

## The Effects of Heating on the Physicochemical and Functional Properties of Acid Whey Compared to Sweet Whey

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**Abstract** In this study, we investigated the effects of heating (80°C, 30 min) on the physicochemical and functional attributes of acid (cottage) and sweet (Edam and Cheddar) whey powders. The water holding capacity (WHC) of the whey powders was not affected by heating or pH value. The heated Cheddar whey powder had a significantly lower ( $p < 0.05$ ) WHC than that of the other wheys. Heating detrimentally impacted the emulsifying and foaming properties. On the other hand, heating significantly enhanced the heat stabilities (HS) of all powders. This was best demonstrated at the acidic pH values of 3.0 and 4.5, where the HS increased by 57 and 53, 181 and 167, and 31 and 48%, for the cottage, Edam, and Cheddar, respectively. Overall, this data provides useful insights into the manufacture of pasteurization and retort-stable whey powders.

**Keywords:** heating, whey powder, heat stability, functionality, water holding capacity

### Introduction

Whey is a byproduct of cheese manufacture, and is rich in proteins, minerals, lactose, and functional peptides (1). Sweet whey, also referred to as cheese whey, is the liquid that separates from the cheese curd when starter cultures and rennet are applied to milk during the manufacture of cheeses like Edam and Cheddar. The pH of sweet whey ranges from 5.2 to 6.7. Acid whey, also known as sour whey, includes whey that is a byproduct of quark, cottage-cheese, and acid casein manufacture. Acid whey is high in lactic acid (pH range 3.8 to 4.6) due to extensive lactic culture-induced fermentation. In 2004, approximately 4.03 million metric tons (MT) of cheese of all types were manufactured in the U.S. (2). Whey has been dumped into rivers, lakes, or similar water reservoirs, and sprayed onto agricultural land ever since the industrialization of cheese production (3). Unfortunately, whey arising from cheese and casein manufacture has a biological oxygen demand (BOD) of 35-45 kg/m<sup>3</sup> (4) making its disposal an environmental dilemma.

Problems associated with whey utilization include the variability in desired functional attributes (5), and the high lactic acid content of acid whey, which interferes with dehydration and negatively impacts food applications and acceptability. The inconsistency in functionality can be caused by differences in cow herd breeds and the processing conditions (6, 7). Also, it may be due to high bacterial and enzyme contents, owing to long periods of starter culture activity prior to whey separation and drying. Peptides generated by protease activity can profoundly affect functionality (8, 9) and subsequent digestibility (10). Therefore, many whey processing plants include a heat treatment or heating stage in the range of 75-85°C for 30-

60 sec following whey separation, using a high temperature short time (HTST) system. Sufficient information about the heating step is not available in the literature since it varies from product to product and plant to plant, and is proprietary. Researchers have reported significant reductions of enzyme activity in the 75-80°C range (11, 12). Furthermore, a heating step has been recommended to the dairy industry where raw milk is transported and stored for extended periods of time prior to processing (13). Recently, the impact of pasteurization at 82.2°C for 23 sec using the HTST system (Mueller, Springfield, MO, USA), on Cheddar whey (from mixed and Jersey herd) functionality was evaluated, and significant changes were noted (6).

The impact of longer heating times at high temperatures (80°C) on desired functional attributes has not been investigated. This is particularly true for acid whey, which is mostly discarded today in spite of being an excellent source of nutrients. Thermal treatment alters protein structure and this structure plays a critical role in the protein's functionality in food (14, 15). Functional attributes like emulsification (16-18), heat stability (HS) (19), oil-holding capacity, water-holding capacity (WHC) (20), foaming (21), and solubility (22) are key attributes that influence the effective utilization of proteins in food systems.

The objective of this study was to evaluate the effects of heating in acid whey from cottage cheese manufacture, and in sweet whey from Edam and Cheddar cheese manufacture, on key functional attributes, including WHC, HS, emulsion activity (EA), creaming stability (CS), foam stability (FS), and foam density (FD).

### Materials and Methods

**Materials** Fresh milk was obtained from a Mississippi State University mixed herd (16.7% Jersey and 83.3% Holstein, Mississippi State University South Dairy Farm,

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Starkville, MS, USA). Diethyl ether and petroleum ether were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Imidazole, sodium dodecyl sulfate (SDS), disodium dihydrogen ethylenediamine tetraacetate (EDTA), and calcein were from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were analytical grade.

