1963) and lactose according to Barrnet and Tawab, (1957).

Emulsifying properties

Emulsifying activity index (EAI) and emulsion stability index (ESI) were determined by the turbidometric technique as described by Manoi and Rizvi (2009).

Creaming Index

The creaming index was evaluated as described by Firebaugh and Daubert (2005) with some modifications. Immediately after emulsification, ten millimeters of each emulsion was filled into a glass test tube (1.5 cm internal diameter × 12cm height) and then stored at ambient temperature. The height of the serum (H_s) and the total height of emulsions (H_t) were recorded after storage at ambient temperature for 1, 2, and 3 days. The mean of three replicates was reported. The creaming index was reported as:

$$\text{Creaming index (\%)} = \frac{H_s}{H_t} \times 100$$

Statistical analysis

The data were analyzed according to Statistical Analysis System (SAS, 1998). Duncan multiple range tests were carried out for separation among means. All experimental were replicated three times.

RESULTS AND DISCUSSION

The chemical composition of whey protein concentrate (WPC)/sodium caseinate (SC) composite blends are shown in table 2. Protein contents varying between 1.99 to 2.27% of WPC and WPC-SC6. Increase in protein content refers to increase in the sodium caseinate protein content and the substitute ratios. pHs varying between 6.60 to 6.80 in the same order. All treatments containing 3% total solids with different ratios between WPC and SC blends.

Table (2): Chemical composition of whey protein concentrate (WPC) / sodium caseinate composite blend solutions of different protein content.

Treatment	T.N	TP	Lactose	pH
WPC	0.312	1.99	1.00	6.60
WPC-SC 1	0.322	2.05	0.95	6.67
WPC-SC2	0.329	2.10	0.90	6.70
WPC-SC3	0.336	2.14	0.85	6.73
WPC-SC4	0.343	2.19	0.80	6.78
WPC-SC5	0.350	2.23	0.75	6.79
WPC-SC6	0.357	2.27	0.70	6.80

Emulsifying activity index (EAI) of WPC/SC treatments as affected by heat treatments is shown in table (3). Treatments substituted WPC with sodium caseinate showed the greatest emulsifying capacity with increase in the sodium caseinate substitution ratios and with advanced in heat treatment. Unheated emulsions substituted WPC by sodium caseinate showed improvement in emulsifying activity index EAI of 45.14 for WPC emulsion increased to 58.30, 50.60, 55.75, 59.28, 64.90 and 67.10 m^2/g with sodium caseinate substitution of 5, 10, 15, 20, 25 and 30% respectively. Unheated WPC samples showed EAI 45.14 m^2/g increased to 47.77, 55.38 and 60.20 m^2/g after heat treatment of pasteurization, boiling and sterilization respectively.

Table (3): Effect of heat treatment on the emulsifying activity (m^2/g) of WPC/sodium caseinate blends.

Temperature / **Treatment**	Unheated	Pasteurization 70°C/15 sec	Boiling 100°C/3 sec	Sterilization 121°C/ 15 min
WPC	45.14	47.77	55.38	60.20
WPC-SC 1	58.30	59.00	59.79	64.48
WPC-SC2	50.60	55.10	63.83	69.02
WPC-SC3	55.75	60.87	68.15	73.46
WPC-SC4	59.28	66.30	73.21	79.93
WPC-SC5	64.90	72.05	77.59	85.08
WPC-SC6	67.10	76.80	81.08	89.36

Table 3 shows that the emulsifying activity EAI of WPC/SC samples increased and improved with the increase of heating temperature and with the increase of sodium caseinate addition.

The best treatment for EAI was at sterilization at 121°C pH 7 on 15 psi for 15 min. It has been proposed that a degree of denaturation might promote an improvement in the emulsifying properties by exposing the hidden hydrophobic groups of the globular proteins (Harper, 1992, Voutsinas et al., 1983). An appropriate degree of denaturation is related to a suitable balance of the hydrophilic and hydrophobic groups of the protein molecule after denaturation (Aoki et al., 1981).

Mixed two aqueous protein solutions, WPI ingredient and sodium caseinate, in the appropriate ratio before homogenization so that the two protein types were free to adsorb together at the oil–water interface during emulsification. Particular concentration of caseinate was sufficient to give complete inhibition of heat-induced aggregation, when the system was subjected to heat treatment (Dickinson & Parkinson, 2004).

