Wheat Flour and Defatted Milk Fractions Characterized by Differential Scanning Calorimetry. I. DSC of Flour and Milk Fractions[1]

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ABSTRACT

Differential scanning calorimetry (DSC) thermograms were determined for wheat flour and milk fractions. Compositional and structural differences between prime and tailings starch apparently affected their DSC thermograms and interaction patterns. Tailings starch had a lower gelatinization enthalpy and a higher amylose-lipid complex transition enthalpy than did prime starch. These observations were attributed to the faster rate of heat transfer and higher accessibility to water, presumably related to the smaller granule size of tailings starch. Gluten lowered the temperatures of the amylose-lipid complex endotherm of both prime and tailings starches. Water solubles increased the gelatinization temperatures of the starches due to the competition for available water. Laboratory-prepared acid whey showed higher denaturation temperatures than sweet whey, which is explained by the acid-resistant character of the major whey protein β-lg. A commercial whey protein concentrate showed endotherm temperatures that were considerably higher (>40° C) than those of whey powders. Ultrafiltration in commercial processing could be responsible for this temperature shift. The degree to which protein is concentrated by ultrafiltration or dialysis affects the relative concentration of water solubles and influences the functional properties and interactions of whey protein concentrates with wheat flour components.

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Dairy products are added to foods with the expectation that they will provide specific benefits to the product. Whey preparations can provide several benefits to baked goods. Many of those benefits can be enhanced or reduced by variations in whey processing (Hugunin 1987).

Whey, the liquid that remains after casein and fat are separated from the milk during cheese production, contains most of the milk's salts, lactose, and water-soluble proteins. It varies in composition with the type of cheese from which it derives, as well as heat treatment, handling, and other factors. Sweet whey, which is derived from the manufacture of the ripened cheeses (cheddar, swiss, provolone, etc.), is so named because its pH (~6.6) is only slightly below that of fresh milk (Bassette and Acosta 1988). Acid whey, on the other hand, has a pH of ~4.6 and is a by-product of cottage cheese or casein manufacture. While the protein and lactose composition of wheys are similar with respect to the majority of proteins, acid whey differs significantly from sweet whey in its content of casein-derived peptides and mineral content (Bassette and Acosta 1988).

The major obstacles in commercial processing of whey protein ingredients are the high variability in composition and properties of products that are classified as whey protein concentrates (WPC) (Schmidt et al 1984). WPC produced by ultrafiltration became commercially available in the mid-1970's. WPC with 35% protein was perceived to be a universal substitute for nonfat dry milk (NFDM) because of its similarity in gross composition and dairy character (Hugunin 1987).

In 1990, the U.S. food industry manufactured ~173 million pounds of WPC (Anonymous 1991) that contained 35–75% protein, and a smaller but unknown amount of whey protein isolate (WPI) that contained ≥90% protein. Current industrial procedures for manufacturing WPC usually involve the following processing steps: 1) whey containing only about 0.5–0.7% protein is pasteurized; 2) the pasteurized whey is concentrated by ultrafiltration (UF) to achieve a 20- to 25-fold concentration of protein; 3) the UF retentate is diafiltered against water; 4) the UF retentate is concentrated by vacuum evaporation (optional); and 5) the UF retentate concentrate is spray-dried (Morr 1984). Ultrafiltration reduces the relative concentration of lactose, minerals, and nonprotein nitrogen compounds in whey. Some of the mineral components, however, are associated with the proteins and may be retained with the proteins through hydrogen bonding or other attractive forces. Whey calcium can foul or reduce the flux rate of water through the membranes. Processors have developed techniques, such as heating and adjusting the pH of the whey, for reducing this problem. These pretreatments have an effect on the final mineral content.

The functionality of commercial WPC products is generally related to the relative content of protein to nonprotein components and the extent to which those proteins have been denatured during processing. Ultrafiltration permits the fractionation of whey components without heat. However, heat still is involved in WPC production during pasteurization, thermal evaporation, and spray-drying (Hugunin 1987). There are indications that purified whey protein solutions are more susceptible to heat denaturation than whole whey solutions (de Wit et al 1986).

A survey of commercial WPC and whey protein isolates (WPI) confirmed a high degree of variability in gross composition, physicochemical properties, functionality, and flavor (Morr and Foegeding 1990). Xiong (1992) also reported that limitations in the commercial utilization of WPC are related to the great variability in the functional properties due to the differences in composition and processing.

Differential scanning calorimetry (DSC) has been a widely accepted tool in investigating variations in thermal behavior of food systems and was used in this study to characterize fractions of wheat flour (prime starch, tailings starch, gluten and water solubles) and milk (whey and casein) and their interactions. This article deals with the thermal behavior and interactions among wheat flour fractions. Using DSC, we investigated processing-related variations in the thermal behavior of dairy fractions. An accompanying article (Erdogdu et al 1995) reports the use of DSC to examine the interaction patterns of the wheat flour components with dairy fractions in a model system. The findings from the DSC study were then utilized in interpreting the effects of dairy fractions on physical dough and breadmaking properties.

MATERIALS AND METHODS

Wheat Flour Fractions

Laboratory-milled, hard wheat standard bread flour (70% extraction, 68% mixograph absorption) was obtained from the Western Wheat Quality Laboratory (USDA-ARS, Pullman, WA).

