Physicochemical Behaviors of Sugars, Lipids, and Gluten in Short Dough and Biscuit

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The structure of short dough and biscuit has been characterized at a macroscopic level (dimensions, bulk structure) and a microscopic level (starch damage, protein aggregates, microstructure) by physical and biochemical methods. The baking process of short dough induces a large decrease of the product bulk density from 1.26 to 0.42 (±0.01) g·cm^{-3} for final biscuit, leading to a cellular solid with a thin colored surface and a porous inner structure. Proteins appear aggregated in biscuit when compared to short dough, whereas starch granules remain almost intact in biscuits. The components which are involved in the cohesiveness of short dough and biscuit final structure have been identified. They suggest that short dough is a suspension of solid particles in a liquid phase being an emulsion of lipids in a concentrated sugar solution. The role of sugars in biscuit structure suggest that biscuit structure is a composite matrix of protein aggregates, lipids and sugars, embedding starch granules.

Keywords: *Short dough; biscuit; structure; sugars*

INTRODUCTION

Among cereal-based products, biscuits are characterized on the one hand by their enrichment with two major ingredients, sugars and fats, and on the other hand by their low final water contents (1−5%) compared to cakes (15−30%) and breads (35−40%) (Wade, 1988). However, significant water additions are present in all these technologies, for creating a cohesive dough when starting from dry flour. The baking process of short dough drives the transformation from a viscoelastic dough into a cellular solid, with a characteristic final texture. This process is induced by the expansion of the dough subjected to the vaporization of water and gases from leavening powders. Meanwhile, rheological properties of the matrix (Chevallier et al., 1999) lessen the impact of the expansion phenomenon. These properties are modified by water losses, thermal denaturation, and melting of dough components. These complex physical and chemical changes in the components of the dough system yield a stable structure with desirable flavor, aroma, and textural properties (Taranto, 1983). Studies on the microstructure of biscuits are scarce (Flint et al., 1970; Burt and Fearn, 1983) compared to the literature focused on microstructure of bread and flour−water doughs (Bloksma, 1990; Hoseney and Rogers, 1990; Freeman and Shelton, 1991): the role of the protein matrix has never been enlightened completely in these products. Similarly, the role of fats and sugars in this texturization process has never been investigated. The objective of this work is to characterize the structure of short dough and biscuit at a macroscopic level (dimensions, global structure) and a microscopic level (starch, proteins aggregates, microstructure) by physical (color measurements, X-ray diffractometry, microscopy) and biochemical methods. Comparison with other generic products from wheat (bread, pasta) will also help in determining which components are involved in the cohesiveness of both starting and final products, dough and biscuit.

EXPERIMENTAL PROCEDURES

Materials. A commercial biscuit was provided by the manufacturer (LU, La Haye-Fouassière, France). The corresponding short dough was picked up on the industrial line after the sheeting and forming stages, just before the oven entrance. The basic formula corresponds to a low-moisture short dough (20% on total weight basis) with 60% db (db = dry basis) flour, 26% db added sugars, 12% db lipids, and 2% db chemical leavening agents, dried milk, and flavors. Specifics of the biscuit dough formula are proprietary to LU. Bread and its corresponding dough, based on a standard white french bread formula, were provided by the baker of the experimental bakery on the research center. Dry pasta was purchased at the local supermarket and cooked according to the instructions on the package.

Methods. *Protein Analysis*. Proteins were extracted from the sample (5 g) in suspension in a sodium phosphate buffer (0.1 M, pH 6.9, SDS 2% p/v, 30 mL) and shaken continuously for 12 h at 25 °C. After centrifugation at 39 000*g* for 30 min, insoluble proteins recovered in the sediment were measured by the Kjeldahl method and expressed as the percentage of total dry proteins.

Kinetics of α-Amylolysis. Grounded sample was first suspended (1 mg of dry starch·mL^{-1}) in 5 mM phosphate buffer, pH 7.0; then an α-amylase solution (200 μg·mg^{-1} of substrate) prepared in the same buffer was added. The suspension, at 37 °C, was shaken continuously. Aliquots (0.8 mL) were withdrawn at time intervals (5, 10, 15, 30, 60, 90, and 120 min) and added to an ethanol solution (4 mL) containing 1.5% of acetic acid. After 12 h at 4 °C, these aliquots were centrifugated for 10 min at 5000 *g* and 4 °C. Total soluble sugars were measured in the supernatant by the orcinol−H_2SO_4 method (Planchot et al., 1997) and the extent of degradation was expressed as the percentage of initial dry starch: the

