Influence of Sodium Caseinate and Whey Protein on Baking Properties and Rheology of Frozen Dough

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ABSTRACT

Dairy ingredients are added to bakery products to increase nutritional and functional properties. Sodium caseinate (SC) and whey protein concentrate (WPC) were incorporated into frozen dough. WPC was subjected to heat treatment (WPCHT) to eliminate undesirable weakening of the gluten network. 2% SC or 4% SC decreased proof time, increased loaf volume, and improved texture. Effects of adding 4% SC on baking quality were similar to adding ascorbic acid (AA) and diacetyl tartaric acid esters of monoglycerides (DATEM). WPC increased proof time, decreased volume, and negatively affected texture. Heat treatment of WPC improved baking performance. Bread with WPCHT had volume similar to that of the control without dairy ingredients. Adding 4% SC decreased resistance to extension (R_{5cm} measured with the extensigraph), while adding 4% WPC increased extensibility. Dynamic oscillation testing determined the effects of the ingredients on fundamental rheological properties. WPC decreased storage modulus (G') and loss modulus (G''), while heat treatment of WPC increased G' and G''. Confocal laser scanning microscopy (CLSM) showed that milk proteins affect frozen dough ultrastructure. Frozen doughs with SC had an enhanced gluten network compared with the control, while untreated WPC appeared to interfere with the gluten network.

Additives are used in baked products to facilitate processing, to compensate for variations in raw materials, to guarantee constant quality, and to preserve freshness and eating properties (Schuster and Adams 1984). High levels of additives, especially dough strengtheners and crumb softeners, are used in frozen dough production (Dubois and Blockcolsky 1986a; Hosomi et al 1992; De Stefanis 1995). The gluten network is responsible for viscoelastic properties in wheat dough and for dough structure strength and gas retention. A strong gluten network is important for frozen dough stability. Oxidants improve dough strength by oxidizing gluten SH to inter- and intramolecular SS bonds (Nakamura and Kurata 1997). Dough strengtheners (or conditioners) promote aggregation of the gluten network by neutralizing positive charges on the surface of the gluten (Kamel and Ponte 1993).

Consumers in recent years have become very conscious of ingredients and additives used in foods and are becoming increasingly opposed to use of additives. Dairy ingredients are used in bread for nutritional benefits including increasing calcium content and protein efficiency ratio, and functional benefits including flavor and texture enhancement and storage improvement. Dairy ingredients that increase water absorption can also improve dough-handling properties (Stahel 1983; Kinsella 1984; Cocup and Sanderson 1987).

Dairy proteins are highly functional ingredients with unique properties and are incorporated in many food products. Whey proteins can be processed to exhibit particular functional properties desirable in a food system (Mangino 1984; Kester and Richardson 1984; Melachouris 1984; Sherwin 1995). Researchers have shown that the extent of whey protein denaturation has the greatest influence on functionality in breadmaking (Harper and Zadow 1984). Heat treatment of whey protein changes its structure from the native, compactly folded, stable structure that is soluble in water to a denatured, unfolded structure with reduced solubility. The native whey protein interferes with gluten development and, therefore, has negative effects in breadmaking. Denaturation of whey protein eliminates this negative effect (Harper and Zadow 1984; Kadharmestan et al 1998a; Erdogdu-Arnoczky et al 1996). Jacobson (1997) suggested that whey proteins may confer a protective effect on the gluten network in frozen dough. Caseinates are ampiphilic proteins with surfactant properties that are exploited in many foods (Kinsella 1984). Van Dam et al (1948) reported positive effects of calcium caseinates in breadmaking, whereas Gordon et al (1954) reported negative effects of caseinates in breadmaking.

Incorporation of dairy ingredients into frozen dough would improve baking quality and be more beneficial than synthetic additives because of the high nutritional value and natural origin of dairy products. The objective of this study was to investigate the influence of whey protein and sodium caseinate on baking performance of frozen dough.