The flour was hand-washed with distilled water into prime starch (PS), tailings starch (TS), gluten (G), and water solubles (WS) (Hoseney et al 1969). The 100-g flour was made into a

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relatively tough dough (60% absorption), covered with water at 15°C in a porcelain basin, relaxed for 30 min, and manually washed with five 200-ml portions of water into gluten and a mixture of starch and water solubles. The water solubles plus starch were screened (metal screen with 0.5-mm openings) to recover small gluten pieces and centrifuged for 15 min at 1,600 × *g*. Four fractions were obtained from top to bottom: water solubles, tailings starch, insoluble fiber (very thin layer), and prime starch. The water solubles were decanted, and the starch plus fiber were resuspended in 500 ml of water from washing of gluten and centrifuged. The resuspension and centrifugation were repeated two more times, and all water solubles were combined. After the tailings starch plus insoluble fiber were removed from the centrifuge tube, the prime starch was resuspended in 250 ml of water and centrifuged. The water solubles were discarded. The flour and the fractions are described in Table I. A total of 28 fractionations were performed on 100-g flour lots. Prime starch was dried at room temperature (23°C) until a moisture of ~10% was reached. Tailings starch, gluten, and water solubles were freeze-dried to an average moisture content of ~5%. Dry prime starch and tailings starch were ground with a Udy grinder to pass a 0.50-mm sieve; gluten was ground to pass a 0.25-mm sieve; and water solubles were manually ground in a mortar. Ground samples were kept in sealed containers at room temperature for further analysis.

Dairy Fractions

Commercial dairy fractions were: 1) acid whey protein concentrate (C-AWPC), 2) sweet whey protein concentrate (C-SWPC), 3) acid casein (C-AC) and sweet casein (C-SC), and 4) lactose (La). Laboratory prepared fractions (L) were: 5) acid (L-AWP) and sweet (L-SWP) whey powders, 6) simulated whey protein concentrate (L-WPC) prepared by heating (L-AWP1, L-AWP2) to 80 or 95°C, respectively, or dialysis (L-DAWP, L-DAWP1, L-DAWP2) of acid whey protein, and 7) sweet (L-SC) and acid (L-AC) caseins.

Sources of Commercial Dairy Ingredients

C-AWPC and C-SWPC were obtained from Main Street Ltd. (LaCrosse, WI). C-AWPC was a by-product of cottage cheese, and C-SWPC was derived from other white cheeses (such as mozzarella). Whey protein concentrates products were heated, ultrafiltered, and spray-dried according to manufacturers' information. C-SC, C-AC, and C-AWP were obtained from New Zealand Milk Products, Inc. (Santa Rosa, CA) and lactose (La) was obtained from Sigma Chemical Co. (cat. L-3625, lot. 29F-0115, St. Louis, MO).

Laboratory Preparation of Dairy Ingredients

Fresh raw milk, obtained from the Washington State University creamery, Pulman, WA, was used to prepare the fractions. The milk averaged 3.9% fat, 3.2% protein, 4.8% lactose, 0.8% ash, and 87.8% water. Milk was skimmed by centrifugation at 3,000 × *g* for 20 min at 4°C. Skimmed milk was fractionated into whey and casein by acid precipitation and centrifugation to produce whey powder and casein (McKenzie 1971).

Acid Precipitation

Hydrochloric acid (1 *M*) was slowly added to skim milk at 20°C. As a pH of 4.6 was reached, precipitation was allowed to continue for another 30 min. The precipitate was collected by centrifugation at 1,300 × *g* for 30 min. Precipitate and supernatant were designated acid casein and acid whey.

Dialysis or heat was included in precipitation of laboratory acid whey to simulate some of the preparation steps in commercial whey processing. The L-DAWP was produced by dialysis of fresh acid whey against distilled water at 4°C for 36 hr. The whey-to-dialysis water ratio was 1:10, and the water was replaced five times during dialysis. Spectra/Por, a regenerated cellulose membrane with a molecular weight cutoff of 12,000–14,000, was used for dialysis. The heated acid whey powders were prepared by heating the fresh acid whey at 80 or 95°C for 10 min in a water bath.

Centrifugation

Skim milk was centrifuged at 20,000 × *g* at 4°C for 90 min for sweet whey and sweet casein preparations according to McKenzie (1971). Centrifugation resulted in separation into three layers from bottom to top: a pale yellow opalescent gel, an opalescent viscous liquid, and the supernatant. The supernatant was decanted from the combined two bottom layers. Centrifugation was selected over rennet precipitation to avoid the formation of casein-derived peptides.

All laboratory-prepared fractions were freeze-dried to a moisture of ~5%, ground on a Udy mill to pass a 0.25-mm sieve, and kept in sealed glass jars at 4°C for further analysis.

Analytical Methods

Moisture and ash were determined in wheat flour fractions according to approved methods 44-15A and 08-01 (AACC 1983) and in dairy fractions according to methods 16.212 and 16.216 (AOAC 1990). Protein was determined with a Leco nitrogen analyzer using the conversion factors of N × 5.70 for wheat flour and 6.38 for dairy fractions. Amylose content of prime starch was determined by a colorimetric method according to Hovenkamp-Hermelink et al (1988).