**Whey manufacture** The fresh milk was skimmed using a decreaming separator, and pasteurized (Yunior SS, AVP Crepaco, Tonawanda, NY, USA) at 71°C for 15 sec at the Mississippi State University Dairy Plant and processed without delay. Cottage (23), Edam (24), and Cheddar cheese (25) were manufactured at the same plant. Fresh cultured buttermilk (5%) from the plant was used as the starter culture for cottage cheese. For both Edam and Cheddar, a mesophilic homo-fermentative culture (Chr. Hansen's Lab. Inc., Milwaukee, WI, USA) was used (165 g/1,000 kg milk). Single strength rennet (Chr. Hansen's Lab. Inc.) was used at a concentration of 115 g/1,000 kg. After the cheese curd was removed, the whey samples were clarified by passage through multiple layers of cheese cloth, and were then centrifuged (3,200×g for 10 min) to remove aggregated casein and divided into 2 equal portions. One portion was immediately flash frozen in liquid nitrogen and lyophilized (Labconco, Kansas City, MO, USA) to obtain the native acid (AWP), Edam (EWP), and Cheddar (CWP) whey powders. The other portion was batch heated in a water bath at constant temperature (80 ±0.1°C) for 30 min prior to flash freezing, and lyophilized to obtain the heated acid (HAWP), Edam (HEWP), and Cheddar (HCWP) whey powders. All powders were stored in sealed containers at 4°C until needed.

**Proximate analysis** Moisture content and total solids: Moisture content was determined by the AOAC vacuum-oven method (16.192) for whey powders (26).

**pH measurement:** The pH values of the whey powders were measured using an Orion 210A pH meter (Orion Research, Inc., Boston, MA, USA). The pH meter was standardized by buffer solutions (pH 4.1 and 7.0) (Fisher Scientific) before measuring the pH of the sample.

**Titrateable acidity (TA):** The TA was determined by AOAC method number 947.05 for whey powders (26). A 17.6 mL amount of prepared sample and 0.5 mL of phenolphthalein (1.0%) indicator were used to titrate with a 0.1 N sodium hydroxide (NaOH) solution. The total acidity, which was expressed as lactic acid, was calculated by the following formula:

$$\% \text{ Total acidity} = \frac{[\text{mL of } 0.1 \text{ N NaOH} \times 0.009] / \text{weight of sample}}{1} \times 100$$

**Ash content:** The ash contents of the whey powders were determined by the AOAC method (16.196) (26).

**Total fat:** Fat contents were determined using the Mojonnier method according to AOAC number 989.05 for whey powders (26).

**Total protein:** The total protein contents of the whey powders were measured by determining total nitrogen content using the Kjeldahl method according to AOAC number 955.04 (26), and the conversion rate of 6.38 for dairy proteins.

**Calcium content:** The calcium contents of the whey powders were determined as described by Ntailians and Whitney (27), where calcein was used as an indicator for the determination of total calcium in milk. This method is based on the spontaneous and tenacious binding of calcium with a chelating agent, EDTA. Standard curves were obtained from a standard CaCl<sub>2</sub> solution at pH 13.0 with calcein.

**Functional properties Water holding capacity (WHC):** The measure of WHC was conducted as described by Sternberg *et al.* (28) with some modifications. Two mL of 10%(w/v) whey solutions adjusted to pH 3.0, 4.5, 7.0, and 9.0 were pipetted into tared centrifuge tubes (T1). The tubes were covered with marbles and heated in a boiling water bath (97) for 10 min. After cooling in tap water for 5 min, the tubes were wiped with filter paper, the marbles removed, and the tubes weighed (T2). Next, the tubes were centrifuged at 1,000×g for 10 min and then weighed (T3) after inverting to drain for 10 min. The WHC was calculated by the following formula:

$$\text{WHC (\%)} = 100 (T3 - T1) / T2 - T1$$

**Heat stability (HS):** The HS of the whey was determined using the method of Haque and Mozaffar (29). The whey powders were vortexed (10 sec) in test tubes with 4 mL of 10 mM imidazole (2%, w/v), adjusted to pH levels of 3.0, 4.5, 7.0, and 9.0 with 0.1 N HCl, and sealed and heated in a thermostated water bath at 82°C (±0.1) for 0, 1, 5, 10, and 20 min under mild agitation. The tubes were quickly brought to 22°C under running water, and the transmittance of visible light (600 nm) (model UV-1201; Shimadzu Co., Kyoto, Japan) through the samples in cuvettes was compared with that of the unheated control. The change in transmittance was expressed as the HS of the sample.