MATERIALS AND METHODS

Dough ingredients included commercial wheat flour (Odlum Group, Dublin, Ireland) 12.7 % protein; instant active dried yeast (Mauri, Sao Paulo, Brazil); and fat (baking margarine, Van den Bergh Foods Ltd., Crawley, UK). Dairy ingredients included sodium caseinate (SC) 87% protein, manufactured at University College Cork, Ireland; and whey protein concentrate (WPC) 75% protein, a commercial product supplied by Carbery Food Ingredients, Ballineen, Cork, Ireland.

Heat-Treated Whey Protein Concentrate

WPC (500 g) was dissolved in distilled water to make a 5% (w/w) solution. The pH level was measured and adjusted using 0.1% NaOH to pH 6.7 for heat treatments at 75–82°C and to pH 6.9 for heat treatment at 84°C to avoid protein aggregation and gelling. Solution (150 mL) was placed in each of eight beakers and covered with aluminum foil. Heat treatment was performed in a water bath at 75, 80, 82, and 84°C for 10 min. Heat-treated WPC solutions were cooled by placing the beakers of solution in ice. The heat-treated WPC (WPCHT) solutions were then freeze-dried (Freezemobile 12SL, The Virtis Company, Inc., Gardiner, New York). Protein content of the freeze-dried powder was determined using a Kjeldahl method (IDF 1993).

Farinograph Test

Doughs containing flour, water, and additions of dairy powder or additives (100 ppm of ascorbic acid [AA] and diacetyl tartaric acid esters of monoglycerides [DATEM]) were tested according to the ICC standard method 115/1 (ICC 1995) using a farinograph (Brabender, Duisburg, Germany) to determine water absorption.

Baking Test

Frozen dough formulas are shown in Table I. Flour was chilled at 4°C and water temperature was controlled to achieve a dough temperature 21 ± 0.5°C after mixing. All ingredients (based on 1,500 g of flour) were mixed for 2 min in a high-speed mixer (Stephan u

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Söhne GmbH & Co., Hameln, Germany). The dough was then rounded by hand and rested for 10 min at room temperature (19°C). After resting, the dough was divided into 65-g portions and molded (Holtkamp Almelo, Holland). Doughs were placed in baking tins (48 × 39 × 33 mm) and blast-frozen to a core temperature of –7°C. Frozen doughs were removed from baking tins, packaged in polyethylene bags, and placed in the freezer at –18 ± 2°C. This procedure was performed for each of the eight formulations and repeated on two consecutive days so that three replicate batches of dough were tested for each of the formulations.

Frozen doughs were thawed and proofed in a retarder-proofer (Koma BV, Roremond, The Netherlands) using a dough conditioning program. Thaw time was set at 7 hr, conservation temperature was 2°C, and proofing temperature was 25°C. Thaw time was essentially the time taken for the retarder temperature to increase from 2°C (conservation temperature) to 25°C (proofing temperature). Thawed doughs were proofed to a standard proof height at 25°C and 75% rh. Baking was done in a deck oven (MIWE, Arnstein, Germany) at 200°C top heat and 220°C bottom heat for 22 min. Bread loaves were cooled at room temperature.

Specific volume was measured 2 hr after baking using rapeseed displacement. For each batch of bread, four loaves were measured three times. At 3 hr after baking, four loaves were each cut into slices 25-mm thick in a specially designed cutting frame. The two center slices of each of the four loaves were used for texture measurement. Bread crumb texture was evaluated using a texture profile analysis with a texture analyzer (TA-XT2i, Stable Micro Systems, Surrey, UK) with a 25-kg load cell. This test involved compressing a bread slice by 40% (10 mm) with a 20-mm cylindrical aluminum probe at a speed of 1 mm/sec.

Extensigraph Measurements

Measurements were performed with an Extensograph (Brabender, Duisburg, Germany). Unyeasted doughs containing 1,500 g of flour and water, salt, sugar, shortening, dairy ingredient, and additives (Table I) were mixed for 2 min in a high-speed mixer. After 10 min of resting at room temperature (19°C), doughs were divided into 150-g pieces and molded in the molding unit of the extensigraph. Doughs were blast-frozen to a core temperature of –7°C, vacuum-packaged and stored at –18 ± 2°C. Similar to baking tests, three batches of dough were produced on three consecutive days for each recipe. After each storage time, for each batch of dough, four frozen dough pieces were placed in extensigraph dough holders and thawed in the retarder-proofer using the dough conditioning program described for the bake test. Resistance to extension (R_{5cm}) and extensibility (E) were calculated from extensigrams.