Differential Scanning Calorimetry

DSC assays were performed as described by Czuchajowska and Pomeranz (1989) on a Perkin-Elmer DSC-2 (Perkin Elmer Corp., Norwalk, CT) instrument with large volume stainless steel capsules (cat. 0319-0218). Water (20 μl) was added to 10-mg samples (water-free basis) and kept in the capsules for 20 min to 4 hr (depending on the nature of the material and rate of water absorption). Increasing the equilibrium time from 2 to 4 hr did not affect enthalpy curves. For DSC analysis of interactions among wheat flour fractions, 1-g samples of each component were blended in a glass vial and vortexed for 3 min.

For each endotherm curve, onset (T_o) and peak (T_p) temperatures and transition enthalpies (ΔH) were computed. Standard deviations of temperature values were <1.0°C; standard deviations of enthalpy values were <10% of the mean.

Statistical Analysis

All experiments were done at least in duplicate and were averaged. Data were analyzed using the statistical analysis system of the SAS Institute (1985).

RESULTS AND DISCUSSION

The distribution of nitrogen in the wheat flour fractions (Table I) is an indication for the efficiency of the fractionation process as well as of compositional differences among the fractions. The high protein content in gluten and the low protein content in prime starch indicate a good separation. The dry matter of gluten can contain 75–85% protein, depending on the thoroughness of the washing, and 5–10% lipids, with occluded starch making up most of the remainder of the dry matter (Khan and Bushuk 1979). The tailings starch was relatively higher in protein than was the prime starch. The tailings fractions is composed of starch (mainly small granules), pentosans, protein, lipids, and ash (Baker et al 1943).

TABLE I
Some Characteristics of Hard Wheat Standard Flour and Flour Fractions

Flour or Fraction	Yield[a]	Protein[a,b]	Ash[a]	Amylose[c]
Flour	100	13.7	0.68	...
Prime starch	66	0.4	0.21	24.8
Tailings starch	14	2.9	0.29	...
Gluten	15	84.0	0.39	...
Water solubles	5	13.3	...	...

[a] Water-free basis (%).
[b] N × 5.7.
[c] In the starch.

DSC Thermograms of Individual Wheat Flour Fractions

The prime starch (Fig. 1a) and tailings starch (Fig. 2a) showed two endotherms, corresponding to gelatinization (~64°C) and amylose-lipid complex (~103°C melting). The starch-to-water ratio of 1:2 used in this DSC study was considered as limiting for gelatinization by some researchers (Münzing 1989, 1991) and sufficient by other researchers (Ghiasi et al 1982, 1983; Liu et al 1991). According to Eberstein et al (1980), in systems with water contents above 60%, enthalpy changes are related to starch concentration independent of the properties of starch. Endotherm temperatures of gelatinization for prime and tailings starches were similar to those reported by Donovan et al (1983) and Russel (1987a,b).

Transition enthalpies of prime and tailings starches differed widely. Tailings starch contains small, damaged granules admixed with lipids, enzymes and pentosans. Prime starch consists of large, relatively pure and undamaged granules (Pomeranz 1988). The tailings starch showed endotherms of fibrous material and hemicellulose complexes at ~150 and 170°C, respectively (Sievert and Pomeranz 1990). Prime starch contained about 75% amylopectin (ordered structure) (Table I) and gelatinized at 64°C with over twice as high a transition enthalpy as that of tailings starch (Table II).

Several factors can explain the ΔH differences between prime starch and tailings starch gelatinization: size of the starch granule, total starch content, mechanical damage to the starch, amylose-to-amylopectin ratio in the starch granule, and interaction between starch and other tailings starch components. The amylose-lipid complex of tailings starch, on the other hand, showed a ΔH that was much higher than that of prime starch. An explanation for that may be the higher amount of lipid in tailings starch available for amylose-lipid interaction.

Gluten showed no ΔH, indicating that the preparation contained little, if any, residual starch (Eliasson and Hegg 1980, Schofield et al 1984). Lacking a tertiary-ordered structure, gluten cannot be put into a disordered form by heat, which explains the absence of DSC thermograms (Hoseney and Rogers 1990).

The aqueous supernatant that is separated from starch after gluten washing contains a mixture of water-soluble components. The mixture showed no transition endotherms under the conditions of this study.

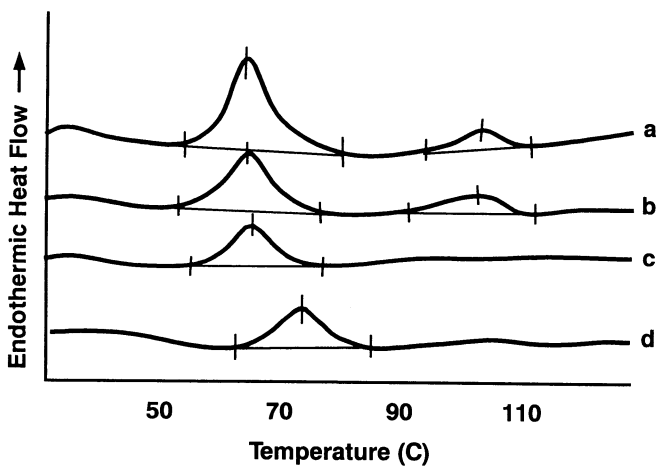

Fig. 1. Differential scanning calorimetry of: a) prime starch, b) a 1:1 mixture of prime starch and tailings starch, c) a 1:1 mixture of prime starch and gluten, and d) a 1:1 mixture of prime starch and water solubles.

