Functionality of Whey and Casein in Fermentation and in Breadbaking by Fixed and Optimized Procedures

N. ERDOGDU-ARNOCZKY, Z. CZUCHAJOWSKA,[1] and Y. POMERANZ

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ABSTRACT

The effects of 4% dairy ingredients on dough absorption and mixing time, parameters of fermentation, loaf volume, and bread characteristics were determined. Dairy ingredients, generally, increased water absorption and decreased mixing time. The decrease in mixing time was to some extent reversed by heat treatment (at 80 or 95°C) of nonfat dry milk (NFDM), casein, or whey. Dialysis of whey did not improve its poor mixing stability. Untreated dairy ingredients lowered the dough height at maximum development time (H_m, measured by the Rheofermentometer). The drop was reversed by heat treatment or dialysis. H_m was positively correlated ($r = 0.87$) with time of H_m (T_1) and negatively correlated ($r = -0.88$) with drop in volume after 2 hr. Caseins drastically reduced the loaf volume of bread baked in the bread machine; heat treatment of the caseins counteracted the loss. Heat-treated acid whey protein increased the loaf volume and lowered the rate of staling, as measured by universal testing machine (UTM) crumb firmness measurements and differential scanning calorimetry enthalpy changes. In bread baked by the optimized procedure, heat treatment alone, or in combination with dialysis, counteracted the deleterious effects of adding nontreated whey protein, but not of caseins. Baking performance could be predicted by the Rheofermentometer time of maximum gas formation. Heat-treated whey proteins lowered the rate of staling in optimally baked bread as in bread baked by the fixed formula.

Dairy ingredients such as nonfat dry milk (NFDM), whey, and casein are widely used in the preparation of bakery products. The nutritional, organoleptic, and some functional properties of bread enriched by dairy products are improved. Increased water absorption, reduced staling rate, and increased crust color are some of the advantages of dairy ingredients in breadbaking (Dubois and Dreese 1984). On the other hand, dough slackening and volume-depressing effects with nonheated dairy fractions have been reported frequently. The performance of dairy ingredients in baking has been the subject of many publications, and almost every milk fraction has been described as loaf volume-depressing. Such fractions include whey proteins (powders or concentrates), casein, and lactose (Harland et al 1943, Larsen et al 1949, Ashword and Kruger 1951, Gordon et al 1953, Swanson and Sanderson 1967, Zadow 1981, Harper and Zadow 1984).

In the context of dairy ingredient performance in baking, several conclusions emerge from the published data. First, the functionality of a dairy ingredient or product is related to its individual components and to its production conditions (Claypool 1984). Second, the interaction patterns between dairy and wheat flour components depend on the dairy fractionation method and wheat flour characteristics. Third, some baking parameters such as mixing time and water absorption govern the performance of a particular dairy ingredient in the final product. Dairy products, such as NFDM, caseins, and whey protein powders or concentrates vary in overall composition and the extent of denaturation. Those variations in starting materials combined with variations in functionality and performance in different foods make it difficult to categorize the dairy products in a simple and straightforward manner (Kinsella 1984).

The complexity of the breadmaking system, including several stages of processing and interaction among the components, makes it difficult to predict the performance of a particular dairy product based on its behavior in a model system. Thus, for example, the performance of a dairy ingredient may vary with flour composition and strength, presence of additives, breadmaking system (straight or sponge; fixed or optimized), and tested parameter (mixing tolerance, fermentation requirements, oxidation requirements, loaf volume, and crumb grain firmness).

In a previously reported model system (Erdogdu et al 1995a,b), we found that, as assessed by differential scanning calorimetry (DSC), dialyzed acid whey powder and commercial acid whey protein concentrate interacted with wheat flour components in a similar manner. Dialysis, rather than heat treatment, caused the most pronounced changes in the interaction patterns of acid whey powder (AWP) with wheat flour components. In this study, we investigated the effects of whey powder, casein, and NFDM on physical dough properties of nonyeasted doughs (by the mixograph), on fermentation parameters of yeasted doughs (by the Rheofermentometer), and on breadmaking in fixed (bread machine) and optimized (100-g flour pup loaf) system (AACC 1983).

MATERIALS AND METHODS

NFDM, AWP, and acid casein (AC) were prepared from fresh raw milk. Milk from the Washington State University Creamery contained 3.9% fat, 3.2% protein, 4.8% lactose, 0.8% ash, and 87.8% water. Milkfat was removed by centrifugation at $3,000 \times g$ for 30 min (4°C). Casein was separated from whey by acid precipitation at pH 4.6 (20°C) (McKenzie 1971). NFDM, AWP, and AC were heat-treated at 80 or 95°C for 10 min. Dialysis and/or heat treatment were included in preparation of laboratory acid whey to simulate preparation of commercial whey protein concentrate (CWPC). The fresh AWP was dialyzed against distilled water at 4°C for 36 hr. Dialyzed AWP (DAWP) was heat-treated at 80°C for 10 min. All fractions were frozen, freeze-dried, and ground to pass a 0.25-mm (round openings) sieve, and kept at 4°C for further analysis (Table I).

Commercial acid whey protein concentrate (CAWPC), a by-product of cottage cheese production, was obtained from Main Street Ltd. (La Crosse, WI). Commercial acid casein (CAC) was obtained from New Zealand Milk Products, Inc. (Santa Rosa, CA). Lactose (CL) was obtained from Sigma Chemical Co. (St. Louis, MO).

