

# Thermal Behavior of Whey Protein Concentrate Treated by Heat and High Hydrostatic Pressure and Its Functionality in Wheat Dough<sup>1</sup>

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## ABSTRACT

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Solutions of commercial whey protein concentrate (CWPC, 82% protein) at 5, 10, 20, and 30% were treated with heat at 90°C or with high hydrostatic pressure (HHP) at 85 Kpsi (Kpsi = 6.9 MPa) for 30 min. A CWPC solution at 20% also was treated for 30 min with heat at 60, 70, 80, and 90°C and HHP at 20, 40, 60, and 85 Kpsi. Differential scanning calorimetry (DSC) thermograms of untreated CWPC (82% protein) showed two endothermic peaks: the first had an enthalpy value of 4.72 J/g between 57 and 86°C, and the second had an enthalpy value of 2.36 J/g between 120 and 143°C. The first enthalpy peak disappeared after heat treatment at 90°C for 30 min and HHP treatment at 85 Kpsi for 30 min, whereas the second peak remained, independent of concentration. The results indicate that HHP treatment caused changes in the protein of CWPC, and the changes were comparable to those caused by high-temperature treatment. Differential scanning calorimetric analysis of CWPC, heat treated at 60°C, showed an enthalpy value for the first peak of 3.34 J/g,  $\approx$ 1.41 J/g lower than for untreated CWPC. A sharp decrease in enthalpy to 0.52 J/g for the first peak was observed at 70°C, with complete disappearance at 80°C. The second enthalpy peak was present at all temperatures

studied, with significantly higher enthalpy values at 90°C than at lower temperatures. DSC value for the first enthalpy peak for CWPC decreased significantly as HHP treatment level increased from 20 to 85 Kpsi. CWPC treated with HHP at 20 Kpsi had an enthalpy value for the first peak that was  $\approx$ 2 J/g higher than for the untreated sample. It can be postulated that low HHP treatment of 20% of CWPC solution for 30 min promotes the formation of covalent or noncovalent cross-links and strong protein-protein interactions, hence the higher enthalpy values. Scanning electron micrographs showed that spray-dried, untreated CWPC was a globular form, whereas heat- and HHP-treated CWPC was a solid glasslike, porous or spongy form. Incorporation of 10% untreated CWPC into wheat flours decreased mixograph water absorption, extended mixing time, and caused rapid breakdown of gluten after optimum dough development. Incorporation of 10% heat- or HHP-treated CWPC significantly increased mixograph water absorption and extended mixing time compared to the control but decreased mixing time compared to dough fortified by untreated CWPC. Mixing tolerance of dough was restored by both heat- and HHP-treated CWPC.

Whey is defined as the “watery component removed after the setting of the curd in cheese manufacture” (Varnam and Sutherland 1994). Whey contains 93.5% water, 5% lactose, 0.5% minerals, 0.2% milk fat, and  $\approx$ 0.6–0.8% protein (Morr 1989, Huffman 1996). The major protein fractions of whey are  $\beta$ -lactoglobulin ( $\beta$ -Lg),  $\alpha$ -lactalbumin ( $\alpha$ -La), bovine serum albumin (BSA), immunoglobulins, and polypeptide protease peptones (Marshall 1982).  $\beta$ -Lg and  $\alpha$ -La are primarily large globular proteins that make up  $\approx$ 80% of the total whey proteins (Schmidt et al 1984).

Advances in food technology allow concentration of whey proteins. Products with a range of protein contents from 10 to  $>$ 90% have been obtained. Manufacturing processes for whey protein products affect their composition and functionality (Jacobson 1997). Products are classified based on their protein content: whey powder is 12–15% protein; whey protein concentrate is 35–85% protein; and whey protein isolate is  $\geq$ 90% protein (Huffman 1996, Jacobson 1997). As the percentage of protein increases in whey products, the percentage of fat increases, and lactose and ash contents decrease (Huffman 1996).

The properties of whey protein products can vary due to differences in their composition and the extent of protein denaturation (Dybing and Smith 1991). Undenatured (native) whey proteins are highly soluble. Because of this, they are easily emulsified, able to form foams, and able to form gels when heated. Common examples of uses of undenatured whey proteins include incorporation as an egg replacer in cakes and candies, a stabilizer in yogurt, and an extender in luncheon meat (Varnam and Sutherland 1994). Denatured whey proteins, on the other hand, are nearly insoluble and cannot be emulsified or form foams. Heat can alter protein conformation and, thus, significantly change the functional properties of proteins (de Wit and Klarenbeek 1984, Schmidt et al 1984, Morr 1989). Denatured

whey proteins are mostly used as protein supplements in wheat-based products that have a low protein efficiency ratio (PER) value or are lacking some essential amino acids, such as lysine (Stahel 1983).

Undenatured whey protein can adversely affect the viscoelastic properties and baking quality of dough by significantly decreasing water absorption and interfering with formation of the gluten network (Ashworth and Krueger 1951, Melachouris 1984, Damodaran 1996, Erdogdu-Arnoczky et al 1996). Sulfhydryl groups in whey protein (albumin and globulin types) that cause this interference can be altered or lost with heat treatment (Ashworth and Krueger 1951, Larson et al 1951, Schmidt et al 1979, Kilara and Mangino 1991, Damodaran 1996). As a result, heat treatment improves the performance of dairy ingredients used by the baking industry (Erdogdu-Arnoczky et al 1996, Jacobson 1997).