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Table 1. Physical Characteristics of Dough Pieces and Biscuits

	dough piece	biscuit[a]
dimensions (mm)		
length	64.8	67.7 (±1.0)
width	57.0	58.5 (±0.8)
thickness	2.00	5.85 (±0.25)
weight (g)	9.20	7.29 (±0.25)
density (g·cm^{-3})	1.26	0.42
lightness L^*	73	62 (±2)
humidity (% wb)	20.1	1.0 (±0.2)

[a] Values in parentheses are accepted limits on the industrial production line before conditioning.

initial rate of hydrolysis corresponds to the rate (% total starch·min^{-1}) of starch solubilization during the first 5 min whereas the easily hydrolyzed starch fraction is defined by extrapolation to zero time of the linear part of the α-amylolysis curve.

Mono- and Disaccharides Analysis. Oligosaccharides were analyzed by a Dionex BioLC model 4000i (Sunnyvale, CA) and a pulsed amperometric detector. A CarboPac PA-100 column was used. Eluents A and B consisted of solutions containing 150 mM NaOH and 600 mM NaOAc, respectively, and the flow rate was 1 mL·min^{-1}. The percentage of buffer B in buffer A changed from 0 to 40% in 4 min. The column was calibrated with standard glucose, fructose, lactose, and sucrose.

X-ray Diffractometry. Diffraction diagrams were recorded using an Inel X-ray equipment operating at 40 kV and 30 mA. Cu Kα_1 radiation ($\lambda = 0.154\ 05$ nm) was selected using a quartz monochromator. A curved position-sensitive detector (Inel CPS 120) was used to monitor the diffracted intensities during 2 h exposure periods. Samples (50 mg) were sealed between two pieces of aluminum foil to prevent any significant change in water content during measurement.

Light Microscopy. Damaged starch granules were detected by loss of birefringence and increased absorption of Congo Red in excess of water using an Olympus light microscope (model Vanox) able to work in the polarization mode.

Color Measurements. Surface browning of biscuits was measured by the CIE $L^*a^*b^*$ colorimetric system using a Minolta Chroma Meter CR-300 tristimulus colorimeter (Minolta, Carrieres s/Seine, France) and the L^* value accounting for lightness has been retained to characterize biscuit surface color.

Density Measurements. The density ρ_s is calculated by weighing a sample (M_s) and by measuring its volume through the displacement of glass beads (bulk density ρ_b, diameter 500 μm). Assuming that the volume of beads is equal to the volume of the sample, if M_b is the mass of beads excluded on introduction of sample, its bulk density verifies as

$$\rho_s = \rho_b(M_s/M_b) \quad (1)$$

RESULTS AND DISCUSSION

Macroscopic Level. Dimensions of dough pieces change during the baking stage. Mean dimensional values characteristic of dough pieces and biscuits are reported in Table 1. Thickness increases from 2.00 to 5.85 (±0.25) mm, length from 64.8 to 67.7 (±1.0) mm, and width from 57.0 to 58.8 (±0.8) mm. Concomitantly, biscuit weight decreases from 9.20 to 7.29 (±0.25) g. The baking stage induces (i) an increase of the product volume by production of gases from chemical leaveners and water vaporization and (ii) a decrease of the product weight corresponding to its drying (water content drops from 20% wb (wb = wet basis) in the dough to 1% wb in the final product). The result of these simultaneous modifications is a large decrease of the product density during the baking stage, from 1.26 to 0.42 (±0.01) g·cm^{-3}.

Table 2. Biochemical Characterization of Dough and Biscuits[a]

	dough	biscuit center	biscuit surface
insoluble proteins (% total proteins)	6.0 (±0.2)	65.7 (±1.5)	67.3 (±1.5)
initial rate of starch hydrolysis (% total starch·min^{-1})	1.5 (±0.6)	5.0 (±0.6)	1.3 (±0.2)
easily hydrolyzed starch fraction (% total starch)	9.3 (±1.4)	49.8 (±2.6)	17.7 (±1.0)
solubility (% total starch)	1.6 (±0.5)	1.2 (±0.3)	0.9 (±0.2)
swelling (g.g. dry matter^{-1})	1.6 (±0.1)	3.0 (±0.1)	1.7 (±0.1)
sugar content (% db)	27.2	27.0	26.5
glucose	1.2	1.2	0.7
fructose	1.0	1.0	0.7
lactose	0.4	0.4	0.2
sucrose	24.4	24.0	24.5
others	0.2	0.4	0.4

[a] Values in parentheses are standard error deviations.