Commercial AWP contains 11–15% protein, 7.1–12.5% ash, 61–75% lactose, and 3.5–7.5% water (Hugunin 1987). Commercial WPC with 35% protein is "perceived" to be a universal substitute for NFDM because of the similarity in gross composition and its dairy character (Hugunin 1987). Dried WPC are usually

[1]Washington State University, Dept. Food Science and Human Nutrition, Pullman, WA 99164-6376. Corresponding author. E-mail: zuza@wsuvmi.csc.wsu.edu

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ultrafiltered to increase the protein content from 13 to 30% or more; at the same time, ash and lactose concentration is reduced proportionately. WPC can contain 36–83% protein, <7% ash, <60% lactose, and 3–4% water. The compositional characteristics of the dairy fractions of this study are in agreement with these specifications (Table I). The laboratory-dialyzed AWP and CAWCP were similar in composition. The difference in the ash contents of AWP and AC was due to the modification of the casein structure by acidic pH. In the native state, casein structure is associated with colloidal calcium. The acid treatment disrupts the native structure of the casein and casein micelles. Thus, adjustment to pH 4.6 dissociates the colloidal calcium phosphate structure, solubilizes the calcium, and, consequently, the casein precipitate contains only low amounts of calcium (Leman and Kinsella 1989). Laboratory-prepared AC was higher than commercial acid casein in its lactose contents.

Commercial bread flour was obtained from Fisher Milling Co. (Seattle, WA). Protein content was 14.7% (N × 5.70); ash content was 0.83% (water-free basis); water absorption was 65% (14% mb).

Analytical Methods

Moisture and ash of dairy ingredients were determined according to AOAC methods 44-16.212 and 16.216, respectively (AOAC 1984). Flour moisture and ash were determined according to AACC approved methods 44-16 and 08-01, respectively (AACC 1983). Protein in milk fractions (N × 6.38) and flour (N × 5.70) were determined with a nitrogen analyzer (Leco Corp. St. Joseph, MI). Moisture content of the bread crumb was determined as weight loss after drying in a forced-draft oven at 130°C for 4 hr. Bread crumb water activity was measured by the CX-1 Water Activity System (Decagon Devices Inc., Pullman, WA).

DSC Measurements

DSC measurements (DSC-2, Perkin-Elmer Corp., Norwalk, CT) were done according to Czuchajowska and Pomeranz (1989).

Dough Mixing Properties

Dough mixing properties and water absorption were determined by the method of Finney and Shogren (1972) for doughs in which 4% dairy product (flour basis) was added to the flour.

Rheofermentometer Gas Formation and Retention

Gas formation and retention in fermenting doughs were determined according to the procedure of Czuchajowska and Pomeranz (1993a,b) using a Rheofermentometer F1 (Tripette & Renaud, Tans, France). Yeasted dough was prepared with 4% dairy ingredient, 6% sugar, 3% shortening, 1.8% salt, and 1.8% instant yeast in 200 g of flour (14% mb). An optimum amount of water, based on mixograph data, was added. The dough was mixed to optimum in a Hobart mixer, and 200 g of dough was placed in the Rheofermentometer vat. The dough was tested under 1,746-g weight constraint at 30°C (±1) for 2 hr. The Rheofermentometer simultaneously measured and recorded the parameters related to dough development, gas production, and gas retention. Measured Rheofermentometer parameters included: H_m = height (mm) under constraint of dough at maximum development time; T_1 = time (hr) of H_m; dough volume loss = drop in the dough volume (%) at the end of the test (2 hr); T_1' = time of maximum gas formation (hr); TG = total gas production in the dough (ml); gas retention = volume of the gas retained in the dough at the end of the test.

TABLE I
Protein, Ash, Lactose Content, and pH of Investigated Dairy Products[a]

Blends[b]	Protein[c]	Ash	Lactose[d]	pH
NFDM (1,2)	34.62	7.02	58.3	6.6
AWP (1,2)	13.49	10.65	75.9	4.6
DAWP (1)	33.80	5.92	60.3	4.6
CAWPC	36.10	7.11	53.5	4.6
AC (1,2)	74.64	3.71	21.7	4.6
CAC	96.90	2.00	0.1	4.6
CL	0.00	0.00	99.9	. . .

[a] % water-free basis.
[b] NFDM = nonfat dry milk; AWP = acid whey powder; D = dialyzed; AC = acid casein; CAWPC = commercial acid whey protein concentrate; CAC = commercial acid casein; CL = commercial lactose; (1,2) = heat-treated at 80 or 95°C.
[c] N × 6.38.
[d] Calculated by difference.

Breadmaking

Fixed procedure. A bread machine, (model ABM350, Welbilt Appliance Inc., New York) was used for the fixed procedure baking experiment. The bread formula included (fwb): 4% dairy ingredient, 6% sugar, 1.8% salt, 1.8% shortening, and 1.2% yeast. All ingredients were dry blended with 200 g of flour (14% mb) in the bread machine. A constant amount of water was added (65% at 30°C), based on the mixograph data of the test flour. The baking program of the machine included kneading for 30 min, rising for 75 min, and baking for 30 min. Temperature was controlled by the settings of the machine throughout baking (Czuchajowska and Pomeranz 1993a,b). Bread weight and volume by dwarf rapeseed replacement were determined immediately after baking. Loaves were cooled to room temperature (21 ± 2°C) for 1 hr, double-packed in polyethylene bags, and stored for six days at room temperature. All subsequent measurements were taken on the central bread crumb. Water activity, moisture, universal testing machine (UTM, Instron), and DSC measurements were made 1 hr and six days after baking. The force (N) required to compress 1 cm of a slice 7-cm thick was determined with a UTM probe (0.5 in. dia.) at 1 mm/sec.