In recent years, high-pressure treatment has received attention as a new alternative to heat processing for food product modifications (Farr 1990, Mertens and Knorr 1992). As reported by Farr (1990), high-pressure treatment can affect product functionality. Okamoto et al (1990) stated that high-pressure treatment can introduce production of a new food material with a novel, desirable texture. Other benefits of high-pressure processing may include retention of flavor, color, and nutritional quality. This low-energy process does not cause environmental pollution and eliminates the use of chemical additives in food products (Hayashi 1989, Hayashi 1992, Pothakamury et al 1995). Because the process is not time or mass dependent, the time required to process large amounts of food is reduced (Pothakamury et al 1995). Many studies have discussed the use of high-pressure technology in the food industry, including production of fresh grapefruit juice without bitterness, production of jams, jellies, and fruit sauces, reduction of bacterial counts in milk, and applications in processing yogurts and salad dressings (Farr 1990, Hori et al 1992, Kanda et al 1992, Mertens and Deplace 1993, Hayakawa et al 1994, Galazka and Ledward 1995, Pothakamury et al 1995).

Galazka and Ledward (1995) postulated that high pressure, when applied to food containing protein, may modify protein structure. Farr (1990) and Okamoto et al (1990) concluded that protein denaturation under high pressure is caused by destruction of nonco-

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valent bonds, such as hydrogen and ionic bonds and hydrophobic interactions, while covalent bonds, such as S-H groups, are not affected. High pressure, instead of heat denaturation, rarely has been used to denature protein and has not been applied to whey protein modification. Therefore, data is lacking about the effect of high hydrostatic pressure (HHP) on whey protein denaturation. The objectives of this study were to evaluate the effects of HHP on modification of commercial whey protein concentrate (CWPC) and

to compare changes to CWPC heat treated by conventional methods; investigate the effect of concentration, time, and level of HHP on modification of CWPC; and evaluate changes in CWPC structure and properties after heat and HHP treatments, using differential scanning calorimetry (DSC) and scanning electron microscopy (SEM), and evaluate their functionality when incorporated into wheat flour dough.

## MATERIALS AND METHODS

Hard white spring (HWS) wheat cv. Klasic and club wheat cv. Moro were milled to 60% extraction levels (Miag Mill, Bühler, Braunschweig, Germany). CWPC, containing 82.0% protein, 5.3% ash, 6.0% lipids, and 3.5% lactose, was produced by an ultrafiltration technique and obtained from AMPC (Ames, IA). The CWPC was a spray-dried powder with a bland flavor. According to the manufacturer, the CWPC possessed high heat stability, strong heat-induced gelling characteristics, and high solubility (AMPC product information).  $\beta$ -Lg that contained genetic variants B,  $\alpha$ -La, and BSA were purchased from Sigma Chemical Co. (St. Louis, MO).

### Analytical Method

Moisture, ash, and free lipids contents in wheat flour were determined according to Approved Methods 44-16, 08-01, and 30-25, respectively (AACC 1995). Moisture and ash contents in CWPC were determined according to AOAC methods 16.212 and 16.216, respectively (AOAC 1984). Protein in CWPC ( $N \times 6.38$ ) and wheat flours ( $N \times 5.70$ ) was determined with a nitrogen analyzer (Leco Corp., St. Joseph, MI).

### Preparation of CWPC Solutions for Heat and HHP Treatments

For heat and HHP treatments, solutions of 5, 10, 20, and 30% CWPC in deionized water were prepared and kept overnight at 4°C prior to treatment.

### Heat Treatment

Two experiments were performed using heat treatment (Fig. 1). In the first experiment, 5, 10, 20, and 30% solutions of CWPC were heated in a water bath at 90°C for 30 min. In the second experiment, a 20% solution of CWPC was heated at 60, 70, 80, and 90°C for 30 min. During heating, the solution was stirred at five rotations per second with a mixer (Con-Toque, Eberbach Corp., Ann Arbor, MI) to achieve uniform heating. After treatment, samples were cooled to room temperature and freeze-dried.

### HHP Treatment

Three experiments were performed using HHP (Fig. 1). In the first experiment, 5, 10, 20, and 30% solutions of CWPC were treated for 30 min by HHP at 85 Kpsi (Kpsi = 6.9 MPa). In the second experiment, a 20% solution of CWPC was treated by HHP at 20, 40, 60, and 85 Kpsi for 30 min. In the third experiment, 5, 10, 20, and 30% solutions of CWPC were treated by HHP at 85 Kpsi for 1, 2, 4, 10, and 30 min. This experiment was performed to investigate whether changes occur in whey proteins after shorter time HHP treatment at 85 Kpsi.

For each of the HHP experiments, 100 mL of CWPC solution (at each concentration) was poured into flexible polyethylene bags (Consolidated Plastic Co., Twinsburg, OH) and heat-sealed. A single bag or several bags (depending on the experiment) were placed in a larger bag filled with water as a pressure media, and the larger bag was sealed and placed inside the chamber of an engineered pressure system instrument (National Forge Co., Willington, MA) and subjected to high-pressure treatment. After treatment, pressure was released automatically, and samples were removed and freeze-dried.