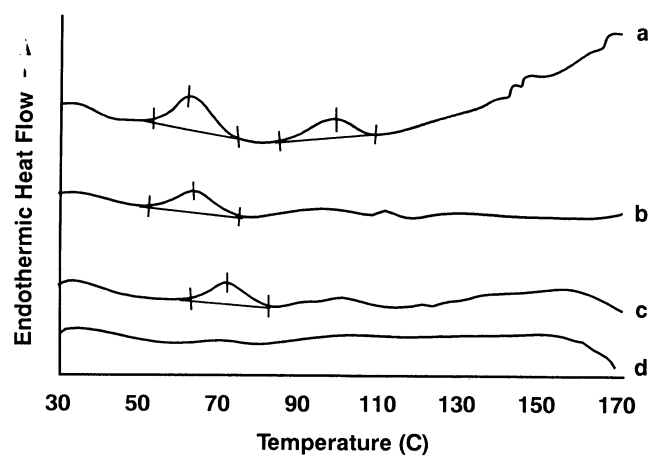

Fig. 2. Differential scanning calorimetry of: a) tailings starch, b) a 1:1 mixture of tailings starch and gluten, c) a 1:1 mixture of tailings starch water solubles, and d) a 1:1 mixture of gluten and water solubles.

DSC Thermograms of Blended (1:1) Wheat Flour Fractions

The interactions between the individual flour fractions (prime starch, tailings starch, gluten, and water solubles) blended in a 1:1 ratio were analyzed by DSC. The T_o, T_p, and ΔH are listed in Table II.

Prime Starch Interactions

The endotherm temperatures of the prime starch-tailings starch mixture (Fig. 1b) were the same as those of their parent fractions, indicating that the order of rate of gelatinization was unchanged. The ΔH values of the gelatinization and amylose-lipid complex of the prime starch-tailings starch mixture were proportional to the amount of parent starches (1:1) present in the mixture. This indicates that the concentrations of intact crystalline regions of the starches governed the transition enthalpy values.

The endotherm temperatures of the amylose-lipid complex for the prime starch-tailings starch mixture were the same as those for tailings starch. A complex T_o, lower by 7°C in comparison to the prime starch might be due to the compositional and structural differences between the two starches. Amylose leaches from the granule at the last stage of gelatinization, after melting of the crystalline part of amylopectin (Lineback and Rasper 1988), and subsequently forms the amylose-lipid complex. Münzing (1991) reported that water accessibility becomes higher as the starch granule gets smaller. Thus, based on the last two observations, it can be postulated that tailings starch, with a higher water binding capacity and smaller granule size than prime starch, can release amylose faster to associate with lipids (Bushuk 1966).

Gluten did not interact with prime starch (Fig. 1c) or with tailings starch (Fig. 2b). Endotherm temperatures of the amylose-lipid complexes of both starch fractions were significantly reduced by gluten (Table II and Fig. 1c and Fig. 2b).

The water-soluble fraction of wheat contains minerals, albumins, globulins and pentosans (Pomeranz 1988). Water solubles significantly raised the T_o and T_p (by ~8°C) of prime starch gelatinization (Table II and Fig. 1d). ΔH was actually decreased (probably a dilution effect). An increase in the gelatinization temperature of prime starch due to water solubles could have two causes. First, water solubles competed with starch for water,

TABLE II
Differential Scanning Calorimetry Characteristics[a] of Wheat Flour Fractions

	1st Peak			2nd Peak			3rd Peak		
Fractions[b]	T_o (°C)	T_p (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	ΔH (J/g)
PS	57.8	64.1	10.47	97.9	102.9	1.47	—	No peak	—
+TS	56.4	64.7	7.32	90.7	102.9	2.58	—	No peak	—
+G	58.2	64.6	4.61	82.7	94.9	0.68	—	No peak	—
+WS	65.9	71.8	4.32	98.6	105.0	1.06	—	No peak	—
TS	54.5	64.6	5.02	91.7	102.9	2.47	152.6	154.0	0.72
+G	55.8	64.7	2.64	84.5	97.3	1.07	—	No peak	—
+WS	64.5	73.7	2.91	87.4	102.6	1.41	—	No peak	—

[a] T_o = onset temperature, T_p = peak temperature, ΔH = enthalpy.
[b] 1:1 Mixtures of prime starch (PS), tailings starch (TS), gluten (G), and water solubles (WS).

resulting in an increased gelatinization temperature. As the water-starch ratio decreases, the transition temperature increases (Münzing 1991). Second, a component of the water solubles might interact with part of the starch at low temperatures. The interaction may be later overcome by the rising temperature. Spies and Hoseney (1982) postulated that interaction of sugar with starch chains in the amorphous regions of the starch granule stabilizes those regions, thus increasing the gelatinization temperature. No significant effect of water solubles on the amylose-lipid complex of prime starch was observed.

Interactions of tailings starch with prime starch and gluten were described previously. Water solubles did not interact differently with tailings than with prime starch (compare Figs. 1d and 2c). The temperature of tailings starch gelatinization was raised by ~10°C. The onset temperature of the amylose-lipid complex endotherm of tailings starch, however, was somewhat lowered.

No transition endotherm was recorded in the mixture of gluten and the water soluble fraction.