Optimized procedure. Bread (100-g flour pup loaf) (14% mb) was also baked by the optimized straight-dough method (AACC 1983). The full bread formula included malt plus the same ingredients and amounts that were used in the Rheofermentometer experiment. An optimum amount of water was added, and the dough was mixed to an optimum. The straight-dough procedure involved 90 min of fermentation, 36 min of proofing at 20°C, and 24 min of baking at 218°C. Three punchings and sheetings followed by molding and panning took place before baking. The proof height was determined at the end of fermentation. Immediately after baking, each loaf was weighed and the volume was measured by dwarf rapeseed displacement. Bread texture was determined 1 hr and 72 hr after baking with a UTM probe (0.5 in. dia.) at 1 mm/sec (slice 2.5 in. thick representing half of a loaf). Loaves cooled to 21 ± 2°C (room temperature) were packed in plastic wrap and sealed for storage.

Statistical Analysis

Determinations were made in at least two replicates and averaged. Data were analyzed using the Statistical Analysis System (SAS 1985).

RESULTS AND DISCUSSION

Physical Dough Properties Determined by Mixograph

Mixograph patterns of the doughs are shown in Figure 1. Mixograph mixing times and absorption levels and optimum bake mixing times are compared in Table II.

NFDM and caseins, independent of heat treatment, required higher water absorption than did the control. Dough prepared with AWP required 1% more water than did the control when heated at 80°C and 2% more when heated at 95°C.

All dairy ingredients, except for lactose, shortened the mixograph mixing times of the dough. Heating and dialysis of dairy fractions increased mixing times, compared to the nontreated forms. Optimum bake mixing times, judged by an experienced baker, were linearly correlated to mixograph mixing times (r = 0.83).

The addition of untreated NFDM, and especially AWP, reduced the mixing time. Heat treatment increased mixing time and improved dough stability beyond the point of minimum mobility (Skovholt and Bailey 1931, 1932, 1938; Eisenberg 1940; Larsen et al 1949, 1951; Gordon et al 1953, 1954; Harper and Zadow 1984).

An analysis of the mixograph data indicated some trends. The nonheated NFDM, and especially AWP, contained ingredients that reduced the mixing tolerance. The mixing tolerance of the dough was restored to that of the control by heat treatment of the NFDM at 80°C. Heat treatment of the NFDM at 95°C did not result in further improvement. The mixing tolerance of AWP, however, was restored to the mixing tolerance of the control flour in part only by heat treatment at 80 and 95°C. Volpe and Zabik (1975) postulated that the reduced mixing tolerance was a function of uncomplexed β-lactoglobulin (whey protein) and κ-casein (casein subunit) in nonheated milk. The κ-casein and β-lactoglobulin, when heated together, interact through disulfide linkages (Morr et al 1962, Morr 1965, Sawyer 1969). This interaction occurs over a narrow pH range of 6.7–7.0 (de Wit 1981) and at 85–90°C (Smits and Brouwershaven 1980). Complex formation is favored by calcium salts. This interaction is a possible explanation for the better mixing tolerance after addition of heated NFDM that contains all milk proteins at a neutral pH, rather than of AWP, which contains only whey proteins at acidic pH.

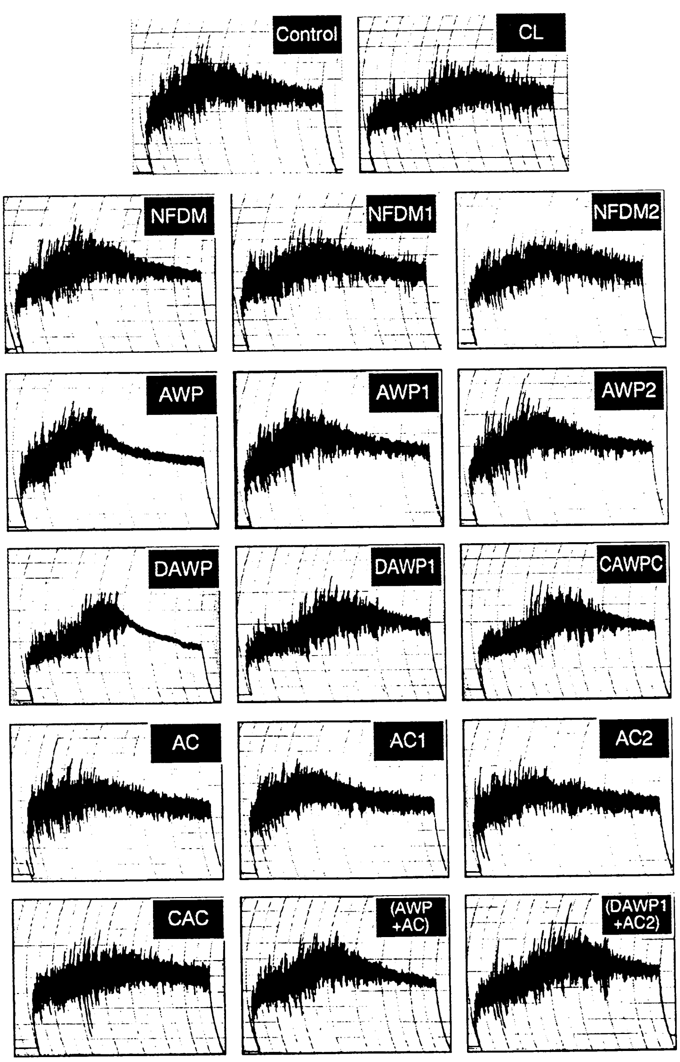

Fig. 1. Mixograph patterns of control flour and flour with 4% dairy ingredient. C = control; CL = commercial lactose; NFDM = nonfat dry milk; AWP = acid whey powder; D = dialyzed; CAWPC = commercial acid whey protein concentrate; AC = acid casein; CAC = commercial acid casein; 1 = heated at 80°C; 2 = heated at 95°C.