Freeze-dried samples (at different concentrations), after both heat and pressure treatments, were ground to a fine powder with mortar and pestle to pass through a screen with 0.25-mm openings. Dry samples were stored at room temperature for further analysis.

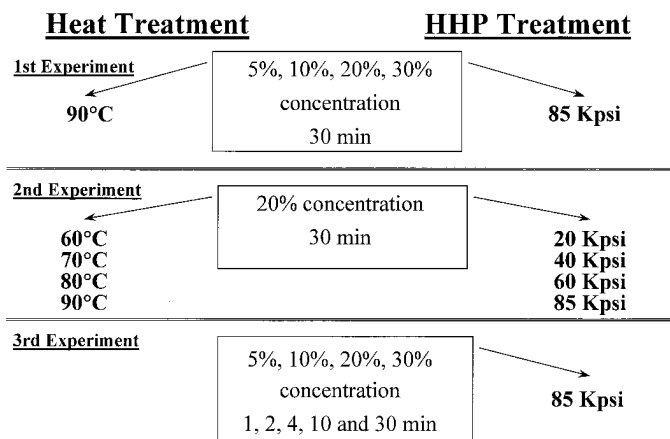


Fig. 1. Schematic of heat and high hydrostatic pressure (HHP) treatments.

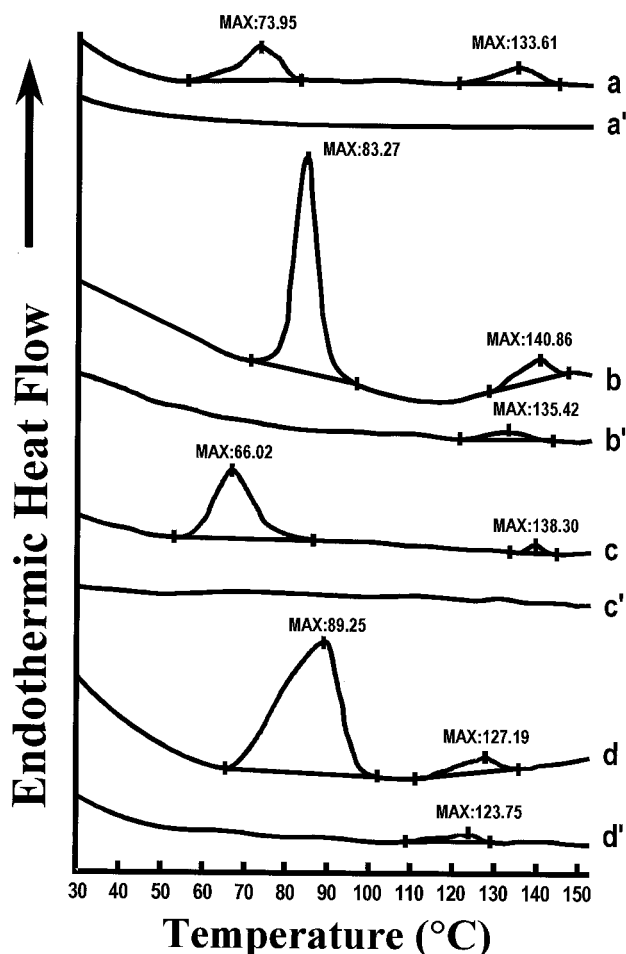


Fig. 2. Differential scanning calorimetry curves of untreated commercial whey protein concentrate (CWPC) and its individual proteins: first scan of untreated CWPC (a); rescan of untreated CWPC (a'); first scan of  $\beta$ -lactoglobulin ( $\beta$ -Lg) (b); rescan of  $\beta$ -Lg (b'); first scan of  $\alpha$ -lactalbumin ( $\alpha$ -La) (c); rescan of  $\alpha$ -La (c'); first scan of bovine serum albumin (BSA) (d); rescan of BSA (d').

## Differential Scanning Calorimetry

The differential scanning calorimetry (DSC) characteristics of CWPC were determined with a DSC instrument (Pyris 1, Perkin Elmer Corp., Norwalk, CT) according to the method of Czuchajowska and Pomeranz (1989). Fine-powder sample (10 mg, dry basis) was mixed with water (20  $\mu$ L) and kept in sealed DSC sample pans overnight. Samples were scanned from 20 to 180°C at a heating rate of 10°C/min. Indium standards were used for temperature and energy calibrations. For each endotherm curve, initial ( $T_i$ ), completion transition ( $T_c$ ), onset ( $T_o$ ), and peak ( $T_p$ ) temperatures and transition enthalpies ( $\Delta H$ ) were computed. All samples were analyzed at least twice.

## Scanning Electron Microscopy

A scanning electron microscope was used to examine CWPC before and after treatment. A thin layer of CWPC powder was sprinkled onto an aluminum stub covered with transparent two-sided adhesive tape. The stub was coated with carbon and gold ( $\approx 15$ – $20$  nm [ $\approx 150$ – $200$ Å] coat thickness) with a sputter device (Hummer V, Technics, San Jose, CA) and viewed in a scanning electron microscope (S-570, Hitachi Corp., Tokyo, Japan). Film (type 55, Polaroid Corp., Cambridge, MA) was used to obtain micrographs.