Furthermore, once the milk proteins become adsorbed at the oil–water interface, little free reversible exchange occurs between the adsorbed and the proteins located at the aqueous phase (Dickinson et al. 1990).

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It has been documented (Dickinson & Matsamura,1994) that when globular proteins such as β-lactoglobulin are adsorbed, displacement from the surface layer by other proteins, even very flexible ones like β-casein or as κ- casein, is rather unlikely to occur.

It has been documented that the amount of protein adsorbed at the emulsion droplet increases with increasing temperature for whey protein-stabilized emulsions (Sliwinski, et al. 2003). They concluded that initially oil droplets aggregate and upon further heating disaggregation takes place, leading to the formation of smaller, more compact emulsion droplets.

On the other hand, when caseinate was substituted by WPI at levels of up to 60%, there was also found to be no significant change in the measured droplet size. Also, at low levels of caseinate, where presumably the whey protein starts to predominate at the interface of oil droplets. (Dickinson and Parkinson 2004)

Table 4 shows that the stability (ESI) of formulated emulsion resulting from pasteurized whey protein/sodium caseinate. Emulsifying stability index (ESI) decreased during storage reached to 72 hrs. Both emulsifying activity EAI and emulsifying stability ESI were significantly improved in the emulsion with addition of sodium caseinate to WPC compared to emulsion prepared with WPC only. EAI increased with increase in the sodium caseinate substitution ratio. Emulsions containing 5% sodium caseinate showed EAI of 43.79 m2/g decreased as ESI to 31.81, 23.68, and 18.18 m2/g after 24, 48 and 72 hrs of storage. Moreover, increased the portion of sodium caseinate to 30% led to enhancement in the EAI of 62.41 m2/g decreased as ESI to 54.23, 41.63, and 33.69 m2/g after 24, 48 and 72 hrs of storage

Table (4): Emulsifying capacity and stability (m^2/g) of emulsion prepared using pasteurized WPC/sodium caseinate blends.

Stability / Treatment	EAI	ESI		
		24 h	48 h	72 h
WPC	38.08^{G}	27.19^{G}	21.91^{G}	15.49^{G}
WPC-SC 1	43.79^{F}	31.81^{F}	23.68^{F}	18.18^{F}
WPC-SC 2	46.83^{E}	37.65^{E}	26.22^{E}	20.47^{E}
WPC-SC 3	50.01^{D}	42.47^{D}	30.56^{D}	24.61^{D}
WPC-SC 4	54.17^{C}	45.17^{C}	32.97^{C}	26.76^{C}
WPC-SC 5	57.82^{B}	51.00^{B}	35.72^{B}	29.81^{B}
WPC-SC 6	62.41^{A}	54.23^{A}	41.63^{A}	33.69^{A}

The effect of boiling treatment of WPC/SC solutions on the emulsifying activity and stability index of resultant emulsions are shown in table 5.

Table (5): Emulsifying capacity and stability (m^2/g) of emulsion prepared using boiled WPC/sodium caseinate blends.

Stability / Treatment	EAI	ESI		
		24 h	48 h	72 h
WPC	37.47^{G}	30.84^{G}	21.24^{F}	20.56^{E}
WPC-SC 1	40.07^{F}	35.87^{F}	24.37^{E}	23.82^{D}
WPC-SC 2	44.24^{E}	42.27^{E}	26.50^{D}	25.59^{DD}
WPC-SC 3	49.14^{D}	44.27^{D}	29.34^{C}	27.91^{C}
	53.51^{C}	47.52^{C}	31.14^{CC}	29.75^{CC}

WPC-SC 4				
WPC-SC 5	58.27^{B}	53.02^{B}	34.15^{B}	32.95^{B}
WPC-SC 6	61.49^{A}	56.60^{A}	44.99^{A}	35.06^{A}

Emulsion prepared from boiling treatment substituted 30% of WPC by sodium caseinate showed significantly highest values of EAI of 61.49 m2/g decreased to 56.60, 44.99 and 35.06 m2/g after 24, 48 and 72 hrs of storage compared to that treatment substituted 5% of sodium caseinate which showed the lowest EAI values of 40.07 m2/g which decreased to 35.87, 24.37 and 23.82 m2/g in the same order.