**Emulsifying properties:** The turbidimetric method of Pearce and Kinsella (30) was used to determine the emulsion activities (EA) and creaming stabilities (CS) of the whey powders.

**Emulsion activity (EA):** Twenty mL of (0.1%, w/v) each powder dispersion at pH levels of 3.0, 4.5, 7.0, and 9.0, and 0.1 M of sodium phosphate were mixed with 6 mL of peanut oil in a commercial mixer (Hamilton Beach, Washington, NC, USA) at maximum speed. A 50 µL aliquot of the emulsion was pipetted from the bottom of the container at 0 and 10 min and mixed with 5 mL of 0.1%(w/v) sodium dodecyl sulfate (SDS). The emulsion absorbance was measured at 600 nm with a spectrophotometer (model UV-1201; Shimadzu Co.). The absorbance measured immediately after emulsion formation (0 min) was expressed as the EA.

**Creaming stability (CS):** The CS was determined by the height of bottom clear liquid, which was measured after 24 hr. Just after emulsion preparation, measured amounts of the emulsions were poured into graduated cylinders and stored in a refrigerator (4) for 24 hr. The height of the clear liquid at the bottom was recorded as CS.

**Foaming properties:** The foaming properties were determined by modifying a method that was developed by Phillips *et al.* (31). The whey powder samples were

dispersed in distilled water to provide a 5%(w/v) protein concentration and adjusted to the pH levels of 3.0, 4.5, 7.0, and 9.0 with 0.1 N HCl or NaOH. The dispersions (200 mL) were whipped in a Sunbeam Mixmaster mixer (Memphis, TN, USA) for 20 min at full whipping speed.

**Foam stability (FS):** The FS was indicated by the length of time required for the first drop of liquid to drain from the funnel at definite intervals. The FS was calculated by the following equation:

$$\% \text{ Foam stability} = \frac{\text{vol. of final liquid (mL)} - \text{vol. of liquid drainage (mL)}}{\text{vol. of final (mL)}} \times 100$$

**Foam density (FD):** FD was calculated by the following equation:

$$\text{Foam density} = \frac{\text{wt.(g) of 100 mL of foam}}{\text{Foam (100 mL)}} \times 100$$

**Statistical design and analysis** The experiments were conducted using a completely randomized design with three replications. The data were analyzed using the general linear models (PROC GLM) procedure. Means were separated using Fisher's protected least significance test at  $p < 0.05$  (32). The statistical analysis was conducted using the SAS Statistical Program, version 8.1 (SAS Institute, Cary, NC, USA) for Windows (33).

## Results and Discussion

**Proximate analysis** Table 1 shows the proximate analysis data of the whey powders. The moisture contents of the whey powders varied from 4.32 to 5.10%. The moisture contents between some native and heated wheys increased, and their differences were significant. A previous report indicated that the moisture contents of dried acid, sweet, and Cheddar whey protein concentrates were 4.5% (34).

The pH ranged from 4.74 to 5.02 for the acid whey, and from 6.35 to 6.74 for the sweet wheys. There were significant differences in pH between the native and

heated samples of both the acid and sweet whey. The pH of the Cheddar whey was lower than that of fresh milk, which has a range of 6.6 to 6.8, but the pH range of the Edam whey was very similar to that of fresh milk. Acid whey, in which lactose is converted to lactic acid by fermenting lactose bacteria during the manufacture of cheese products, has a pH of 4.7. Heating can change the pH values of whey powders.