Oscillation Measurements

Unyeasted doughs were prepared with 10 g of flour, salt, dairy ingredient, and additives (Table I). The control and AA+DATEM doughs were prepared with 58% water absorption (optimum level for baking, farinograph water absorption minus 4%). Doughs containing 4% SC, 4% WPC, and 4% WPCHT were prepared with the optimum water absorption levels used in baking and also with 58% water absorption (constant water absorption used in the control dough). Dough was mixed for 70 sec using a Glutomatic 2200 (Falling Number AB, Stockholm, Sweden) without washing and using a modified mixing chamber without perforation. This system allowed reproducible mixing of small quantities of dough. One 4.5-g dough piece from each mix was lightly rounded and rested for 10 min at room temperature (19°C). The dough piece was placed in a plastic container, blast-frozen, and stored at –18 ± 2°C. Doughs were thawed for 25 min at 4°C and 25 min at 25°C to simulate a gradual thawing and transferred to the rheometer for measurement.

A controlled stress rheometer (Bohlin Rheology AB, Lund, Sweden) was used for dynamic oscillation measurements. The geometry consisted of parallel plates 40 mm in diameter with a gap of 2 mm between them. A target strain of 10^{-3} (0.001%), which is within the linear viscoelastic region for wheat dough was used. A frequency sweep was used to characterize doughs. Measurements were performed at 16 frequencies in 0.01–10 Hz (on a logarithmic scale). Temperature was kept constant at 25°C. The dough sample was placed between parallel plates and excess dough protruding from the edge of the plate was carefully trimmed. Before starting the oscillatory measurement, the dough sample was rested for 5 min, allowing any normal stresses induced during sample loading to relax. During resting and measuring, the system was covered and water-saturated cotton strips lined the inside of the cover to prevent drying out of the dough rim. A Bohlin software package was used to calculate phase angle (δ) storage modulus (G'), loss modulus (G''), complex modulus (G^*), and complex viscosity (η^*). Each result is the average of four to six measurements.

Confocal Scanning Laser Microscopy

Confocal scanning laser microscopy (CSLM) was used to visualize the protein and starch in frozen dough. A 65-g frozen dough piece from each of the batches of dough prepared for the baking test was removed from frozen storage after 10 weeks. Disks (≈20 mm diameter, 5 mm thick) were cut from the dough piece with a razor blade and transferred to a cryostat held at –25°C. Sections (15 µm thick) were then cut and placed on a microscope slide. One drop of Nile blue dye (0.1 % aqueous, w/v) was added to the section and a coverslip placed on top. After 5 min, stained sections were ex-

TABLE I
Frozen Dough Recipes[a]

	Control	AA+ DATEM	2% SC	4% SC	2% WPC	4% WPC	2% WPCHT	4% WPCHT
Flour	100	100	100	100	100	100	100	100
Water[b]	58	58	61	67	57.5	57	58	60
Fat	3	3	3	3	3	3	3	3
Yeast[c]	2	2	2	2	2	2	2	2
Salt	2	2	2	2	2	2	2	2
Sugar	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
AA[d]	...	0.01	...	...	...	...	...	...
DATEM[e]	...	0.5	...	...	...	...	...	...
SC[f]	...	...	2	4	...	...	...	...
WPC[g]	...	...	...	...	2	4	...	...
WPCHT[h]	...	...	...	...	...	...	2	4

[a] % Flour wt.
[b] Water addition for each formula was % farinograph water absorption minus 4%.
[c] Instant active dried yeast.
[d] Ascorbic acid.
[e] Diacetyl tartaric acid esters of monoglycerides.
[f] Sodium caseinate.
[g] Whey protein concentrate.
[h] Whey protein concentrate heat-treated at 82°C for 10 min.