## Dough Mixing Properties

The physical properties of dough (optimum water absorption and mixing time) and mixograms of control flours and flours fortified (replacement method) with 10% untreated and modified CWPC were evaluated with a mixograph, according to the method of Finney and Shogren (1972). Wheat flour fortified with untreated or modified CWPC was well mixed and kept overnight in a tightly sealed glass jar before testing.

## Statistical Analysis

All determinations were replicated at least twice and averaged. Least significant difference was calculated at the 5% level. Data were analyzed using Statistical Analysis System (SAS Institute: Cary, NC).

## RESULTS AND DISCUSSION

The composition of wheat flours and CWPC is shown in Table I. Protein content of HWS wheat Klasic and club wheat Moro was 15.90 and 10.56%, respectively. Flours had ash contents of 0.43 and 0.41% for HWS and club wheat, respectively. The low ash con-

**TABLE I**  
Composition (%) of Wheat Flours and Commercial Whey Protein Concentrate (CWCP)<sup>a</sup>

Sample	Protein	Ash	Lipids	Lactose
Wheat flour <sup>b</sup>				
cv. Klasic (HWS)	15.90 <sup>c</sup>	0.43	0.74	...
cv. Moro (CW)	10.56 <sup>c</sup>	0.41	0.77	...
CWPC	82.46 <sup>d</sup>	5.33	6.00	3.50

<sup>a</sup> Values are means of two determinations.

<sup>b</sup> HWS = hard white spring; CW = club wheat.

<sup>c</sup> N  $\times$  5.70.

<sup>d</sup> N  $\times$  6.38.

**TABLE II**  
Thermal Characteristics of Individual Proteins in Commercial Whey Protein Concentrate (CWCP)

Protein Sample	First Peak <sup>a</sup>			Second Peak		
	$T_o$	$T_p$	$\Delta H$	$T_o$	$T_p$	$\Delta H$
Untreated CWPC <sup>b</sup>	66.9	74.0	4.7	124.6	133.6	2.4
$\beta$ -Lg	79.1	83.3	14.6	133.5	140.9	2.6
$\alpha$ -La	60.3	66.0	8.5	137.2	138.3	0.2
Bovine serum albumin	74.5	89.3	15.2	118.1	127.2	2.3

<sup>a</sup>  $T_o$ ,  $T_p$ , and  $\Delta H$  = onset and peak temperature (°C), and transition enthalpy (J/g), respectively.

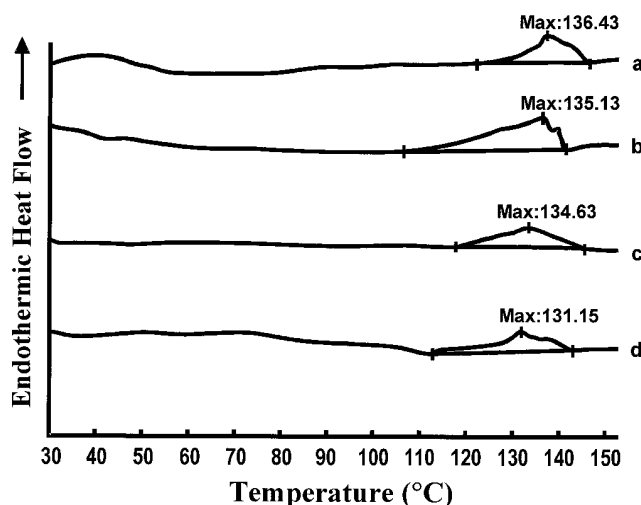
<sup>b</sup>  $\beta$ -Lg =  $\beta$ -lactoglobulin;  $\alpha$ -La =  $\alpha$ -lactalbumin.

centration was due to the low (60%) extraction rate. Free lipids content of 0.74–0.77% was similar for both flours. CWPC contained 82.46% protein, 5.33% ash, 6.00% lipids, and 3.50% lactose. The high protein content in CWPC indicates a large portion of minerals and lactose were removed during the concentration process.

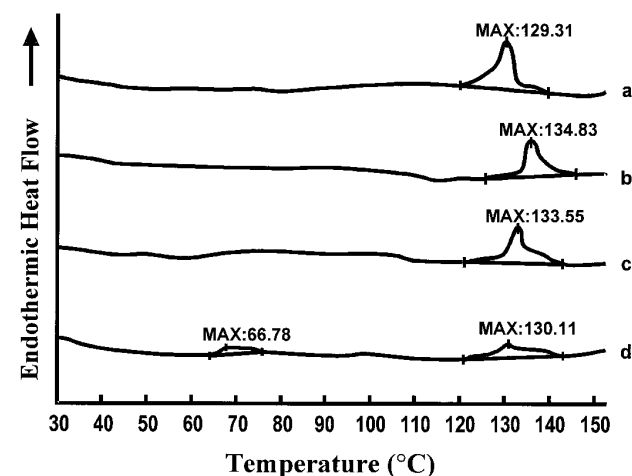
## DSC Thermograms of CWPC and Individual Proteins

The DSC curves of untreated CWPC and some individual whey proteins, such as  $\beta$ -Lg,  $\alpha$ -La, and BSA, are shown in Fig. 2. Details of thermal characteristics and enthalpy values are summarized in Table II. The DSC curve of untreated CWPC had two distinct enthalpy peaks. The first peak, with an enthalpy value of 4.7 J/g, occurred between 57 and 86°C; the second peak, with an enthalpy value of 2.4 J/g, occurred between 120 and 143°C. Onset temperature for the first peak was 66.9°C, with a maximum temperature of 74.0°C and, for the second peak, was 124.6°C, with a maximum temperature of 133.6°C. These two enthalpy peaks for untreated CWPC reflect the temperature enthalpy of individual whey proteins.