DSC Thermograms of Dairy Fractions

Whey preparations consist of lactose, proteins β-lactoglobulin (β-lg), α-lactalbumin (α-la), immunoglobulins, and serum albumin, in decreasing order of abundance, as well as minerals (Marshall 1982). The compositional characteristics of commercial and laboratory dairy fractions are summarized in Table III. The composition of the fractions varied depending on the preparation methods used. For example, laboratory-prepared L-AWP contained more ash (10.7%) than the L-AC (3.7%). On the other hand, L-SWP and L-SC were similar in ash content (average 8%). These variations in the mineral contents can be explained by pH-dependent stability of the metallo-proteins of milk. Caseins, for instance, consist of mostly calcium-phosphoproteins (α_{s1}, α_{s2}, β, κ) that precipitate from raw skim milk upon acidification to pH 4.6 at 20°C (Leman and Kinsella 1989). The calcium caseinate-calcium phosphate complex of casein is dissociated from casein at pI 4.6), where precipitation is completed. Thus, the complex is found in the whey portion. This explains the higher ash content of AWP than SWP. By centrifugation, however, caseins (L-SC) are precipitated as the intact calcium caseinate-calcium phosphate complex. Therefore, L-SC had a higher ash content than did L-AC. Commercial acid and sweet C-WPC were similar in protein, ash, and lactose probably because of the pre- or postprocessing adjustments made. The commercial casein preparations had a higher protein content (90.2–96.1%) than those that were laboratory isolated (74.6–77.2%). The higher protein content was probably achieved by additional commercial processing steps.

DSC Thermograms of Commercial Dairy Ingredients

Commercial acid whey powder gave an endotherm peak at 64.7°C (Fig. 3a). The transition endotherms of commercial sweet (C-SWPC) and acid (C-AWPC) whey protein concentrates appeared at ~92°C (Table IV and Fig. 3g,i). These results are consistent with the data obtained by Bernal and Jelen (1985), who reported the highest denaturation temperature of 88°C for an AWPC prepared by ultrafiltration. On the other hand, lower denaturation temperatures (~70°C) have been reported by many researchers. The differences (~20°C) were attributed to methods

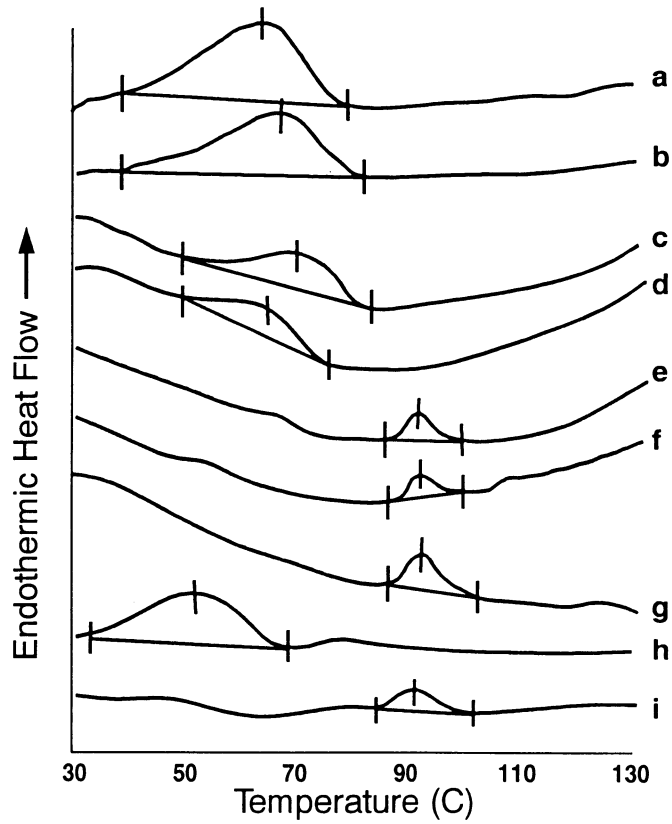

Fig. 3. Differential scanning calorimetry of laboratory (L) versus commercial (C) whey fractions: a) C-acid whey powder, b) L-acid whey powder, c) L-acid whey powder heated at 80°C, d) L-acid whey powder heated at 90°C, e) L-dialyzed acid whey powder, f) L-dialyzed and acid whey powder heated at 80°C, g) C-acid whey protein concentrate, h) L-sweet whey powder, and i) C-sweet whey protein concentrate.

TABLE III
Some Characteristics of Dairy Fractions

Fraction	Protein[a]	Ash[a]	Lactose[a]	pH
Commercial products[b]				
Lactose (Sigma)	0.0	0.0	99.9	...
Acid whey powder (Sigma)	16.7	9.1	74.2	4.7
Sweet whey protein concentrate (Main Street Ltd.)	36.0	7.1	53.4	6.7
Acid whey protein concentrate (Main Street Ltd.)	36.0	7.1	53.4	4.5
Sweet casein (New Zealand Milk Products)	90.2	8.7	0.1	7.1
Acid casein (New Zealand Milk Products)	96.1	2.0	0.1	4.6
Laboratory preparations				
Sweet whey protein	14.2	7.6	78.1[c]	6.6
Acid whey protein	13.5	10.7	75.9[c]	4.6
Dialyzed acid whey protein	34.9	4.9	60.2[c]	4.6
Sweet casein	77.2	8.5	15.5[c]	6.6
Acid casein	74.6	3.7	23.3[c]	4.6

[a]Water-free basis (%); N × 6.38.
[b]Manufacturer's data.
[c]Calculated by difference.