amined using a Zeiss LSM310 confocal scanning laser microscope (Carl Zeiss Ltd., Welwyn Garden City, Herts, UK). Images of representative areas of each sample were taken at ×40, 1.3 N.A. Confocal illumination was provided by a Ne/Ne laser (633 nm laser excitation) fitted with a band pass 670–810 nm filter. The confocal pinhole was set to give an *x*-*y* resolution of ≈0.2 µm and an axial resolution of ≈1.0 µm. Projections consisting of a *z*-series of ≈30 optical sections (overall *z*-depth ≈50 µm) were acquired.

Statistical Analysis

Using a statistical software package (SPSS Inc., Chicago, IL), dynamic oscillation data were analyzed using one-way analysis of variance (ANOVA) and Tukey's posthoc test to detect significant differences. A blocked split-plot design was used for all other data. In this design, each trial was divided into five or eight main plots, with the five or eight formulations assigned randomly to these main plots. Subsequently, each main plot was divided into four smaller plots (split plots) for the frozen storage time (1, 5, 10, and 15 weeks). Different storage times were randomly assigned to these plots (Mead et al 1993). Multiple comparisons were performed with Tukey's posthoc to identify significant differences.

RESULTS AND DISCUSSION

Effect of WPCHT on Baking Properties

Table II shows the effects of heat treatment of WPC on water absorption and frozen dough baking properties measured after one week of frozen storage. Heat treatment at 75°C had no effect on water absorption or baking properties. Heat treatment at 82 or 84°C increased water absorption from 61 to 64%. Kadharmestan et al (1998a) also observed an increase in water absorption measured with the mixograph when whey protein was denatured by heat or hydrostatic pressure. Water absorption is increased after protein denaturation because there is an increase in the availability of water binding sites due to changes in protein conformation (Jacobson 1997). Heat treatments at 82 and at 84°C improved baking performance of frozen dough by decreasing proof time, increasing specific loaf vol-

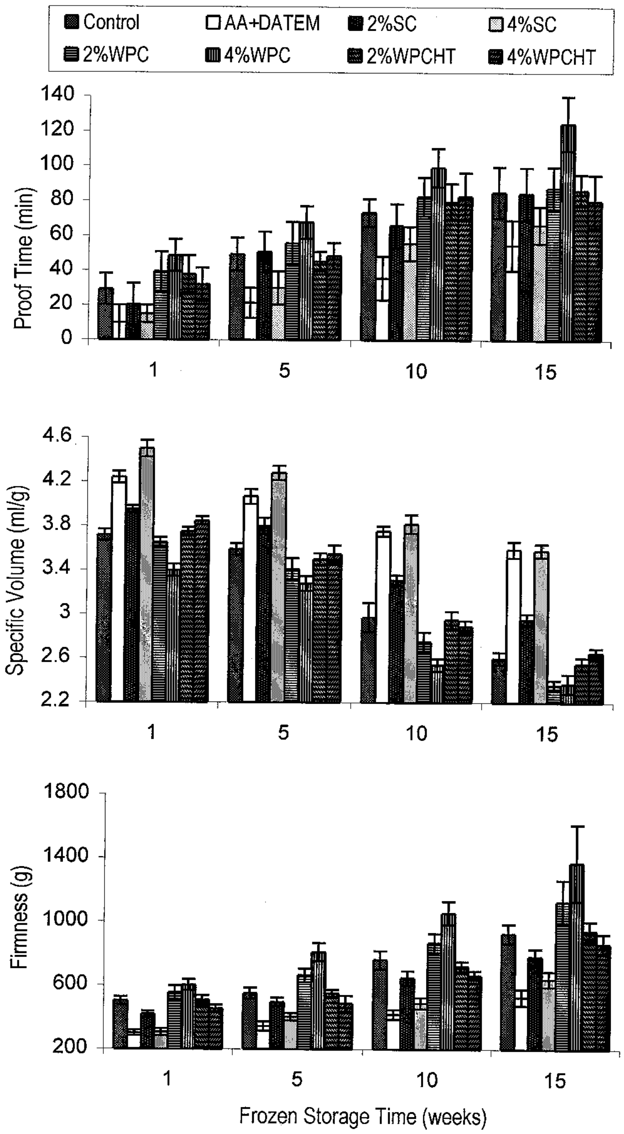

Fig. 1. Effects of dairy proteins on frozen dough baking properties proof time, specific volume, and crumb firmness ($n = 3$, measured 3 hr after baking).