$\beta$ -Lg,  $\alpha$ -La, and BSA also exhibited enthalpy peaks. The first peak of  $\beta$ -Lg had a maximum temperature of 83.3°C and an enthalpy value of 14.6 J/g. The maximum peak for  $\alpha$ -La occurred at 66.0°C and had an enthalpy value of 8.5 J/g. BSA had an enthalpy value



**Fig. 3.** Differential scanning calorimetry curves of heat-treated commercial whey protein concentrate, four concentrations at 90°C for 30 min: 5, 10, 20, and 30% (a–d, respectively).



**Fig. 4.** Differential scanning calorimetry curves of high hydrostatic pressure (HHP)-treated commercial whey protein concentrate, four concentrations at 85 Kpsi for 30 min: 5, 10, 20, and 30% (a–d, respectively).

as high as 15.2 J/g and a maximum temperature of 89.3°C. The maximum temperature of the second peaks for these three proteins was 127–141°C. The enthalpy values of the second endothermic peaks were much lower than the values for the first peaks. The enthalpy values of the second peaks of  $\beta$ -Lg and BSA are comparable to the enthalpy value of untreated CWPC, whereas  $\alpha$ -La had an enthalpy value of only 0.2 J/g.

The thermal characteristics of CWPC indicate that endothermic peaks of CWPC are due to the major individual proteins, namely  $\beta$ -Lg and  $\alpha$ -La. The thermal behaviors of individual proteins obtained in this study agree with those reported in previous studies that showed denaturation of  $\beta$ -Lg at  $\approx$ 80°C and denaturation of  $\alpha$ -La at 60–70°C (de Wit and Klarenbeek 1984, Hollar et al 1995).

Rescanning of CWPC and individual proteins showed no first

peak, which in the first scan had occurred at 53–104°C. Also, the second peak, which existed in the first scan at 110–149°C, disappeared in CWPC and  $\alpha$ -La.  $\beta$ -Lg, and BSA showed second peaks with lower enthalpy values (0.62 and 0.31 J/g, respectively) compared to those in the first scan.

#### DSC Thermograms of CWPC Heat or HHP Treated

The extent of CWPC denaturation by heat and HHP was determined based on the enthalpy value of the DSC curve. DSC is an excellent tool for basic or in-depth investigation and measurement of the thermal response of a food system, particularly protein denaturation (de Wit and Swinkels 1980, de Wit and Klarenbeek 1981, Park and Lund 1984, Relkin and Launay 1990, Dybing and Smith 1991, Kilara and Mangino 1991, Relkin et al 1992, Erdogdu et al 1995).

The DSC enthalpy curves of CWPC solutions at 5, 10, 20, and 30%, each treated with heat at 90°C or HHP at 85 Kpsi for 30 min, are presented in Figs. 3 and 4, respectively. At all concentrations, except 30%, which was treated by HHP, the first enthalpy peak disappeared with both treatments, while the second peak remained, independent of concentration. A very small, yet detectable, enthalpy peak of  $\approx$ 0.4 J/g for 30% CWPC solutions was observed in samples subjected to HHP treatment. This may have been due to promotion of protein-protein interactions, which could have increased protein resistance to denaturation. The results further indicate that HHP-denatured CWPC protein is comparable to CWPC protein denatured

**TABLE III**  
Thermal Characteristics of Commercial Whey Protein Concentrate (20% concentration) Treated with Different Temperatures for 30 min<sup>a</sup>

Temp. (°C)	First Peak <sup>b</sup>			Second Peak		
	<i>T</i> <sub>o</sub>	<i>T</i> <sub>p</sub>	$\Delta H$	<i>T</i> <sub>o</sub>	<i>T</i> <sub>p</sub>	$\Delta H$
60	72.1b	77.4b	3.3a	125.2ab	132.8bc	2.2b
70	78.0a	84.5a	0.5b	125.2ab	132.6c	2.1b
80	0.0c	0.0c	0.0c	126.2a	133.2b	2.3b
90	0.0c	0.0c	0.0c	124.2b	134.6a	3.8a

<sup>a</sup> Means with different letters in the same column within each treatment are significantly different at the 5% level.

<sup>b</sup> *T*<sub>o</sub>, *T*<sub>p</sub>, and  $\Delta H$  = onset and peak temperature (°C) and transition enthalpy (J/g), respectively.