TABLE IV
Differential Scanning Calorimetry[a] Characteristics of Commercial and Laboratory Dairy Preparations

	1st Peak			2nd Peak		
Materials	T_o (°C)	T_p (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	ΔH (J/g)
Commercial products						
Acid whey protein	57.5	64.7	15.21	—	No peak	—
Acid whey protein concentrate	87.6	92.0	1.85	—	No peak	—
Sweet whey protein concentrate	81.0	93.0	1.82	—	No peak	—
Lactose	56.7	88.5	65.35	—	No peak	—
Laboratory preparations						
Acid whey protein	47.0	66.8	10.82	101.3	107.2	0.42
Heated to 80°C	55.8	68.3	3.95	—	No peak	—
Heated to 95°C	49.3	61.7	2.51	—	No peak	—
Dialyzed acid whey protein	86.6	90.3	1.23	—	No peak	—
Heated to 80°C	87.2	90.6	0.95	—	No peak	—
Sweet whey protein	34.0	51.5	8.76	101.1	108.9	0.72

[a]T_o = onset temperature, T_p = peak temperature, ΔH = enthalpy.

of preparation (Lapanje and Poklar 1989). Commercial C-SC and C-AC showed no transition peaks in this study (data not shown). Caseins form micelles lacking a tertiary-globular structure and, consequently, do not show endotherm transitions in DSC experiments (Paulsson and Dejmek 1990).

DSC Thermograms of Laboratory-Prepared Dairy Ingredients

L-AWP and L-SWP showed transition endotherms at 66.8°C and 51.5°C, respectively, which represent the aggregation of α-la and β-lg, the major proteins of whey (Table IV, Fig. 3b,h). L-AWP with a T_p 15.3°C higher than that of L-SWP, was more resistant to denaturation. Higher denaturation temperatures of acid whey in comparison to sweet whey proteins have been reported earlier and these observations were attributed to the acid-resistant denaturation profile of the major whey protein, β-lg. Apparently, β-lg governs the thermal behavior of whey preparations (Bernal and Jelen 1985, Relkin et al 1992). de Wit (1981) and de Wit and Klarenbeek (1981) analyzed by DSC the thermal behavior of major whey proteins up to 150°C. They observed two distinct heat effects: 1) near 70°C, attributed to denaturation; 2) near 130°C, attributed to unfolding of the remaining protein structure. The second transition endotherm of both laboratory whey powders (Table IV) can be attributed to β-lg. According to de Wit (1981), β-lg exhibits a second thermal change above 100°C caused by breakdown of disulfide bonds and additional unfolding of the molecule. Similar to the results with commercial caseins, laboratory L-SC and L- AC, lacking the tertiary-globular structure, showed no DSC transition peaks.

Thus, endotherm temperatures of laboratory whey powders and commercial whey protein concentrates differed widely. Difference in T_p between C-SWPC to L-SWP was >40°C. Differences in mineral content between laboratory and commercial whey powders can possibly explain the large differences in denaturation temperatures. The denaturation temperature of whey proteins is strongly dependent on the calcium content. Elevated denaturation temperatures corresponding to reduced calcium concentrations were reported by Relkin et al (1992). The laboratory whey powders were processed into simulated L- WPC to study the variation in endotherm temperatures among whey preparations.

Simulated Whey Powders

To identify the processing step in acid L-WPC preparation that causes the shift in the endotherm temperatures, dialysis and heat application (corresponding to UF and pasteurization) were included in the preparation procedure. The characteristics of the simulated fractions are presented in Table IV. Dialyzed laboratory AWP (L-DAWP) was similar to commercial WPC (C-AWPC) in composition (Table III). Dialysis resulted in a relative reduction of lactose and ash. Heating had no influence on the compositional characteristics of whey fractions (data not shown).

DSC Analysis of Simulated Whey Protein Concentrates

The variations in DSC thermograms of whey powders when processed into L-WPC are presented in Table IV and Figure 3.

Endotherms of heat treated (at 80 or 95°C) L-AWP1 or L-AWP2 (Fig. 3c,d) appeared with similar denaturation temperatures but significantly lower enthalpy transitions than nonheated L-AWP. Reappearance of these endotherms after heating is a result of reversible, heat and pH-dependent denaturation properties of whey proteins. If the heating time at a temperature higher than the denaturation temperature is sufficiently long, irreversible aggregation takes place (Relkin and Launay 1990). Relkin et al (1992) postulated that "during the first heating run, the intramolecular S-H/S-S interchange reactions have sufficient time to be completed and the denaturation is partly reversible; soluble aggregates are formed and the peak observed during the second heating corresponds to the dissociation of these aggregates".

Acid whey showed a shift in the peak temperature from 66.8 to 90.3° after dialysis (Fig. 3e). This increase of ~24°C produced an endotherm that is analogous to that of commercial C-AWPC at 92°C (Fig. 3g). Heat treatment of acid whey did not change the endotherm temperatures of the dialyzed acid whey (Fig. 3f). Ultrafiltration, therefore, seems to be responsible for the variations in the DSC thermograms.