Titrateable acidity (TA) indicates the amount of acid present in foods. The TA was in the range of 0.39 to 0.40 for the acid whey and 0.06 to 0.11 for the Edam and Cheddar whey. There were no significant differences in TA between the native acid wheys and heat-denatured acid wheys. The TA of whey differs depending on the composition of the original milk, the method of curd coagulation, and the cheese type. The acidity of whey may vary from 0.11 to 0.75.

Total ash content was higher in the acid whey powders compared to the Edam and Cheddar whey powders. However, there were no significant differences in ash content between the native and heated whey.

Crude protein (CP) was significantly higher in the Cheddar whey powders compared to the acid whey powders. Also, there was significantly higher CP in the heated whey powders compared to the native whey powders ( $p < 0.05$ ).

The crude fat (CF) contents of the whey powders were significantly affected by whey type. The acid whey powders had significantly lower CF content compared to the Edam and Cheddar whey powders.

Calcium is one of the major mineral components of whey. The acid whey powders had significantly higher calcium contents compared to the sweet whey powders. A previous report indicated that acid whey generally has higher calcium content compared to rennet whey (35, 36).

**Functional properties** The CWP had the highest water-holding capacity (WHC) and HCWP showed the lowest WHC (Fig. 1). There was a significant difference ( $p < 0.05$ ) in WHC between the CWP and HCWP. However, the WHCs of the acid and Edam wheys were not affected by heating. The WHC was not significantly different between the pH levels. The WHC of AWP was lower than that of

**Table 1. Proximate analysis of whey powders<sup>1)</sup>**

	Whey powder <sup>2)</sup>					
	AWP	HAWP	EWP	HEWP	CWP	HCWP
Moisture (%)	4.32 <sup>e3)</sup>	5.05 <sup>a</sup>	4.74 <sup>b</sup>	5.10 <sup>a</sup>	4.41 <sup>c</sup>	4.35 <sup>c</sup>
pH	4.74 <sup>f</sup>	5.02 <sup>e</sup>	6.67 <sup>b</sup>	6.74 <sup>a</sup>	6.35 <sup>d</sup>	6.51 <sup>c</sup>
Titrateable acidity (TA) (%)	0.39 <sup>b</sup>	0.40 <sup>a</sup>	0.07 <sup>d</sup>	0.06 <sup>d</sup>	0.11 <sup>c</sup>	0.11 <sup>c</sup>
Ash (%)	9.90 <sup>b</sup>	11.7 <sup>a</sup>	7.69 <sup>d</sup>	8.09 <sup>c</sup>	7.81 <sup>d</sup>	8.01 <sup>c</sup>
Total protein (%)	11.7 <sup>d</sup>	12.9 <sup>c</sup>	12.8 <sup>c</sup>	13.4 <sup>b</sup>	13.3 <sup>b</sup>	14.4 <sup>a</sup>
Fat (%)	0.57 <sup>c</sup>	0.71 <sup>c</sup>	1.03 <sup>b</sup>	1.38 <sup>a</sup>	1.19 <sup>b</sup>	1.49 <sup>a</sup>
Calcium (mg/100 g)	1580.0 <sup>b</sup>	1786.5 <sup>a</sup>	683.4 <sup>e</sup>	738.1 <sup>c</sup>	765.6 <sup>d</sup>	876.1 <sup>c</sup>

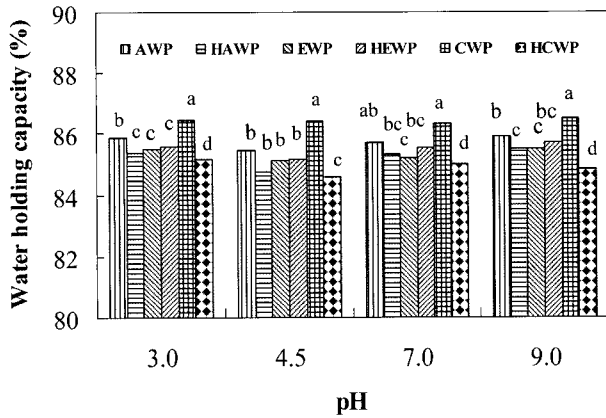
<sup>1)</sup>Values represent means of 3 replications.

<sup>2)</sup>AWP, acid whey powder; HAWP, heated acid whey powder; EWP, Edam whey powder; HEWP, heated Edam whey powder; CWP, Cheddar whey powder; HCWP, heated Cheddar whey powder.