**TABLE IV**  
Thermal Characteristics of Commercial Whey Protein Concentrate (20% concentration) Treated with Different High Hydrostatic Pressures for 30 min<sup>a</sup>

Pressure (Kpsi)	First Peak <sup>b</sup>			Second Peak		
	<i>T</i> <sub>o</sub>	<i>T</i> <sub>p</sub>	$\Delta H$	<i>T</i> <sub>o</sub>	<i>T</i> <sub>p</sub>	$\Delta H$
20	66.6a	75.7a	6.6a	125.8a	133.0a	2.5a
40	60.8b	67.5b	1.6b	132.5a	136.7a	2.3ab
60	61.0b	67.6b	1.2c	128.1a	133.4a	1.7b
85	0.0c	0.0c	0.0d	128.3b	133.5a	2.7a

<sup>a</sup> Means with different letters in the same column within each treatment are significantly different at the 5% level.

<sup>b</sup> *T*<sub>o</sub>, *T*<sub>p</sub>, and  $\Delta H$  = onset and peak temperature (°C) and transition enthalpy (J/g), respectively.

**TABLE VI**  
Mixograph Characteristics of Dough Fortified with 10% Commercial Whey Protein Concentrate (CWPC)<sup>a</sup>

Sample	Water Absorption (%)	Mixing Time (sec)
Hard white spring cv. Klasic (100%)	70.0c	300d
Klasic (90%) + untreated CWPC (10%)	60.5d	535a
Klasic (90%) + heat-treated CWPC (10%) <sup>a</sup>	78.0a	405b
Klasic (90%) + HHP-treated CWPC (10%) <sup>b</sup>	74.0b	390c
Club wheat cv. Moro (100%)	55.0c	95d
Moro (90%) + untreated CWPC (10%)	54.0d	180a
Moro (90%) + heat-treated CWPC (10%) <sup>a</sup>	68.0a	135b
Moro (90%) + HHP-treated CWPC (10%) <sup>b</sup>	60.0b	120c

<sup>a</sup> 20% CWPC treated with 90°C for 30 min.

<sup>b</sup> 20% CWPC treated with high hydrostatic pressure (HHP) at 85 Kpsi for 30 min.

**TABLE V**  
Thermal Characteristics of Commercial Whey Protein Concentrate (CWPC) Treated with High Hydrostatic Pressure at Different Concentrations and Times<sup>a</sup>

CWPC Sample	Time (min)	First Peak <sup>b</sup>			Second Peak		
		<i>T</i> <sub>o</sub>	<i>T</i> <sub>p</sub>	$\Delta H$	<i>T</i> <sub>o</sub>	<i>T</i> <sub>p</sub>	$\Delta H$
5% Concentration	1	59.0a	72.5a	2.7a	127.9ab	135.3a	4.3a
	2	66.1a	75.5a	2.2ab	122.9b	133.6a	2.4a
	4	59.4a	66.9a	1.5ab	123.6b	134.1a	2.1a
	10	56.5a	64.5a	0.9bc	133.3a	134.4a	2.5a
	30	0.0b	0.0b	0.0c	125.0ab	129.3a	2.2a
10% Concentration	1	71.8a	75.2a	1.5a	126.5b	135.3a	1.2a
	2	61.9a	69.8ab	1.5a	120.7c	131.9c	1.2a
	4	59.8a	66.3b	0.8b	128.4b	134.6ab	1.8a
	10	60.1a	64.8b	0.3bc	127.8b	133.5bc	1.0a
	30	0.0b	0.0c	0.0c	133.7a	134.8ab	1.3a
20% Concentration	1	63.0a	67.1a	2.2a	126.3a	134.1a	1.9a
	2	59.5a	66.1a	0.8b	120.9b	133.2a	1.6a
	4	60.1a	66.2a	0.9b	128.8a	133.2a	2.0a
	10	43.7b	55.7b	0.6b	125.6a	134.4a	1.7a
	30	0.0c	0.0c	0.0c	128.3a	133.6a	2.7a
30% Concentration	1	59.5a	65.9a	1.4a	134.7a	141.9a	3.4a
	2	60.2a	66.9a	1.1ab	124.0a	135.0b	1.6a
	4	60.8a	66.1a	0.8bc	132.4a	134.3b	1.6a
	10	41.4b	45.2b	0.2d	127.2a	134.3b	1.5a
	30	62.9a	66.8a	0.4c	126.1a	130.1b	1.5a

<sup>a</sup> Means with different letters in the same column within each treatment are significantly different at the 5% level.

<sup>b</sup> *T*<sub>o</sub>, *T*<sub>p</sub>, and  $\Delta H$  = onset and peak temperature (°C) and transition enthalpy (J/g), respectively.