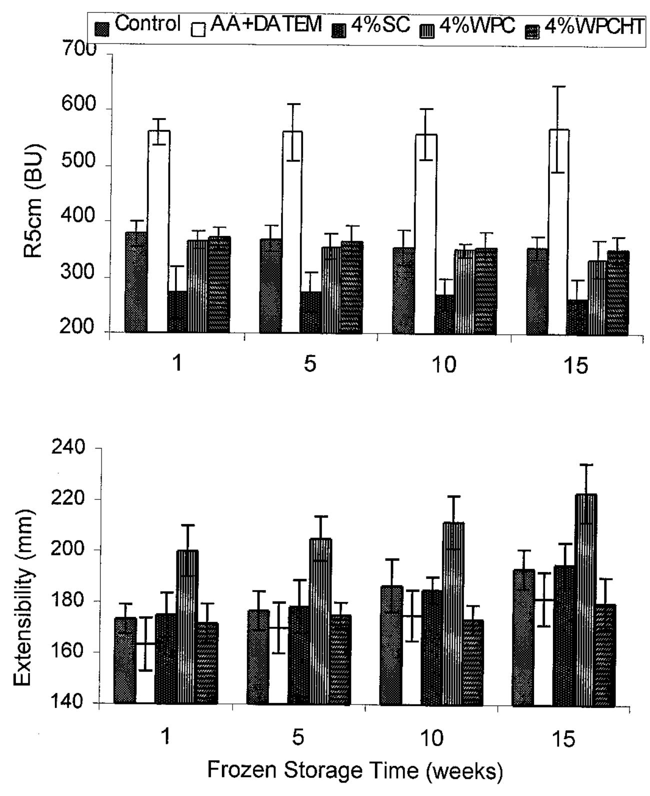

Fig. 2. Influence of dairy ingredients on extensigraph properties resistance to extension and extensibility ($n = 3$).

TABLE II
Water Absorption and Frozen Dough Baking Properties of Heat-Treated Whey Protein Concentrate[a]

	Untreated WPC	75°C[b]	80°C[b]	82°C[b]	84°C[c]
Water absorption (%)	61	61	63.5	64	64
Proof time (min)	45	55	35	30	35
Specific volume (mL/g)	3.4	3.5	3.6	3.8	3.6
Crumb firmness (g)	910	930	825	720	765

[a] Incorporated at 4% flour wt; one week of frozen storage; mean of two baking tests.
[b] pH 6.7, heat-treated for 10 min
[c] pH 6.9, heat-treated for 10 min

ume, and decreasing crumb firmness. WPC heat-treated at 82°C performed slightly better in baking tests than WPC heat-treated at 84°C and was used in frozen dough baking trials and in rheology testing. This observation is in agreement with those of Harper and Zadow (1984), who reported that partial denaturation of whey protein improved bread quality, whereas higher levels of protein denaturation (at 85°C) had negative effects on bread quality.

Baking Test Results

Figure 1 shows the influence of whey protein and sodium caseinate on baking properties of frozen dough. The control dough contained no dairy ingredients or additives. Doughs with dairy ingredients contained WPC and SC at 2% and 4% of flour weight. The formulation AA+DATEM (containing 100 ppm of ascorbic acid and 0.5% DATEM) was tested for comparison.