<sup>3)</sup>Means within the same row with the same letters are not significantly different ( $p < 0.05$ ).

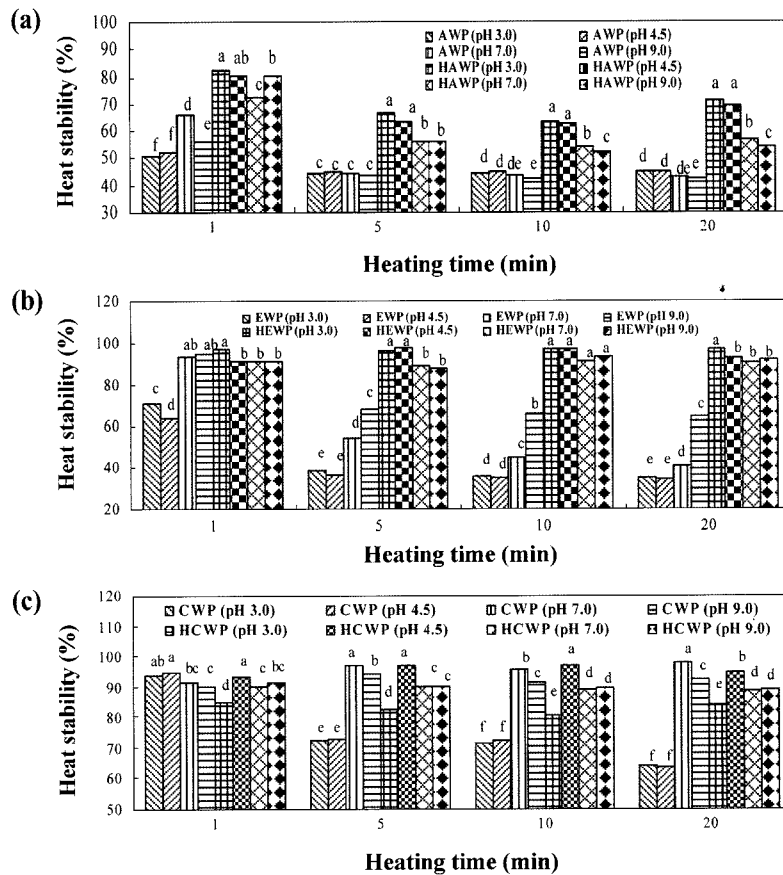
CWP, but 86% of the entrapped water was still retained after 10 min of centrifugation at 1,000xg. The range of

WHC found in this study was somewhat lower than that reported by others (28) who examined egg white and cottage cheese whey protein isolate at pH 7.0 and 9.0. The high WHC of whey protein concentrate results in large water entrapment and reduced gel stress values (20). The objective of the WHC test is to measure the amount of water that differently dried powders would bind after a relatively short time of contact with water, as would be the case under manufacturing conditions in the food industry (37). The WHC may help enhance sensory properties such as flavor retention and mouth feel. It is also significantly correlated with bulk density (38), and important characteristic that influences flow properties and dispersibility. The WHC can be affected by water that is physically entrapped within unfolding proteins, and depends on the degree of denaturation (39). The water holding properties of protein are therefore conceivably related to its swelling, which would impact solubility, viscosity, and gelation. Generally, the WHC of whey powder decreases as bulk density increases (37).



**Fig. 1. Water holding capacities (WHC) (%) of whey powders.** AWP, acid whey powder; HAWP, heated acid whey powder; EWP, Edam whey powder; HEWP, heated Edam whey powder; CWP, Cheddar whey powder; HCWP, heated Cheddar whey powder. Dissimilar letters above bars indicate a significant difference ( $p < 0.05$ ) within the pH group.