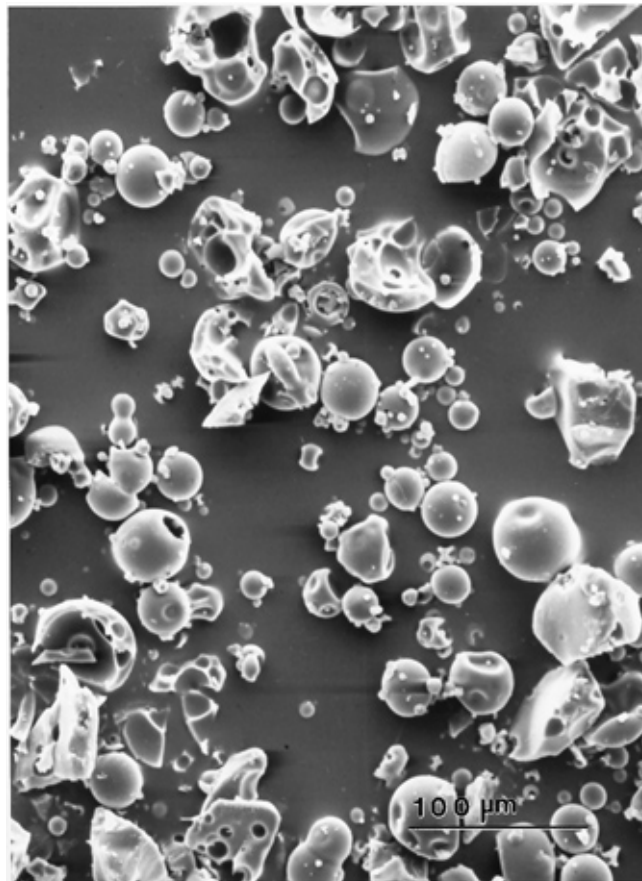
by high-temperature treatments for concentrations of up to 20%.

A 20% CWPC concentration was selected based on the results from the first part of the study, which indicated that a 20% CWPC solution was the highest concentration at which the first enthalpy peak did not exist for both temperature and HHP treatments. The DSC enthalpy values of heat-treated CWPC at 60, 70, 80, and 90°C are summarized in Table III. Heat treatment of CWPC at 60°C resulted in an enthalpy value for the first peak of 3.3 J/g,  $\approx 1.0$  J/g lower than for untreated CWPC. A sharp decrease in enthalpy to 0.5 J/g for the first peak was observed at 70°C, with complete disappearance of the peak at 80°C. The second enthalpy peak was observed at all applied temperatures and had a significantly higher enthalpy value at 90°C than at lower temperatures.

The DSC enthalpy values for CWPC treated by HHP at 20, 40, 60, and 85 Kpsi are presented in Table IV. The value of the first enthalpy peak decreased significantly as pressure was increased from 20 to 85 Kpsi. The first peak of CWPC treated by HHP at 20 Kpsi had an enthalpy value  $\approx 2.0$  J/g higher than untreated CWPC (Table II). Even though the mechanism is not fully understood, these results suggest that treatment with low HHP promotes reversible unfolding of protein, altering the protein-protein interactions, which require more thermal energy to denature. Also, the presence of cross-links of covalent or noncovalent bonds may be the reason for the greater stability of the native protein structure, which makes the molecules resistant to denaturation (Mangino 1984). Thus, it can be postulated that low HHP treatment of 20% CWPC solutions for 30 min promotes the formation of these cross-links and strong protein-protein interactions. HHP at 20 Kpsi or lower is not likely to denature CWPC.

#### DSC Enthalpy Values for CWPC Treated by HHP

The results of DSC analysis of CWPC treated with HHP for 30 min raised the question of whether shorter treatment times ( $<30$  min) at



**Fig. 5.** Scanning electron micrograph of untreated commercial whey protein concentrate.

85 Kpsi can effectively denature CWPC and also how changes in protein are affected by protein concentration. Changes on the molecular level in 5, 10, 20, and 30% CWPC solutions treated by HHP at 85 Kpsi for 1, 2, 4, 10, and 30 min were evaluated by DSC and are summarized in Table V. Independent of concentration, the enthalpy values for the first peak decreased as treatment time increased. After 30 min of treatment, the first peak disappeared, except at a 30% concentration, as previously discussed. As for the second peak, enthalpy values did not differ statistically between treatment time within each CWPC concentration.

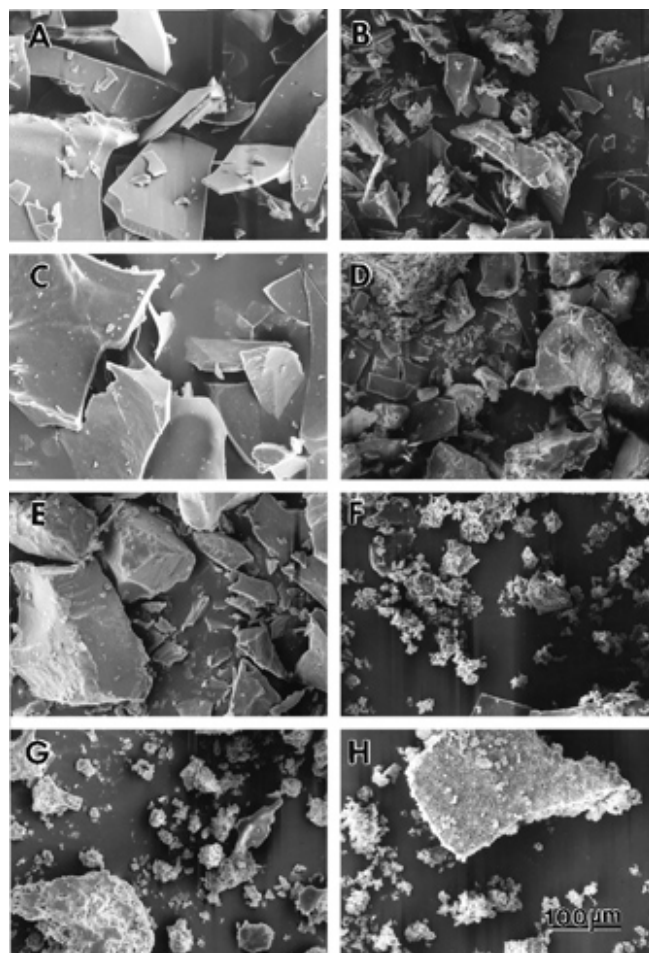
#### Scanning Electron Micrographs of Untreated and Heat- and HHP-Treated CWPC