We are not certain about the identity of a small, dialyzable component that may have caused a shift in the T_p temperature. However, the removal of minerals is likely since they play vital roles in protein conformation and functionality (Xiong 1992). Several studies have reported the effect of calcium ions on heat aggregation of whey proteins. Morr and Josephson (1968) suggested that heat aggregation of whey protein is a multireaction, the third reaction of which involves the formation of large aggregates and depends on Ca^{++} concentration. de Wit (1981) showed that the amount of calcium necessary to induce the aggregation of β-lg was equivalent to its net negative charge. This observation can be related to the suggestion of Zittle et al (1957) that Ca^{++} binding neutralized the net negative charges of whey proteins and caused isoelectric precipitation. Varunsatian et al (1983) showed that denaturation temperature of WPC, determined by DSC, significantly decreased in the presence of $CaCl_2$. When the ionic strength of Ca^{++} was as low as 0.03, the denaturation temperature of the WPC was already suppressed by 4°C.

While this study characterized flour and milk fractions by DSC, the interactions between the two groups of components are reported separately (Erdogdu et al 1995).

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LITERATURE CITED

AMERICAN ASSOCIATION OF CEREAL CHEMISTS. 1983. Approved Methods of the AACC, 8th ed. Method 08-01, approved April 1961, revised October 1981; Method 44-15A, approved October 1975, revised October 1981. The Association: St. Paul, MN.

ANONYMOUS. 1991. Production of whey and modified products. Am. Dairy Products Inst.: Chicago.

AOAC. 1990. Official Methods of Analysis of the Association of Official Analytical Chemists, 15th ed. The Association: Washington, DC.

BAKER, J. C., PARKER, H. K., and MIZE, M. D. 1943. The pentosans of wheat flour. Cereal Chem. 20:267-280.

BASSETTE, R., and ACOSTA, J. S. 1988. Composition of milk products. Pages 39-80 in: Fundamentals of Dairy Chemistry. N. Wong., R. Jeness, M. Keeney, and E. Marth, eds. Van Nostrand Reinhold: New York.

BERNAL, V., and JELEN, P. 1985. Thermal stability of whey proteins—A calorimetric study. J. Dairy Sci. 68:2847-2852.

BUSHUK, W. 1966. Distribution of water in dough and bread. Baker's Dig. 40(5):38-40.

CZUCHAJOWSKA, Z., and POMERANZ, Y. 1989. Differential scanning calorimetry, water activity, and moisture contents in crumb center and near-crust zones of bread during storage. Cereal Chem. 66:305-309.

de WIT, J. N. 1981. Structure and functional behavior of whey proteins. Neth. Milk Dairy J. 35:47-64.

de WIT, J. N., and KLARENBEEK, G. 1981. A differential scanning calorimetric study of the thermal behavior of bovine β-lactoglobulin at temperatures up to 160°C. J. Dairy Res. 48:293-302.

de WIT, J. N., KLARENBEEK, G., and ADAMSE, M. 1986. Evaluation of functional properties of whey protein concentrates and whey protein isolates. 2. Effects of processing and history of composition. Neth. Milk Dairy J. 40:41-56.

DONOVAN, J. W., LORENZ, K., and KULP, L. 1983. Differential scanning calorimetry of heat-moisture treated wheat and potato starches. Cereal Chem. 60:381-387.

EBERSTEIN, K., HÖPCKE, R., KONIECZNY-JANDA, G., and STUTE, R. 1980. DSC- Untersuchungen an Stärken. I. Möglichkeiten thermoanalytischer Methoden zur Stärkecharakterisierung. Starch/Staerke 32:397-404.

ELIASSON, A. C., and HEGG, P. O. 1980. Thermal stability of wheat gluten. Cereal Chem. 57:436-437.

ERDOGDU, N., CZUCHAJOWSKA Z., and POMERANZ, Y. 1995. Wheat flour and defatted milk fractions characterized by differential scanning calorimetry (DSC). II. DSC of interaction products. Cereal Chem. 72:76.

GHIASI, K., HOSENEY, R. C. and VARRIANO-MARSTON, E. 1982.