The commercial AWPC reduced the mixing tolerance slightly (Fig. 1). Heated-dialyzed laboratory DAWP1 produced a mixograph curve that was practically identical to that for CAWPC. Dialysis of nonheated AWP did not improve the poor mixing tolerance. This observation indicates that heating is the essential factor in improving the mixing properties of whey preparations.

Caseins, being insoluble, showed higher water absorption requirements than nonheated wheys. Unlike nonheated NFDM and AWP, caseins did not impair the mixing tolerance of the control flour. The factor that reduces the mixing tolerance, apparently, was absent in caseins. To test this hypothesis, we asked whether AC can counteract, totally or in part, the deleterious effect of AWP on mixing tolerance. Mixtures (1:1) of nonheated AWP+AC and heated DAWP1+AC2, representing the worst and best combinations, respectively, were tested in the mixograph (Fig. 1). Mixograph curves of the mixtures showed that caseins improved the mixing tolerance of wheys significantly either due to casein-whey protein interactions (at room temperature from the introduced mixing energy) or as a result of a dilution effect.

Overmixing of a dough containing nonheated NFDM or AWP for 1 min impaired the dough-handling properties drastically. Volume of bread made from such an overmixed dough was depressed by 20% compared to bread from doughs mixed to optimum (data not reported here). To produce an optimum loaf of bread, dough must be mixed to the peak of the mixing curve (Pomeranz 1987, 1988). The time required to reach this peak varied depending on the type of ingredients. As mixing proceeds, flour becomes hydrated. The resistance to mixing increases, until all the flour is hydrated. Clearly, if flour is not hydrated it cannot be expected to contribute to the properties of the dough (Hoseney and Rogers 1990). Mixing of dough past the optimum lead to different results, depending on the ingredient being mixed with the control flour. Caseins and heated NFDM and AWP resisted overmixing, and the mixing curves remained at the peak with

TABLE II
Mixograph and Baking Characteristics of Doughs Mixed with 4% Dairy Ingredients

	Mixograph		Baking
Blends[a]	Absorption (%)	Mixing Time (min)	Mixing Time (min)
Control	65.0	3:25	3:26
+NFDM	67.0	3:00	2:57
+NFDM1	67.0	3:15	3:22
+NFDM2	67.0	3:15	3:35
+AWP	65.0	2:30	2:31
+AWP1	66.0	2:50	2:55
+AWP2	67.0	3:00	3:06
+DAWP	65.0	3:15	3:15
+DAWP1	66.0	3:15	3:17
+CAWPC	66.0	3:15	3:14
+AC	66.0	2:15	2:13
+AC1	66.0	2:30	2:28
+AC2	66.0	2:45	2:46
+CAC	67.5	3:00	3:39
+CL	64.0	3:25	3:26
LSD[b]	0.0	0:05	0:07

[a] NFDM = nonfat dry milk; AWP = acid whey powder; D = dialyzed; AC = acid casein; CAWPC = commercial acid whey protein concentrate; CAC = commercial acid casein; CL = commercial lactose; (1,2) = heat-treated at 80 or 95°C.

[b] Least significant difference at $P < 0.05$.

little apparent change. Nontreated NFDM and AWP underwent rapid and severe change.

A complete and clear understanding of the overmixing phenomenon is still elusive. The fact that doughs mixed in a nitrogen atmosphere or under vacuum do not overmix infers that overmixing is an oxidation process (Hoseney and Rogers 1990). An effect of activated double-bond compounds on the reduced mixing tolerance has been reported by Jackson and Hoseney (1986). They also showed the same factor in gluten- and starch-controlled mixing tolerance. The nature of this factor has not been determined yet. Apparently, a heat-denaturable factor of NFDM and AWP is involved in factors or reactions that reduce the mixing tolerance.

TABLE III
Rheofermentometer Data[a] for Development of Yeasted Dough Prepared with 4% Dairy Ingredients

Blends[b]	H_m (mm)	T_1 (hr)	Vol. Loss (%)
Control	52.8	1.29	14.0
+NFDM	35.5	1.19	82.5
+NFDM1	48.1	1.20	30.4
+NFDM2	55.2	1.32	13.8
+AWP	41.8	1.05	62.8
+AWP1	53.1	1.26	26.2
+AWP2	58.7	1.34	12.6
+DAWP	50.4	1.21	40.2
+DAWP1	70.0	1.48	2.8
+CAWPC	69.6	1.58	9.7
+(AWP+AC)	47.6	1.27	26.0
+(DAWP1+AC2)	52.8	1.39	14.3
+AC	39.0	1.23	50.5
+AC1	47.7	1.23	25.6
+AC2	52.4	1.32	15.2
+CAC	60.7	1.41	5.8
+CL	53.8	1.37	8.6
LSD[c]	5.8	0.13	9.3

[a] H_m = height (mm) under constraint of dough at maximum development time; T_1 = time (hr) of H_m; volume loss = drop in dough volume at the end of 2 hr.
[b] NFDM = nonfat dry milk; AWP = acid whey powder; D = dialyzed; AC = acid casein; CAWPC = commercial acid whey protein concentrate; CAC = commercial acid casein; CL = commercial lactose; (1,2) = heat-treated at 80 or 95°C.
[c] Least significant difference at $P < 0.05$.