Doughs with untreated WPC took longest to proof. Addition of additives (AA and DATEM) or SC reduced proof time. Addition of 2% SC, 4% SC, or additives (AA+DATEM) significantly increased specific volume ($P < 0.05$), while untreated WPC decreased specific volume ($P < 0.05$). Bread baked form frozen dough with WPCHT had significantly higher volume than with untreated WPC ($P < 0.05$). Crumb firmness of bread baked from frozen dough was measured 3 hr after baking. The 4% SC significantly decreased crumb firmness compared with the control ($P < 0.05$). Addition of 2 or 4% WPC significantly increased crumb firmness ($P < 0.05$). Heat treatment of WPC significantly reduced crumb firmness ($P < 0.05$), which can be seen by comparing doughs containing WPC with doughs containing WPCHT. Overall, SC had a very positive effect on baking properties of frozen dough. It increased water absorption considerably (by 9% for addition of 4% SC). There was a significant improvement in baking properties of frozen doughs containing 2 and 4% SC compared with the control. Frozen doughs with 4% SC had baking properties similar to frozen dough with added AA+DATEM. These data suggest that SC improves the gluten network. The amphiphilic nature of SC (Kinsella 1984) could result in similar effects to surfactants on the gluten structure. No literature has been published recently on the effects of caseinates on fresh or frozen dough. Existing literature on fresh dough reports both positive and negative effects (Van Dam et al 1948; Gordon et al 1954). Untreated WPC had an overall negative influence on baking properties. Heat treatment of WPC eliminated these negative effects and frozen doughs with WPCHT had superior baking properties compared with doughs with WPC. Addition of WPCHT, however, did not improve baking properties of frozen dough when compared with the control. The negative influence of WPC on frozen dough quality can be explained by weakening of the gluten network. Negative effects of WPC in fresh dough have been reported (Harper and Zadow 1984; Kulp et al 1988; Erdogdu-Arnoczky 1996; Kadharmestan et al 1998a). Zadow (1981) postulated that WPC SH groups interfere with the normal SH-SS interchange that occurs during gluten development. This results in weaker and less elastic dough. According to Zadow and Hardman (1981), WPC that performed poorly in fresh dough baking trials had high levels of reactive SH groups. Chemical blocking of SH residues resulted in an improvement in baking characteristics (Zadow et al 1983). Denaturation of whey protein results in conformational changes and protein unfolding, which may reduce the availability of SH groups for interactions (De Wit and Klarenbeek 1984; Jacobson 1997).

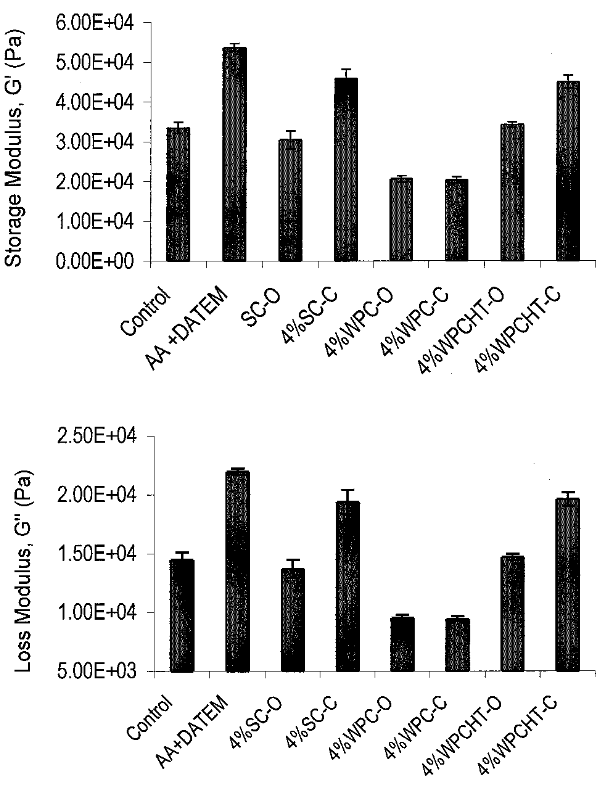

Fig. 3. Effects of dairy proteins on dynamic oscillation properties of dough storage modulus (G') and loss modulus (G'') at 10 Hz after 10 weeks of frozen storage. Water absorption levels: optimum (O, farinograph water absorption minus 4%) and constant (C, 58%).