At the same time, a change in surface color, followed by a decrease in lightness from 73 to 62 (±2), corresponds to a darkening of the biscuit surface. From light microscopy observations, biscuits exhibit a thin colored surface (thickness ≈1 mm, pore diameter ≈100 μm) and a porous inner structure (pore diameter ≈1 mm), in agreement with observations reported by Burt and Fearn (1983). This difference between edge and center leads to a separate analysis of these parts of the biscuit.

Microscopic Level. *Proteins.* By using a sodium phosphate buffer with 2% SDS (w/v), all noncovalent bonds are assumed to be broken and all proteins are assumed to be solubilized, except those aggregated through covalent bonds. Insoluble protein content increases from 6.0% (±0.2) in the dough to 65.7% (±1.5) in the biscuit center and 67.3% (±1.5) in the biscuit surface (Table 2). During baking, proteins undergo a thermal transition that induces a reduction in protein solubility aggregation (Schofield et al., 1984). This temperature-induced phenomenon corresponds to the cross-linking by specific bonding at specific sites on the protein strands or by nonspecific bonding occurring along the protein strands. Both disulfide and hydrogen bonds, as well as ionic interactions, are involved in the cross-linking of protein aggregates (Bottomley et al., 1982; Schofield and Booth, 1983). There is no significant difference between surface and center of biscuit with regards to protein aggregation level.

Starch. Starch organization can be characterized by its susceptibility toward enzymatic hydrolysis (α-amylase). Initial rate of hydrolysis and easily hydrolyzed starch fraction values for the different samples are reported in Table 2. The initial rate of starch hydrolysis is equivalent for samples from dough and biscuit surface, 1.5 and 1.3% min^{-1}, respectively, and appears quite higher for biscuit center samples, 5.0% min^{-1}. This means that starch is more easily hydrolyzed at the center of the biscuit. Similarly, the easily hydrolyzed starch fraction is more important in the center of the biscuit (49.8% of initial starch) than on the biscuit surface (17.7%), meaning that some starch is damaged in the biscuit center. This higher damaged starch level in the biscuit center compared to biscuit surface has been confirmed by polarizing microscopy observations. Starch granules are intact and birefringent in dough and biscuit surface samples, whereas some of them are damaged in biscuit center samples: some granules have partially lost their birefringence and others are slightly swollen. It affects a proportion of the granule population lower than 50%. These observations are in agreement

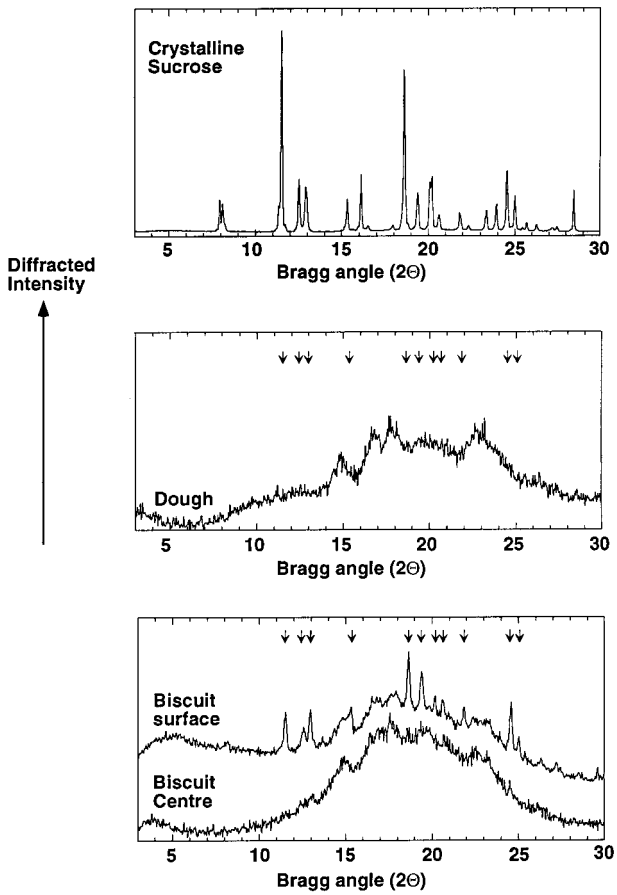

Figure 1. X-ray diffraction diagrams of (a, top) crystalline sucrose, (b, middle) short dough, and (c, bottom) biscuit center and surface.

with other microscopic studies on the same type of biscuits (Flint et al., 1970; Burt and Fearn, 1983; Hoseney, 1994). Most of the starch granules are not gelatinized due to the lack of sufficient water, as well as the presence of sugars (Chevallier et al., 1999).