Heat stability (HS) is a desirable attribute for food proteins, especially for high protein beverages that need heat treatments (9). The HS of the acid whey is significantly lower than that of the sweet whey at all pH levels and heating times studied (Fig. 2A). At the pH



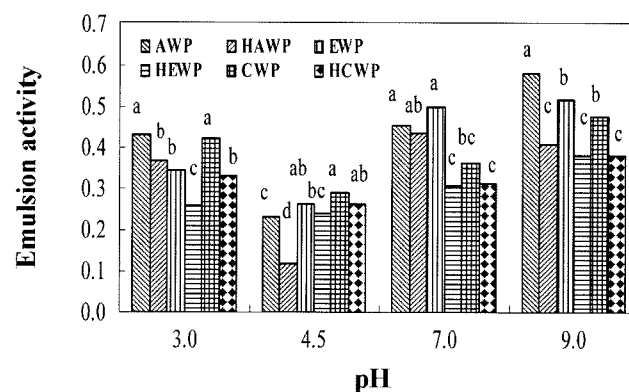
**Fig. 2. Heat stabilities of unheated (native) and heated whey powders (2%, w/v).** Fig. (a), (b), and (c) show acid whey, Edam whey, and Cheddar whey, respectively. AWP, acid whey powder; HAWP, heated acid whey powder; EWP, Edam whey powder; HEWP, heated Edam whey powder; CWP, Cheddar whey powder; HCWP, heated Cheddar whey powder. Dissimilar letters above bars indicate a significant difference ( $p < 0.05$ ) within the pH group.

values of 3.0, 4.5, 7.0, and 9.0, after 1 min of heating, CWP showed the highest HS values at 94, 95, 92, and 90, respectively (Fig. 2B); EWP came next with 71, 64, 93, and 95, respectively (Fig. 2C); and AWP was lowest with 51, 52, 66, and 56, respectively (Fig. 2A). However, these HS values decreased drastically after further heating for 20 min. For EWP, HS decreased by 103 and 85% (in contrast to 1 min) at the acidic pH levels of 3.0 and 4.5, respectively (Fig. 2B), as compared to 45 and 49% for CWP, respectively (Fig. 2C), and only 13 and 16% for AWP, respectively (Fig. 2A). This indicates that the acid whey powder was more resistant to precipitation upon extended heating. This heat resistance property would be useful in foods such as health and sports drinks, which are acidic in nature and must be pasteurized, and in other foods that have to be retort stable. The higher heat stability of the acid whey than that of the sweet whey may be due to differences in their compositional or physicochemical properties; in particular, pH and calcium content. Acid whey typically has a pH of 4.6, while that of sweet whey is about 6.6. At low pH values, the free sulphhydryl (SH) and disulphide (SS) groups of  $\beta$ -LG are not as readily exposed during heating; therefore, enzyme inactivation due to the interaction with denatured  $\beta$ -LG will be reduced (40). The heat denaturation of  $\beta$ -LG in whey increases with increasing pH in the range of 4.5-7.0 (19). Acid whey also contains a high level of calcium, resulting from the solubilization of colloidal calcium phosphate at low pH values, which may influence the heat stability of  $\beta$ -LG (41). However, although acid whey has higher calcium content than sweet whey, it appears that pH was the dominant factor affecting the heat stability of  $\beta$ -LG in the both the acid and sweet whey.

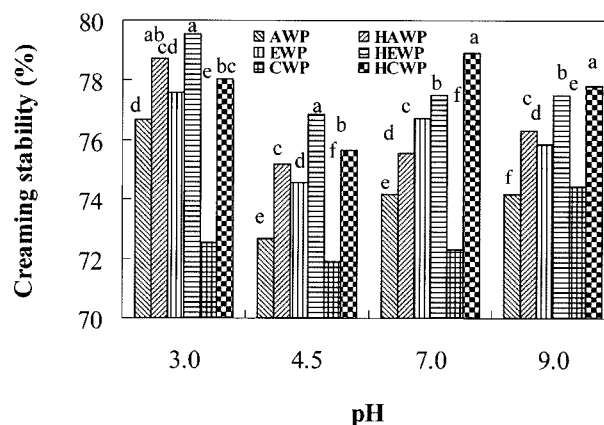
Heating dramatically and significantly increased the HS of all powders at all pH levels for HAWP and HEWP, and at the acidic pH levels for HCWP. The most marked improvement was seen for HEWP, where HS values after 20 min of heating at pH 3.0 and 4.5 were 181 and 167% more, respectively, than the HS of the native EWP. The improvements in HS for the heated as compared to the native whey powders at the same pH levels were 57 and 53%, and 31 and 48% for the acid and Cheddar whey, respectively.