Table 6 showed the properties of emulsions which formulated from sterilized WPC containing different portion substituted with sodium caseinate.

Table (6): Emulsifying capacity and stability (m^2/g) of emulsion prepared using sterilized WPC containing different supplemented ratios of sodium caseinate.

Stability / Treatment	EAI	ESI		
		24 h	48 h	72 h
WPC	46.20^{G}	41.53^{F}	20.97^{G}	15.69^{E}
WPC-SC 1	51.50^{F}	45.30^{E}	27.54^{F}	19.35^{D}
WPC-SC 2	55.24^{E}	47.04^{EE}	32.95^{E}	20.96^{DD}
WPC-SC 3	58.54^{D}	50.01^{D}	37.31^{D}	25.32^{C}
WPC-SC 4	62.50^{C}	56.21^{C}	40.28^{C}	26.83^{CC}
WPC-SC 5	67.33^{B}	59.91^{B}	45.08^{B}	34.38^{B}
WPC-SC 6	71.34^{A}	62.63^{A}	47.66^{A}	37.12^{A}

Emulsions prepared from sterilized WPC solutions containing different ratios of sodium caseinate showed significantly increased of EAI or ESI compared to that preparing from pasteurized or boiling treatment. Sterilized WPC solution containing 5% sodium caseinate showed EAI of 51.50 m2/g decreased as ESI to 45.30, 27.54 and 19.35 m2/g after 24, 48 and 72 hrs of storage. Increasing the sodium caseinate ratio to 30% increased the EAI to 71.34 m2/g and then decreased as ESI to 62.63, 47.66, 37.12 m2/g after 24, 48, 72 hrs of storage.

The results indicated that the emulsion EAI increased significantly with increased the presence of sodium caseinate portions in WPC solutions. Also sterilization treatment showed improvement in the EAI of emulsions than that boiling or pasteurization heat treatment.

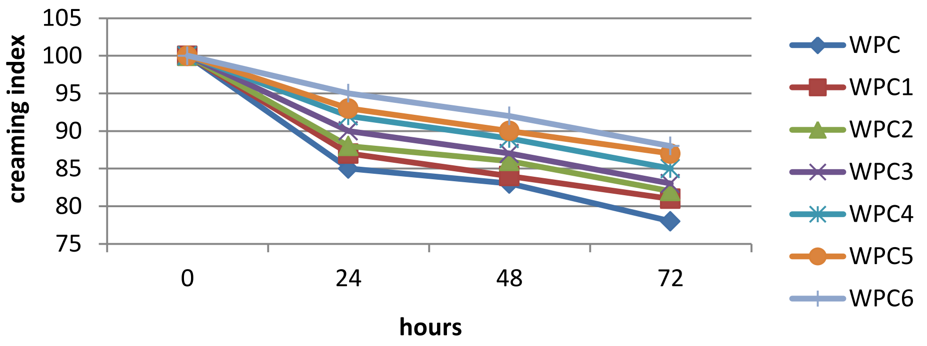
During homogenization of milk protein emulsions, competitive adsorption between the caseins and the whey proteins occurs, which results to the formation of a thin layer consisting of both types of proteins (Millqvist-Fureby et al., 2001). As a general rule, the protein that arrives first at the interface is the one that predominates (Dickinson, 1997).

Creaming Index.

The creaming index of emulsions formed with WPC as a function of heat treatment and sodium caseinate replacement ratio is shown in Fig. 1, 2 and 3.