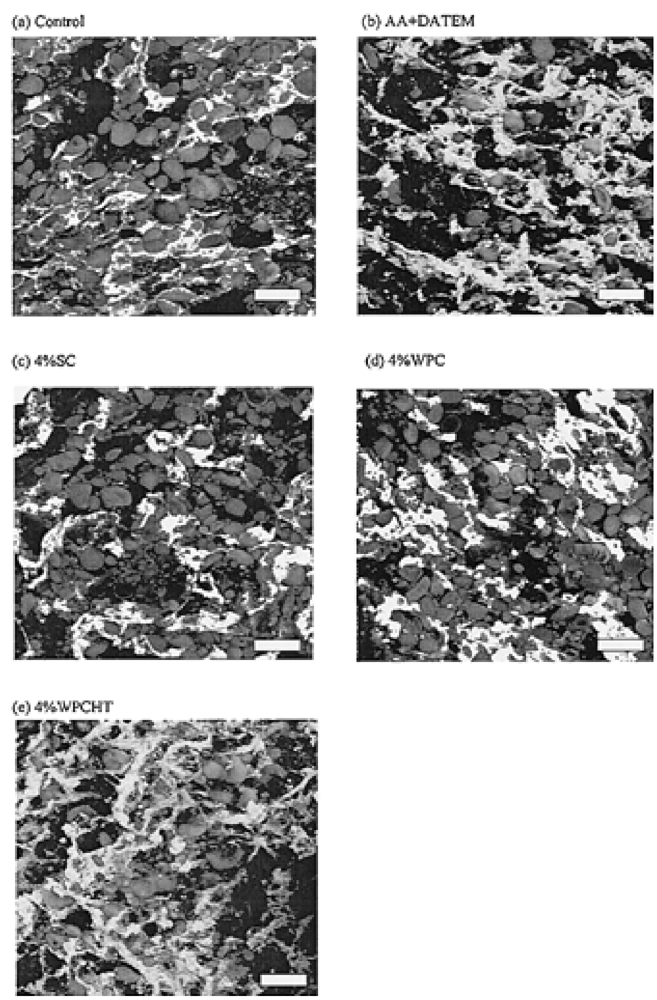

Fig. 4. Confocal scanning laser microscopy of frozen dough ultrastructure after 10 weeks of frozen storage. Protein appears white to light gray. AA+DATEM, ascorbic acid + diacetyl tartaric acid esters of monoglycerides; SC, sodium caseinate; WPC, whey protein concentrate; WPCHT, whey protein concentrate heat-treated.

Extensigraph Measurements

Effects of 4% dairy protein on resistance to extension (R_{5cm}) and extensibility (E) of frozen doughs are shown in Fig. 2. The 4% SC significantly reduced R_{5cm} compared with the control ($P < 0.05$). There were no significant differences in R_{5cm} between doughs with WPC, WPCHT, and the control ($P > 0.05$). R_{5cm} values of frozen dough with additives (AA+DATEM) were significantly higher than R_{5cm} of all other doughs tested ($P < 0.05$) at each storage time. R_{5cm} did not change significantly during frozen storage for any of the formulations tested. SC had no significant influence on E ($P > 0.05$). WPC significantly increased E compared with the control ($P < 0.05$). Doughs with WPCHT had significantly lower E compared with doughs with untreated WPC ($P < 0.05$). A slight increase in E was observed over the frozen storage period tested.

The reduction in R_{5cm} due to addition of SC is a little surprising, considering that SC improves frozen dough baking properties. This may be due to the increase in water absorption when SC is added. Researchers have attributed decreases in R_{5cm} of frozen dough to weakening (Wolt and D'Appolonia 1984a; Inoue and Bushuk 1992). However, 4% SC addition increased water absorption considerably from 62 to 71%, and this increased water content had a negative effect on R_{5cm}, decreasing it by 80–100 BU, relative to the control. The increase in E when untreated WPC was added to frozen dough can be related to its negative effect in baking. An increase in E indicates weakening of the gluten network and a decrease in gas retention properties. Heat treatment of WPC reduced E, indicating that whey protein denaturation reduced dough weakening.