TABLE IV
Rheofermentometer Data for Gas Production and Retention of Yeasted Dough Prepared with 4% Dairy Ingredients

Blends[a]	Maximum Gas Formation (hr)	Total Gas Production (ml)	Gas Retention (%)
Control	1.17	1,451	83.2
+NFDM	0.51	1,532	78.1
+NFDM1	1.17	1,534	79.2
+NFDM2	1.17	1,539	81.3
+AWP	0.54	1,496	79.5
+DAWP	1.23	1,461	84.4
+AWP1	1.17	1,506	82.3
+AWP2	1.06	1,494	84.6
+DAWP1	1.06	1,476	84.5
+CAWPC	1.12	1,475	86.6
+(AWP+AC)	1.06	1,420	84.6
+(DAWP1+AC2)	1.00	1,455	86.9
+AC	0.48	1,546	77.1
+AC1	0.55	1,544	80.6
+AC2	0.54	1,547	82.6
+CAC	1.20	1,476	83.4
+CL	2.07	1,446	85.5
LSD[b]	0.12	46.3	3.15

[a] NFDM = nonfat dry milk; AWP = acid whey powder; D = dialyzed; AC = acid casein; CAWPC = commercial acid whey protein concentrate; CAC = commercial acid casein; CL = commercial lactose; (1,2) = heat-treated at 80 or 95°C.
[b] Least significant difference at $P < 0.05$.

Physical Dough Properties Determined by Rheofermentometer

The effect of heat treatment on dough development and on gas production-retention as measured by the Rheofermentometer is summarized in Tables III and IV, respectively. Figures 2–4 illustrate the effect of heat treatment on the development of doughs prepared with NFDM, AWP, and AC, respectively. Addition of untreated ingredients depressed the dough volume (H_m). H_m increased after heat treatment of NFDM, AWP, or AC (Table III). Controls and doughs that contained heated or dialyzed ingredients, unlike those with untreated ones, maintained the fermentation volume to the end of the test (T_1). Moreover, volume losses decreased from 14.0% in the control to 2.8% in DAWP1 (Table III). The time of maximum dough development (T_1) generally increased as H_m increased for doughs that contained NFDM or wheys. Adding heat-treated caseins, however, had no consistent effects on T_1 values.

The addition of milk ingredients, per se, independent of the heat treatment, had a relatively small effect on total gas production (Table IV). Dough prepared with NFDM and AC showed higher gas production rates than those with wheys. The incorporation of whey solids did not affect the volume of CO_2 produced during proofing of doughs. On the other hand, heat treatment and dialysis of the untreated ingredients improved the amount of gas retained in the dough at the end of the test.

The times of maximum gas formation were also the starting points of gas escape from the dough (Table IV). Those times were significantly shorter with lab-caseins (ave. 0.52) and nonheated NFDM and AWP (ave. 0.53) than with the control (1.17). Shortened gas formation times with nonheated NFDM, AWP, and AC are indicative of a weakened dough structure. In other words, these doughs ruptured earlier than the control dough during the fermentation period (under the weight constraint). Gas retention

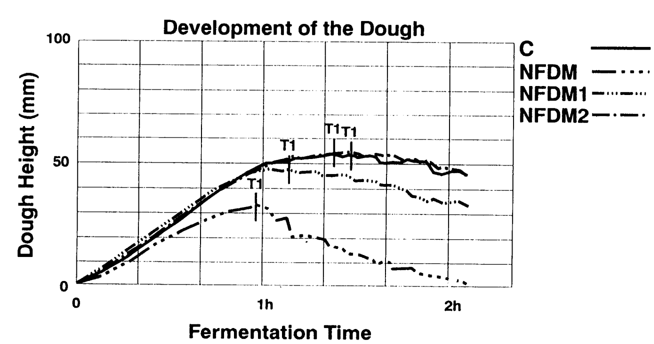

Fig. 2. Dough development in the Rheofermentometer for control (C) plus 4% nonfat dry milk (NFDM) heated at 80°C (1) or 95°C (2).

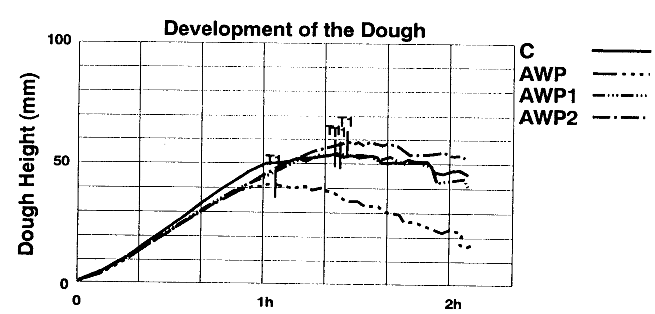

Fig. 3. Dough development in the Rheofermentometer for control (C) plus 4% acid whey powder (AWP) heated at 80°C (1) or 95°C (2).

depends on the ability of dough to be stretched into thin membranes and is attributed to its protein phase (Bloksma and Bushuk 1988). During fermentation, CO_2 and ethanol produced by yeast are lost by diffusion to the external surface of the dough followed by evaporation and rupture of dough membranes at this surface. Comparisons of gas production in fermenting doughs and their volume expansion show that the loss is slow for ≈1 or 2 hr and then accelerates rapidly. The slow initial loss can be explained by diffusion and evaporation, and the rapid increase by rupture of membranes. Under normal bakery conditions, the stage of rapid loss is not reached before baking. This is not true for the Rheofermentometer, in which the loss of gas begins earlier as a result of the mechanical stress of the tested dough. Therefore, gas formation time in the Rheofermentometer indicates the ability of the dough to expand during baking. A long time interval before the rapid release of CO_2 is positively correlated with a long oven rise and with a large loaf volume. Therefore, we can tentatively postulate that different milk solids interact with gluten in different manners and most probably determine the numbers and sizes of gas cells in the dough. Such a mechanism implies that, unlike other ingredients used, caseins possess a volume-depressing factor that cannot be denatured-inactivated by heat treatment. Dough prepared with caseins showed patterns of dough development and gas production that were similar to that of NFDM, but the time of maximum gas formation in AC1 or AC2 did not increase by heating and was significantly lower than in doughs with treated NFDM and wheys (Tables III and IV). The low H_m (Table III) and times of maximum gas formation (Table IV) of AWP (41.8 mm, 0.54 hr) were increased by dialysis (to 50.4 mm and 1.23 hr) and by a combination of dialysis and heat treatment (to 70.0 mm, and 1.06 hr); the latter values were similar to those for CAWPC (Fig. 5; Tables III and IV). The linear correlation coefficients of H_m with percent volume loss were $r = -0.88$, with T_1 $r = 0.87$, and with baking proof height $r = 0.70$. These results prompted us to test whether blends of whey proteins with caseins would improve