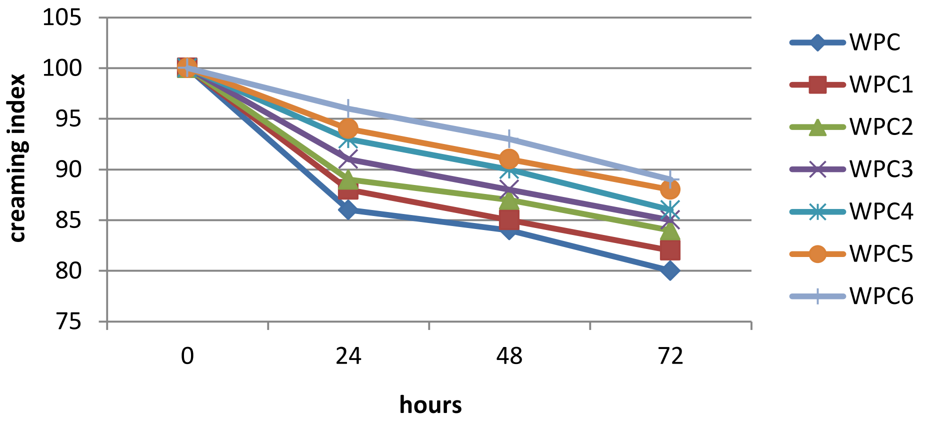
The stability of the emulsions formed with WPC only reached a maximum and then decreased markedly as the sodium caseinate replacement increased further. Emulsions prepared from pasteurized WPC showed decreased in its stability as creaming index to 85, 83 and 78% after 24, 48 and 72 h of storage but increase in this stability reached of 95, 92 and 88% when substituted whey protein by sodium caseinate of 30% in the same order (Fig. 1).

Fig. 1: Effect of pasteurization treatment of WPC/SC on the Creaming Indes of the emulsion resultant.



Increased heat treatment of whey protein mixtures to boiling treatment slightly improvement the emulsion stability compared to pasteurized heat treatment (Fig. 2). Boiling of whey protein containing different substitute of sodium caseinate ratio showed creaming index of 86, 84 and 80% with whey protein emulsion only after 24, 48 and 72 h of storage increased to 96, 93 and 89% of emulsion containing 30% sodium caseinate in the same order. The results in the present study displayed that substitution whey protein by sodium caseinate caused a increase in emulsion stability and decreased of creaming layer formed of resultant emulsions.

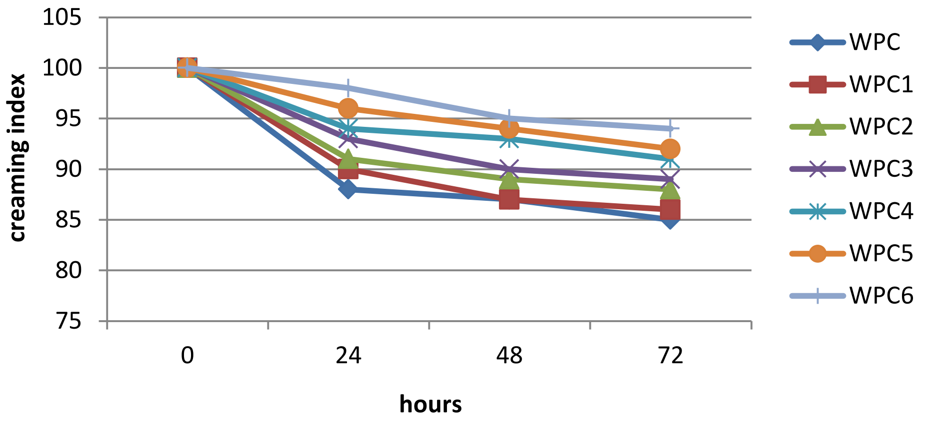
Fig. 2: Effect of boiling treatment of WPC/SC on the Creaming Indes of the emulsion resultant.



At higher temperatures, proteins become fully unfolded and are able to rearrange effectively all non-polar amino acids towards the oil phase, thus reducing the tendency for aggregation. Most likely, at higher temperatures proteins at the interface possibly may form a more compact layer covering the oil droplet, which increases the density of the droplet and lowers the susceptibility to creaming (Monahan et al., 1996).

The results displayed that substitution whey protein by sodium caseinate caused an increase in emulsion stability and decreased of creaming layer formed of resultant emulsions. The best heat treatment for creaming stability was showed at sterilization followed by boiling and pasteurized treatment. It has been proposed that a degree of denaturation might promote an improvement in the emulsifying properties by exposing the hidden hydrophobic groups of the globular proteins (Harper, 1992, Voutsinas et al., 1983). An appropriate degree of denaturation is related to a suitable balance of the hydrophilic and hydrophobic groups of the protein molecule after denaturation (Aoki et al., 1981).

Fig. 3 Effect of sterilization treatment of WPC/SC on the Creaming Indes of the emulsion resultant.



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