Using dynamic light scattering as a tool, it was noted that milk peptide preparations caused a dramatic increase in the degree of facile association of  $\beta$ -LG upon heating (42). Even in native globular whey proteins, apolar side-chain residues (surface hydrophobicity) are exposed to the aqueous phase; and water forms cage-like structures around these to reduce hydrocarbon-aqueous contact (43). As water is heated, its structure breaks down and proteins associate to reduce the hydrocarbon-aqueous interface (44). All other variables being equal, the degree, tenacity, and spontaneity of thermal association depend primarily on surface hydrophobicity (45). This may also explain the dramatic increase in HS that is caused by heating. Partially heat-denatured proteins have greater surface hydrophobicity. Large facile protein clustering protects molecules that are within the cluster. The trace casein contaminants in whey powder may also have enhanced HS, as observed by Parkinson and Dickinson (46) in  $\beta$ -LG stabilized emulsions.

**Emulsifying properties** Emulsion activity (EA) was highest at pH 9.0, followed by 7.0 and 4.5 (Fig. 3). The whey powders had the lowest EA at pH 4.5 (pI region of  $\beta$ -lactoglobulin), and EA increased at pH values above or below this region. The lowest EA at pH 4.5 may be due to increased protein-protein interaction, resulting in low surface hydrophobicity and decreased net charge as well as protein solubility. There were significant differences in EA among the samples at the different pH levels. The native whey powders had higher EAs than the heated whey powders. Heating significantly reduced the EAs of acid whey at pH 3.0, 4.5, and 9.0. The EA is based on the protein's ability to adsorb, spread, and stabilize the oil/water interface. Heat-induced covalent interaction and tenacious aggregation conceivably reduced this ability. A previous report indicated that the surface hydrophobicities



**Fig. 3. Emulsion activities of whey powders.** AWP, acid whey powder; HAWP, heated acid whey powder; EWP, Edam whey powder; HEWP, heated Edam whey powder; CWP, Cheddar whey powder; HCWP, heated Cheddar whey powder. Dissimilar letters above bars indicate a significant difference ( $p < 0.05$ ) within the pH group.



**Fig. 4. Creaming stabilities of whey powders.** AWP, acid whey powder; HAWP, heated acid whey powder; EWP, Edam whey powder; HEWP, heated Edam whey powder; CWP, Cheddar whey powder; HCWP, heated Cheddar whey powder. Dissimilar letters above bars indicate a significant difference ( $p < 0.05$ ) within the pH group.

**Table 2. Foaming properties of whey powders<sup>1)</sup>**

Whey powder <sup>2)</sup>	pH <sup>3)</sup>							
	3.0		4.5		7.0		9.0	
	FS	FD	FS	FD	FS	FD	FS	FD
AWP	91.1 <sup>Aa</sup>	0.17 <sup>Bd</sup>	90.7 <sup>Aa</sup>	0.17 <sup>Bd</sup>	91.9 <sup>Aa</sup>	0.16 <sup>Bb</sup>	89.1 <sup>Bab</sup>	0.21 <sup>Ab</sup>
HAWP	88.5 <sup>Ab</sup>	0.16 <sup>Bd</sup>	86.5 <sup>Bd</sup>	0.16 <sup>Be</sup>	89.5 <sup>Ac</sup>	0.14 <sup>Cc</sup>	89.0 <sup>Aab</sup>	0.20 <sup>Ab</sup>
EWP	90.5 <sup>ABa</sup>	0.25 <sup>Aa</sup>	89.5 <sup>Cb</sup>	0.23 <sup>Ba</sup>	91.3 <sup>Aab</sup>	0.17 <sup>Cb</sup>	89.9 <sup>BCa</sup>	0.23 <sup>ABa</sup>
HEWP	87.8 <sup>Ab</sup>	0.21 <sup>Ac</sup>	88.0 <sup>Ac</sup>	0.19 <sup>Bc</sup>	88.8 <sup>Ad</sup>	0.15 <sup>Cbc</sup>	87.9 <sup>Ab</sup>	0.20 <sup>ABb</sup>
CWP	90.1 <sup>BCa</sup>	0.23 <sup>Ab</sup>	91.9 <sup>Aa</sup>	0.21 <sup>Bb</sup>	90.5 <sup>Bbc</sup>	0.20 <sup>Ba</sup>	90.0 <sup>BCa</sup>	0.23 <sup>Aa</sup>
HCWP	88.7 <sup>Bb</sup>	0.21 <sup>Ac</sup>	89.0 <sup>Bbc</sup>	0.19 <sup>Ac</sup>	89.0 <sup>Bd</sup>	0.17 <sup>Bb</sup>	89.3 <sup>Aa</sup>	0.19 <sup>Ab</sup>