Scanning electron micrographs of CWPC showed that protein in the spray-dried commercial product was globular (Fig. 5). Micrographs of CWPC treated with heat at 90°C for 30 min showed changes in physical appearance as a function of concentration (Fig. 6). The physical appearance of freeze-dried CWPC changed from an easy-to-break, solid, glasslike form at 5% to a hard-to-break, porous or spongy form at 30%.

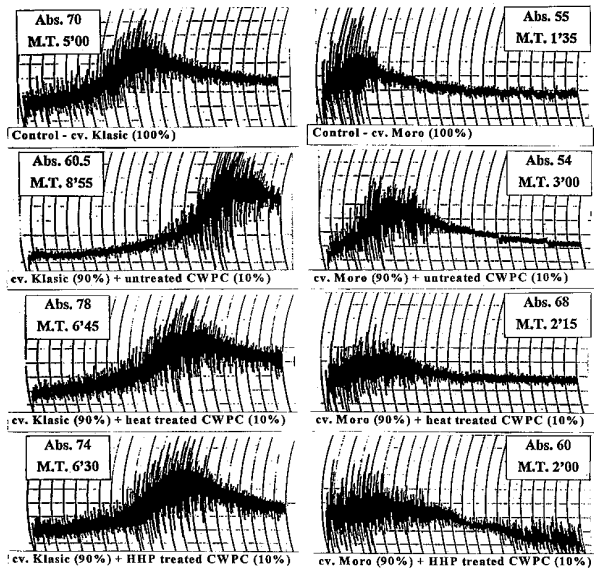
Scanning electron micrographs of HHP-treated CWPC revealed that changes in the physical appearance of CWPC depend on concentration. HHP-treated CWPC had a more porous structure than heat-treated CWPC at the same concentration (Fig. 6).

#### Physical Dough Properties

The physical properties of hard and soft wheat flours (control) and flours fortified by untreated and heat- and HHP-treated CWPC were evaluated with a mixograph. Changes in mixograph mixing



**Fig. 6.** Scanning electron micrographs of commercial whey protein concentrate (CWPC) treated with heat and high hydrostatic pressure, respectively: **A** and **B**, 5% CWPC; **C** and **D**, 10% CWPC; **E** and **F**, 20% CWPC; **G** and **H**, 30% CWPC.



**Fig. 7.** Mixograms of control flours with or without 10% untreated and heat- or high-hydrostatic pressure (HHP) treated commercial whey protein concentrate.

time, water absorption, and mixograph curves were examined before and after fortification. Water absorption and mixing times of both flours are summarized in Table VI; mixograph curves are presented in Fig. 7.

HWS wheat Klasic had a 15% higher water absorption and mixing time 205 sec longer than club wheat Moro. These differences in mixograph parameters were due to differences in protein content and quality between hard and club wheats (Table I). The mixograph curves best illustrate these differences. The mixograph curve of HWS wheat flour showed a well-defined peak of gluten development at 5 min of mixing and high dough resistance to mixing after the peak at optimum water absorption. In contrast, club wheat flour had a very short (<2 min) mixing time and broke down rapidly after the peak.

Large changes in mixograph water absorption and mixing time took place in dough when 10% of each flour was replaced with untreated CWPC. Water absorption decreased and mixing time increased for both flours. Moreover, the large differences between the two flours became more pronounced. Water absorption for hard wheat flour decreased by 9.5% and mixing time increased by 4 min compared to the control (Table VI). Club wheat fortified with 10% untreated CWPC had a 1% decrease in water absorption and 100% increase in mixing time compared to the control. The changes in mixograph curves of both hard and soft wheat flour doughs fortified with untreated CWPC were due to the rapid breakdown of gluten after optimum dough development. Therefore, a decrease in water absorption and reduction in mixing tolerance of dough containing 10% untreated CWPC could be undesirable to the bread baking industry. Similar results were reported by Erdogdu-Arnoczky et al (1996), in which 4% commercial acid whey powder was added to wheat flour.

Incorporation of 10% heat- or HHP-treated CWPC brought about marked changes in mixograph curves in both flours. Optimum water absorption and mixing time increased compared to the control, but mixing time was much shorter than for dough fortified with untreated CWPC (Table VI). The most significant changes in mixing properties were found in mixing tolerance, which was restored by addition of heat- or HHP-treated CWPC to flours (Fig. 7). The similarity in thermal behavior of heat- or HHP-treated CWPC, as measured by DSC and SEM, resulted in similar functional properties in the dough system, as shown by dough rheology. CWPC treated either by HHP for 30 min at 85 Kpsi or by heat at 90°C for

30 min had similar effects on rheological properties of dough, as tested with a mixograph, when added to wheat flours. HHP treatment of CWPC caused changes in protein conformation. This proved that HHP can be used to modify properties of dairy products and increase their use in more areas, including the baking industry.

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