Sugars. Biochemical analysis of dough and biscuit samples yielded a sugar content around 27% db, sucrose being the major one (24% db) for all samples (Table 2). In dough formulation, part of the sucrose is added up in solution (syrup and invert sugar), whereas the other part is crystalline (granulated sucrose). The presence of this fraction of solubilized sugars is sufficient to explain the persistence of starch granule integrity in biscuits (Chevallier et al., 1999). The crystallinity level of sucrose in the different samples has been studied by X-ray diffractometry. Characteristic peaks of crystalline sucrose alone are presented in Figure 1a. None of these peaks was detected on dough diffraction diagrams (Figure 1b), meaning that no crystalline sucrose seems to remain present in dough as crystals. Two hypotheses may explain the absence of characteristic diffraction peaks in dough samples: either all sucrose crystals solubilize during the mixing stage, or the diffraction diagram of the part of sucrose remaining crystalline in the dough may be hidden by the presence of water (20% wb) and other biochemical components.

When examinating diffraction diagrams of biscuit (Figure 1c), samples taken at the center exhibit none of the peaks of crystalline sucrose. The water content of the biscuit sample being lower than 2% db, the absence of peak cannot be attributed to solubilization in water as mentioned in the case of dough. Therefore, the mechanism proposed in that case is an increase in solubilization and/or a melting of crystalline sucrose during baking, followed by a glass transition of this amorphous sucrose during the cooling period. T_g of sucrose hydrated at 2% db is around 50 °C (Roos, 1995). So, in the final product, sucrose would be amorphous and glassy, in agreement with the absence of diffraction peaks. In contrast, surface samples exhibit peaks at the same angles than crystalline sucrose (Figure 1c). The proposed mechanism for biscuit surface is as follows. In the first step, at the beginning of baking, condensation may occur on the biscuit surface (for temperatures below 100 °C) and facilitate molecular mobility and thereby sucrose crystallization. In a second step, surface biscuit dries up with increasing temperature, at a higher rate than the biscuit center since it is exposed to hot air of the oven. So sucrose, insufficiently hydrated, does not melt (melting temperature of anhydrous sucrose is 185 °C (Roos, 1993) and remains crystalline in the baked product. Indirectly, this observation demonstrates that temperature at the biscuit surface is always lower than 185 °C.

The color changes are due to several reasons. In the Maillard reactions, nonenzymatic browning occurs, which involves the interaction of reducing sugars with proteins and produces reddish-brown hues. Color development may also be associated with dextrinization of starch and caramelization of sugars. For biscuits with a very open structure, moisture migration to the surface is slower, so a local increase in surface temperature and therefore coloring could be more easily achieved.

Cohesiveness of Short Dough and Biscuit in Specific Solvents. MEB observations do not allow to discriminate proteins, lipids, and sugars in the dough and biscuit structure. Bread is the most studied cereal-based baked product. Many studies demonstrated that bread structure is based on a gluten network embedding starch granules (Marston and Wannan, 1976; Bloksma, 1990; Hoseney and Rogers, 1990). This network develops during mixing and resting and is thermally set by baking. Could biscuit structure be described by a similar scheme (Figure 2a) with a continuous phase of gluten or any other component in which are embedded isolated particles? In order to determine which components are involved in dough and biscuit cohesiveness, samples have been placed in different solvents, each one presenting specific solubilization capacities and samples bulk structures have been followed for 48 h. Bread and pasta doughs and corresponding baked products have also been studied according to the same procedure. Table 3 presents components solubilities in the different solvents chosen: water, ammonium sulfate solution at 70%, ethanol, and hexane, as well as observations on samples structure after 48 h.