Oscillation Measurements

Results of dynamic oscillation tests on frozen dough are presented in Fig. 3. For each of the recipes tested, rheological data did not change significantly with frozen storage time. Hence, data are presented at one storage time (10 weeks). Dynamic oscillatory measurements were made to examine the effects of dairy proteins on the viscoelastic behavior of frozen dough. When 4% SC and 4% WPCHT were incorporated into frozen dough, optimum water absorption was increased by 9 and 2%, respectively. Addition of 4% WPC decreased water absorption by 1%. Several authors have researched the effects of dough moisture content on dynamic viscoelastic behavior of wheat dough. Hibberd (1970) proposed that water molecules in high-moisture doughs behave as inert fillers. When water content is increased, elastic modulus, viscous modulus, and $\tan\delta$ decrease (Masi et al 1998). For this reason, doughs with dairy proteins (4% SC, 4% WPC, and 4% WPCHT) were prepared at optimum water absorption levels used in baking tests (farinograph water absorption minus 4%) and also at the constant water absorption (58%), used in the control dough and AA+DATEM.

Data for G' shows that addition of AA+DATEM significantly increased G' compared with the control. When SC was added to frozen dough with optimum water absorption, G' was not significantly different from that of the control. G' of dough with SC and constant water absorption (58%) was significantly higher than that of the control ($P < 0.05$). WPC at both optimum and constant water absorptions significantly reduced G' compared with the control ($P < 0.05$). WPCHT significantly increased G' compared with dough with WPC at both optimum and constant water absorptions ($P < 0.05$). Data for G'' shows that addition of AA+DATEM significantly increased G'' compared with that of the control. Addition of SC at constant water absorption significantly increased G'' ($P < 0.05$). The 4% WPC significantly reduced G'' at both water absorptions ($P < 0.05$). WPCHT significantly increased G' compared with dough with WPC at both water levels ($P < 0.05$).

Overall, these results show that doughs with SC at optimum water absorption displayed similar dynamic viscoelastic behavior to dough with AA+DATEM added. These doughs were more elastic and firmer compared with the control. Dough with WPC, on the other hand, was less elastic and softer compared with the control. Heat treatment of WPC eliminated this effect.

Confocal Scanning Laser Microscopy

Confocal scanning laser microscopy (CSLM) was used to visualize frozen dough ultrastructure (Fig. 4). The gluten network of frozen dough with AA+DATEM appears more continuous than the control, which was expected with the addition of oxidant and dough strengthener. Doughs with 4% SC and 4% WPC differ considerably from each other and from the control. The gluten network of dough containing SC appears to be more continuous and connected compared with the control. Images of dough containing WPC show a gluten network that appears less organized and fragmented compared with the control. Dough with WPCHT appears to have a better gluten network than dough with untreated WPC.

CONCLUSIONS

SC improved baking properties of frozen dough. It decreased proof time, increased specific volume, and improved texture. Baking attributes of frozen dough with SC were comparable to those of a control dough containing additives. Therefore, SC can be used in frozen dough as a functional ingredient of natural origin and could reduce the level of synthetic additives required. It was difficult to determine the effect of SC on dynamic oscillation parameters due to the large increase in water absorption as a result of SC addition. Frozen doughs with 4% SC and the same water content as the control dough did appear to be more elastic and firmer than the control. This indicated interaction of SC with the gluten to enhance the gluten network in a manner similar to a dough strengthener. CLSM images also suggested that SC improves the gluten network. This effect combined with the increased water absorption, which makes the dough more expandable, can account for the positive influence of SC on frozen dough quality.

WPCHT performed better in frozen dough trials than the untreated protein. However, the WPCHT does not improve baking performance compared with the control dough without WPC. Jacobson et al (1997) suggested that whey protein might confer a protective effect on frozen dough and render it more stable. It is evident from these data that neither the untreated WPC nor the WPCHT tested improved frozen dough stability over the 15-week frozen storage period.

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