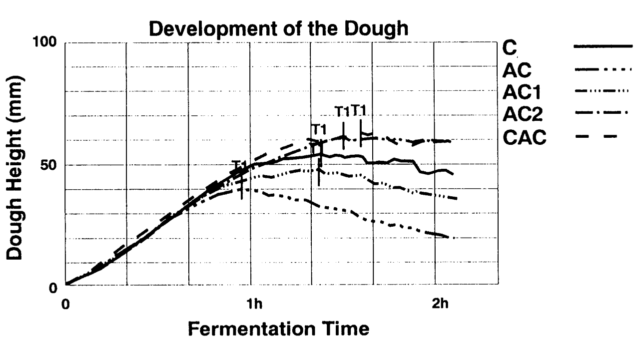

Fig. 4. Dough development in the Rheofermentometer for control (C) plus 4% acid casein (AC) heated at 80°C (1) and commercial acid casein (CAC).

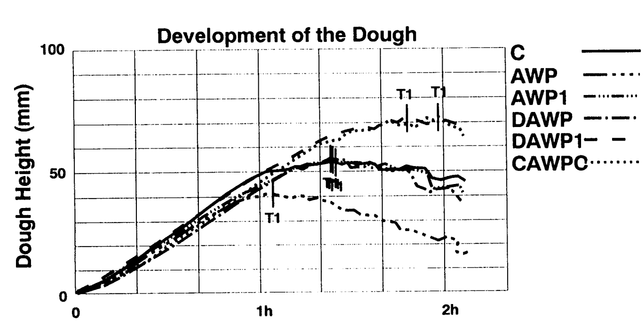

Fig. 5. Dough development in the Rheofermentometer for control (C) plus 4% acid whey powder (AWP) heated at 80°C (1) and commercial acid whey protein concentrate (CAWPC).

the performance of caseins. For this purpose, 1:1 mixtures of caseins and wheys, representing the worst (AWP+AC) and best (DAWP1+AC2) combinations, were tested in the Rheofermentometer. Nonheated casein lowered the volume loss of nonheated AWP from 63 to 26% (Table III) and wheys improved the time of maximum gas formation of the dough made with casein from 0.52 hr to 1.06 hr (Table IV).

Breadmaking: Fixed Procedure

An average loaf of bread weighed ≈336 g, the central crumb contained 43.7% moisture with a water activity of 0.96. Neither the bread weight nor the crumb moisture nor its water activity were significantly affected by the addition of various dairy ingredients or storage for up to six days.

The volume and firmness of the breads varied depending on the type of the dairy fraction included in the formula (Table V). Casein and whey fractions exerted opposite effects. Both SC and AC drastically reduced the loaf volume of the control; heat treatment counteracted the loss, especially for AC. The most pronounced increase in bread volume was observed when AWP, especially its heated form, was incorporated. SWP did not affect the volume. Whey powders did not exert the reported volume-depressing effect (Harland et al 1943; Larsen et al 1949, 1951; Gordon et al 1953; Zadow 1981). Note that Ashword and Kruger (1951) and Harper and Zadow (1984) reported that heating AWP increased loaf volume. A similar effect is shown in Table V.

There have been conflicting reports about the effect of dairy fractions on bread quality. The conflicting results may have resulted, in part at least, from different interactions between milk and wheat flour components. Those interactions differed with the composition of the dairy ingredients, which in turn is governed by the preparation method used.

The kind of baking method used is another factor affecting functionality of dairy fractions. For example, in this part of the study, water absorptions and mixing times were fixed on the basis of the mixograph data and schedule of the automated bread machine, respectively. No adjustments were made for the dairy fractions. In conventional breadmaking, mixing of a dough and its water absorption are critical parameters that influence overall bread quality (Pomeranz 1987). Variations in water activity due to the addition of water-soluble or insoluble materials (e.g., whey or caseins) affect physical dough properties and may result in softening or toughening of the dough (Bloksma and Bushuk 1988). Furthermore, Shelton and D'Appolonia (1985) suggested that wheat starch granules interact with several dough components depending on the water activity and dough development. Those interactions may involve specific and different proteins.

TABLE V
Characteristics[a] of Bread Prepared with Dairy Ingredients in the Bread Machine Under Fixed Conditions

Blends[b]	Vol. (cm^3)	PF_1 (N)[a]	PF_2 (N)	ΔPF (N)
Control	1,250	0.83	2.48	1.65
+AWP	1,433	0.68	1.68	1.68
+AWP1	1,543	0.75	1.95	1.95
+SWP	1,255	1.01	1.70	0.69
+SWP1	1,193	1.10	1.98	1.28
+AC	898	2.50	2.59	0.09
+AC1	1,325	2.62	2.60	0.02
+SC	908	2.71	2.46	0.25
+SC1	1,120	2.72	2.42	0.30
+Lactose	1,398	0.76	2.46	1.70
LSD[c]	124	0.43	0.74	...