Gelatinization of wheat starch. III. Comparison by differential scanning calorimetry and light microscopy. Cereal Chem. 59:258-262.
GHIASI, K., HOSENEY, R. C., and VARRIANO-MARSTON, E. 1983. Effects of flour components and dough ingredients on starch gelatinization. Cereal Chem. 60:58-61.
HOSENEY, R. C., and ROGERS, D. 1990. The formation and properties of wheat flour doughs. CRC Crit. Rev. Food Sci. Nutr. 29:73-93.
HOSENEY, D. C., FINNEY, K. F., SHOGREN, M. D., and POMERANZ, Y. 1969. Functional (breadmaking) and biochemical properties of wheat flour components. III. Characterization of gluten protein fractions obtained by ultracentrifugation. Cereal Chem. 46:126-135.
HOVENKAMP-HERMELINK, J. H. M., DE VRIES, J. N., ADAMSE, P., JAKOBSEN, E., WITHOLD, B., and FEENSTRA, W. J. 1988. Rapid estimation of the amylose-amylopectin ratio in small amounts of tuber and leaf tissue of potato. Potato Res. 31:241-246.
HUGUNIN, A. G. 1987. Applications of U.F. whey protein: Developing new markets. Pages 135-144 in: Bull. 212, Trends in Whey Utilization. Intl. Dairy Fed.: Brussels, Belgium.
KHAN, K., and BUSHUK, W. 1979. Structure of wheat gluten in relation to functionality in breadmaking. Pages 191-206 In: Functionality and Protein Structure. A. Pour-El, ed. Am. Chem. Soc.: Washington, DC.
LAPANJE, S., and POKLAR, N. 1989. Calorimetric and circular dichroic studies of the thermal denaturation of β-lactoglobulin. Thermochim. Acta 34:155-162.
LEMAN, J., and KINSELLA, J. E. 1989. Surface activity, film formation, and emulsifying properties of milk proteins. CRC Crit. Rev. Food Sci. Human Nutr. 28:115-138.
LINEBACK, D. R., and RASPER, V. F. 1988. Wheat carbohydrates. Pages 277-372 in: Wheat Chemistry and Technology, Vol. 2. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.
LIU, H., LELIEVRE, J., and AYOUNG-CHEE, W. 1991. A study of starch gelatinization using differential scanning calorimetry, X-ray, and birefringence measurements. Carbohydr. Res. 210:79-87.
MARSHALL, K. R. 1982. Industrial isolation of milk proteins: Whey proteins. Developments in Dairy Chemistry, Vol. 1. P. F. Fox, ed. Applied Science Publ.: London.
McKENZIE, H. A. 1971. Whole casein: Isolation, properties, and zone electrophoresis. Page 87 in: Milk Proteins. Chemistry and Molecular Biology, Vol. 2. H. A. McKenzie, ed. Academic Press: New York.
MORR, C. V. 1984. Production and use of milk proteins in food. Food Technol. 38(7):39, 40-42, 44, 46-48.
MORR, C. V., and FOEGEDING, A. E. 1990. Composition and functionality of commercial whey and milk protein concentrates and isolates. A status report. Food Technol. 44(4):100, 102-104, 106, 108, 110, 112.
MORR, C. V., and JOSEPHSON, R. V. 1968. Effect of calcium, *N*-ethylmaleimide and casein upon heat-induced whey protein aggregation. J. Dairy Sci. 51:1349-1355.
MÜNZING, K. 1989. Demonstration of changes in structure in cereal starches by heat flux DSC. Thermochim. Acta 151:57-62.
MÜNZING, K. 1991. DSC studies of starch in cereal and cereal products. Thermochim. Acta 193:441-448.
PAULSSON, M., and DEJMEK, P. 1990. Thermal denaturation of whey proteins in mixtures with caseins studied by differential scanning calorimetry. J. Dairy Sci. 73:590-600.
POMERANZ, Y. 1988. Composition and functionality of wheat flour components. Pages 231-241 in: Wheat Chemistry and Technology, Vol. 2. Y. Pomeranz, ed. Am. Assoc. Cereal Chem.: St. Paul, MN.
RELKIN, P., and LAUNAY, B. 1990. Concentration effects on the kinetics of β-lactoglobulin heat denaturation: A differential scanning calorimetric study. Food Hydrocolloids 4:19-32.
RELKIN, P., EYNARD, L., and LAUNAY, B. 1992. Thermodynamic parameters of β-lactoglobulin and α-lactalbumin. A DSC study of denaturation by heating. Thermochim. Acta. 204:111-121.
RUSSELL, P. 1987a. Gelatinization of starches of different amylose-amylopectin content. A study by differential scanning calorimetry. J. Cereal Sci. 6:133-145.
RUSSELL, P. 1987b. The aging of gels from starches of different amylose-amylopectin content studied by differential scanning calorimetry. J. Cereal Sci. 6:147-158.
SAS. 1985. SAS User's Guide: Statistics. The Institute: Cary, NC.
SCHMIDT, R. H., PACKARD, V. P., and MORRIS, H. A. 1984. Effect of processing on whey protein functionality. J. Dairy Sci. 67:2723-2733.
SCHOFIELD, J. D, BOTTOMLEY, R. C., LEGRYS, G. A., TIMMS, M. F., and BOOTH, M. R. 1984. Effects of heat on wheat gluten. Pages 81-90 in: Proc. Int. Workshop on Gluten Proteins, 2nd. A. Graveland and J. M. E. Moonen, eds. TNO: Wageningen, The Netherlands.
SIEVERT, D., and POMERANZ, Y. 1990. Enzyme resistant starch. I. Characterization and evaluation by enzymatic, thermoanalytical, and microscopic methods. Cereal Chem. 66:342-347.
SPIES, R. D., and HOSENEY, R. C. 1982. Effects of sugars on starch gelatinization. Cereal Chem. 59:128-131.
VARUNSATIAN, S., WATANABE, K., HAYAKAWA, S., and NAKAMURA, R. 1983. Effects of Ca^{++}, Mg^{++}, and Na+ on heat aggregation of whey protein concentrates. J. Food Sci. 48:42-48.
XIONG, Y. 1992. Influence of pH and ionic environment on thermal aggregation of whey proteins. J. Agric. Food Chem. 40:380-384.
ZITTLE, C. A., DELLAMONICA, E. S., RUDD, R. K., and CUSTAR, C. H. 1957. The binding of calcium ions by β-lactoglobulin both before and after aggregation by heating in the presence of calcium ions. J. Am. Chem. Soc. 79:4461-4467.

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