<sup>1)</sup>Values represent means of 3 replications; FS and FD represent foam stability and density, respectively.

<sup>2)</sup>AWP, acid whey powder; HAWP, heated acid whey powder; EWP, Edam whey powder; HEWP, heated Edam whey powder; CWP, Cheddar whey powder; HCWP, heated Cheddar whey powder.

<sup>3)</sup>ABC, B-C means within the same row and column with the same letters are not significantly different ( $p < 0.05$ ), respectively.

of  $\beta$ -LG and bovine serum albumin (BSA) decreased with heat treatment, indicating a reduction of the hydrocarbon-aqueous interface due to aggregation (21). The EA and emulsion stability of proteins correlate linearly with surface hydrophobicity.

The emulsion creaming stabilities (CS) of the whey powders, expressed as the stability rating (SR), are shown in Fig. 4. The heated whey powders showed higher CS as compared to the native whey powders ( $p < 0.05$ ). The CS was lowest at pH 4.5 and altering the pH from 4.5 to 3.0, 7.0, and 9.0 increased the CS. These results suggest that the CS of an emulsion strongly depends on the electrostatic nature of the whey protein (47). At pH 5, which is close to the isoelectric pH of major whey proteins, the net charges of proteins are diminished and repulsive forces among the molecules are eliminated.

**Foaming properties** Foam stability (FS) was quite variable in relation to the pH values and the whey powders (Table 2). There were little, although in some cases significant, differences in the FS, which varied from 86.5 for HAWP to 91.9 for AWP. The native acid whey appeared to do better at all pH values except pH 7.0, where it statistically tied with the sweet wheys. The HAWP gave foams with low stability at pH 4.5. The native whey powders had higher FS than the heated whey powders. Heating significantly reduced the FS of all wheys at pH 3.0, 4.5, and 7.0. However, no significant differences were found between the whey powders. Interestingly, there was no detrimental effect in the acid whey and Cheddar whey with heating, although the result was not significant. The pasteurization of whey protein isolate at 80°C for 1 min increases foamability and FS (48). The pH adjustments in the acid whey powders had a beneficial effect on the foam properties at pH 3.0, 7.0, and above. The FS improved when the acid whey was adjusted to these pH levels, but adjusting the pH of Edam and Cheddar had little effect.

Foam density (FD), which reflects the bubble size, was lower for the acid whey as compare to the sweet whey at all pH levels (Table 2). A larger FD value would imply a smaller bubble size, larger number of bubbles, and hence, a greater interfacial area. FD varied from 0.137g/mL for HAWP to 0.240 g/mL for EWP. The acid whey gave an FD that was significantly lower than those of the sweet

wheys at pH 3.0 and 4.5. Heating appeared to decrease FD in all the whey foams.

In conclusion, heating had little effect on whey composition. The WHCs of the whey powders were not affected by heating or pH. Although there were statistical differences, the emulsifying properties and FS of the native acid whey were comparable to the native sweet wheys. However, FD was significantly lower for the acid whey at pH 3.0 and 4.5. The HS was higher for the sweet wheys, although the desired attribute was more consistent across all pH levels and heating regimens for the acid whey. Heating dramatically enhanced the HSs of all whey powders, and the increase was highest for the Edam whey. Our data indicate that this heating step appears to have a significant functional advantage, in that it inactivates bacteria and enzymes that are major causes for variability in functionality, and also markedly improves key attributes that allow for the use of such powders in foods and drinks that need to be heat treated. Related studies have addressed the influence of thermization on the antioxidative properties of sour and sweet whey (49).

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