[a] $PF_{1,2}$ = UTM peak force 1 and 6 days after baking, respectively. ΔPF = difference between PF_1 and PF_2.
[b] AWP = acid whey powder; AC = acid casein; SWP = sweet whey powder; SC = sweet casein; 1 = heat-treated at 80°C for 10 min.
[c] Least significant difference at $P < 0.05$.

Some water-soluble components may reduce the contact points in the continuous phase, which in turn may change the physical properties of the dough. The rheological properties of a dough can vary widely, depending not only on the inherent properties of a flour, but also on the extent of dough development and water activity of the system (Bloksma and Bushuk 1988). Consequently, loaf volume, crumb grain, and rate of staling may differ in bread produced under fixed conditions (as in the bread machine) or under optimized conditions.

Staling Changes

The results of the DSC study are summarized in Table VI. Freshly baked breads showed a DSC endotherm at ≈115°C. The size of this endotherm did not change consistently with storage. After storage for six days, a second endotherm appeared in the DSC thermograms at ≈65°C. Russell (1983) and Czuchajowska and Pomeranz (1989) attributed the endotherm at 115°C to the melting of an amylose-lipid complex and the endotherm at 65°C to starch retrogradation. AC or SWP did not affect the transition enthalpy of the retrogradation endotherm significantly. AWP and its heated form caused a significant reduction. This indicates that AWP, independent of heat treatment, could decrease the rate of bread staling. In assessment of the staling rate, both DSC data (enthalpy of the retrogradation) and UTM firmness measurements taken after storage (Tables V and VI) were in good agreement. The addition of AWP resulted in the lowest bread crumb firmness values before and after storage.

Breadmaking: Optimized Procedure

According to the mixograph results (Fig. 1), exceedingly high dough mixing times in the presence of nonheated NFDM and AWP were detrimental. As stated before, overmixing for 1 min of a dough containing nonheated NFDM or AWP impaired the dough-handling properties drastically. Volume of bread made from such an overmixed dough was depressed by 20% compared to bread from doughs mixed to optimum (data not reported here). Consequently, baking tests in this part of the study were for bread from optimally mixed doughs.

Generally, heat treatment of dairy fractions increased dough mixing times. Adding laboratory caseins shortened mixing times, with some increase after heat treatment (Table II).

The results of baking experiments (Table VII) are in general agreement with mixograph and Rheofermentometer data (Tables II–IV). Thus, heat treatment of all dairy ingredients resulted in larger loaf volumes. For instance, the loaf volume was larger for NFDM1 and AWP1 than for their nonheated counterparts. DAWP1 yielded loaf volume similar to that of CAWPC. The low bread volume of loaves containing AWP was improved by dialysis as well as by heat treatment of AWP and restored, after heat and dialysis were combined, to the volume of CAWPC. The effect of dialysis and heat treatment of the wheys on bread volume is possibly a function of the calcium content of the whey fractions. Nondialyzed AWP is high in minerals, especially in calcium (Leman and Kinsella 1989). DAWP is significantly lower in ash and lactose but higher in protein than the AWP (Table I). When the protein concentration is increased by dialysis (ultrafiltration), the mineral content is reduced. The status and amount of the minerals in the whey preparations depend on the isolation and concentration methods used (Hugunin 1987). For instance, whey obtained by acid precipitation contains more minerals than rennet whey. Ionic calcium, at a level of 0.002*M*, comprises <10% of the total calcium in milk, while the bulk of the calcium is retained in nondissociated complexes with the phosphate, citrate, and protein components. Under the influence of increasing hydrogen ion concentration, calcium phosphate progressively dissociates, and at a pH value of 5.2, practically all the calcium and phosphate of casein is soluble. The mineral content of commercial WPCs ranges between 0.8 and 12% and influences the dough formation properties of gluten proteins. Inclusion of ≈2% sodium chloride in bread formulations increases dough rigidity. However, different ions at different concentrations may improve both association or dissociation of dough components and change the properties of dough and bread. The low charge density on the surface of the gluten proteins combined with hydrophobic areas and abundance of residues capable of forming hydrogen bonds (both hydrophobic interactions and hydrogen bonds could maintain an interaction once formed) result in a high sensitivity to salt concentration (Bernardin 1978). Even a low (0.005*M* NaCl) salt concentration effectively masks the repulsion between gluten molecules of like charge. The effect of salt concentration directly follows ionic strength. For example ionic calcium, a divalent cation, is more effective than monovalent ions. Preston (1981) reported that, whereas at low salt concentrations the solubility and aggregation properties of gluten proteins are largely determined by ionic interactions, at higher salt concentrations (0.500*M*) hydrophobic interactions predominate. Holmes and Hoseney (1987) reported

TABLE VI
Differential Scanning Calorimetry of Bread Prepared with 4% Dairy Ingredients in the Bread Machine Under Fixed Conditions

Blends[b]	Amylose-Lipid Complex, ΔH[a] 1st Day	Amylose-Lipid Complex, ΔH[a] 6th Day	Retrogradation, ΔH 6th Day
Control	1.85	1.75	2.20
+AWP	1.06	1.20	1.37
+AWP1	1.52	1.27	1.15
+SWP	1.96	1.86	1.54
+SWP1	2.00	1.75	1.78
+AC	1.84	2.10	1.70
+AC1	1.83	2.10	1.85
+SC	1.75	2.00	1.82
+SC1	1.99	1.85	2.02
+Lactose	1.55	1.85	2.05
LSD[c]	0.46	0.54	0.71

[a] ΔH = enthalpy difference, J/g.
[b] AWP = acid whey powder; AC = acid casein; SWP = sweet whey powder; SC = sweet casein; 1 = heat-treated at 80°C for 10 min.
[c] Least significant difference at $P < 0.05$.

TABLE VII
Characteristics[a] of Bread Prepared with 4% Dairy Ingredients

Blends[b]	Height (cm)	Vol. (cm³)	PF_1 (N)[a]	PF_2 (N)	ΔPF (N)
Control	7.50	830	0.80	1.81	1.01
+NFDM	7.63	780	0.67	1.78	1.11
+NFDM1	7.63	830	0.73	1.68	0.95
+NFDM2	7.77	823	0.64	1.76	1.12
+AWP	7.30	797	0.73	1.81	1.08
+DAWP	7.60	838	0.73	1.80	1.07
+AWP1	7.40	841	0.63	1.74	1.11
+AWP2	7.60	827	0.58	1.61	1.03
+DAWP1	8.00	858	0.65	1.95	1.30
+CAWPC	7.85	858	0.70	1.95	1.25
+(AWP+AC)	7.80	830	0.78	1.82	1.04
+(DAWP1+AC2)	7.67	843	0.76	1.93	1.17
+AC	7.47	722	0.72	1.85	1.13
+AC1	7.53	765	0.76	1.79	1.03
+AC2	7.40	787	0.68	1.72	1.04
+CAC	7.90	808	0.79	2.32	1.53
+CL	7.65	*[c]	0.73	1.55	0.82
LSD[d]	0.20	19.9	0.09	0.19	...

[a] $PF_{1,2}$ = UTM peak force 1 and 72 hr after baking, respectively. ΔPF = difference between PF_1 and PF_2.
[b] NFDM = nonfat dry milk; AWP = acid whey powder; D = dialyzed; AC = acid casein; CAWPC = commercial acid whey protein concentrate; CAC = commercial acid casein; CL = commercial lactose; (1,2) = heat-treated at 80 or 95°C.
[c] Inconsistent, varying between 800 to 900 cm³.
[d] Least significant difference at $P < 0.05$.

that addition of NaCl to bread containing NFDM improved the loaf volume. This is most probably due to a reduced interaction between NFDM and flour proteins by charge shielding (Roach et al 1992).

Heating drives calcium phosphate into the colloidal state due to the inverse temperature-solubility property of the salt and its concentration in milk, which is close to saturation. The situation is similar with heating of raw whey: calcium is readily precipitated with the denatured serum proteins. The precipitated calcium phosphate is highly resistant to resolution upon cooling (Tumerman and Webb 1964). Reduction in the amount of ionic calcium in heated milk and whey is the most probable explanation for the improved baking characteristics of the heated ingredients.

Addition of casein reduced loaf volume. Some improvement in volume was observed as a result of heat treatment. Sanderson (1966) postulated that the volume-depressing factor in casein differs from the one affecting mixing tolerance. Our results show that AC is a volume-reducing ingredient, and its effect cannot be predicted by the mixograph and Rheofermentometer. The shorter time of maximum gas formation is the only Rheofermentometer parameter that indicated that AC is a potentially detrimental ingredient. According to the results of the DSC interaction study (Erdogdu et al 1995a,b), caseins do not seem to interact with starch. Although the interaction of casein with gluten could not be followed by DSC, we can tentatively postulate that caseins interact with a component of wheat flour other than starch, probably the gluten fraction. The large reduction in bread volume is a possible indication of casein-gluten interaction.

Swanson and Sanderson (1967) reported that adding acid-precipitated caseins caused severe loaf volume losses. We found that the loaf volume was restored up to that of the control when casein was blended with wheys (AWP+AC and DAWP1+AC2) (Table VII). This increase in loaf volume may reflect either a reduction in the amount of included casein or a more favorable interaction of caseins with whey instead of gluten. Thus, the functionality of casein, especially when unheated, can be improved by blending with whey.

Baking proof height values did not correlate well with the loaf volume ($R = 0.56$). The baking performance of NFDM and whey, but not of caseins, was predicted by the Rheofermentometer dough development data (Table III). Baking performance of all ingredients, including caseins, was predicted well by the Rheofermentometer time of maximum gas formation data (Table IV). AC1 and AC2, which demonstrated dough development and gas production patterns similar to those of NFDM1 and NFDM2, were significantly lower in the time of maximum gas formation than were NFDM1 and NFDM2 and AWP1 and AWP2 (Table IV and Fig. 5). This finding confirmed that doughs containing AC have an easily ruptured, weak structure and cannot maintain the Rheofermentometer proof volume or baking stress. The effect of lactose on bread quality was erratic. When evaluated in three flour samples, the effects ranged from beneficial to detrimental.

Crumb softness improved as a result of heat treatment of AWP, and some improvement was obtained with lactose. Kulp et al (1988) and Zadow (1981) reported that the breads with added whey were perceived by panelists to stay fresh longer than controls.

CONCLUSIONS

Proof height measurements did not consistently reflect the contribution to baking performance of dairy ingredients. Rheofermentometer T'_1 could predict the effect of milk ingredients on baking. The volume-depressing factor resided in the casein fraction and could be eliminated, in part only, by heat treatment.

Heat treatment improved the performance of NFDM and AWP in baking. Loaf volume was increased significantly by heating the dairy ingredient at 80°C. Heating at 95°C did not result in additional improvement.

Although dialysis did not influence the mixing characteristics of AWP, per se, it improved the Rheofermentometer properties of the yeasted dough and the loaf volume. Dialysis plus heat treatment of AWP improved mixing time, Rheofermentometer H_m and bread quality and yielded a product with functional properties comparable to those of a commercial acid whey protein concentrate. There may be advantages of casein-whey blends (at yet-to-be-determined optimum ratios) over the use of individual ingredients. Caseins improved the mixing tolerance of wheys significantly. The dough retention volume of AWP was significantly increased by the addition of AC.

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