Protein Contribution. Bread and pasta samples retain their structures in water and ammonium sulfate solution. Their structures remain soft in water, but become rigid in ammonium sulfate solution. These products are known to develop a gluten network during mixing and forming (Hoseney and Rogers, 1990; Bloksma, 1990), which is reinforced during baking by temperature due to protein aggregation. Gluten proteins are hardly soluble in water and completely insoluble once they are thermally aggregated. Cohesiveness of these samples in water can therefore be attributed to a gluten network, whereas it is reinforced in ammonium sulfate solution which precipitates proteins, explaining the observed

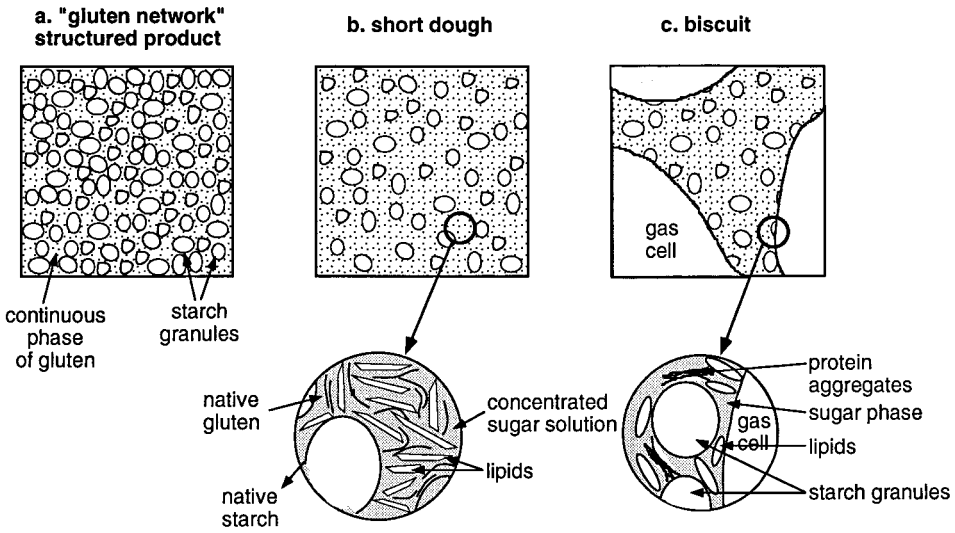

Figure 2. Schematic representation of matrix structure: (a) "gluten network" structured product, (b) short dough, (c) biscuit. Representation takes into account volume fractions evaluated for each component of the dough and biscuit.

Table 3. Components Solubility in Different Solvents at Ambient Temperature and Observations Made on Samples Structure and Rigidity after 48 h

sample		ammonium sulfate solution at 70%	water	hexane	ethanol
native starch		none	none	none	none
gelatinized starch		partial 5%	partial 10%	none	none
native proteins		none	partial 10−20%	none	none
aggregated proteins		none	none	none	none
lipids		none	none	complete	partial
sugars		complete 1 g in 0.8 mL	complete 1 g in 0.5 mL	none	partial 1 g in 170 mL
bread dough	structure	intact	intact	intact	intact
	rigidity[a]	+	−	++	++
pasta dough	structure	intact	intact	intact	intact
	rigidity	+	−	++	++
biscuit dough	structure	broken on	broken by itself	intact	intact
	rigidity	touch		−	++
bread crumb	structure	intact	intact	intact	intact
	rigidity	+	−	+	++
cooked pasta	structure	intact	intact	intact	intact
	rigidity	+	−	+	++
biscuit	structure	broken on	broken on touch	intact	intact
	rigidity	touch		++	++

[a] + means an increase in rigidity, and − means a steady state.

increase in rigidity with this solvent. Short dough loses its structure in water and appears as a suspension of swollen particles after 48 h. In ammonium sulfate solution, short dough is destructured on touch. The same behavior is observed for biscuits in water and ammonium sulfate solution: a complete destructuration on touch. These observations suggest that no gluten network can be responsible for the macroscopic cohesiveness of short dough and biscuit samples.

Lipids Contribution. Bread and pasta samples retain their structures and even appear more rigid in ethanol and hexane. This increase in rigidity can be explained by the drying of samples over the experiment time range (48 h). Hexane may invade samples by capillarity and ethanol reduces the partial pressure of water which, then, evaporates.

Short dough retains its structure that becomes also more rigid in ethanol, whereas it is soft enough in hexane to be broken with a spatula. The same drying phenomenon as described previously in ethanol occurs. In hexane, even though, concomitant solubilization of lipids prevents hardening of the sample. Since no suspension is observed in hexane, as compared to water, lipids could be involved in short dough cohesiveness but may not be totally responsible for it. Therefore, short dough cannot be considered as a suspension of proteins, starch, and sugars in a matrix of lipids.

Biscuit samples retain their structure in ethanol and hexane, solvents that solubilize lipids. Two hypotheses can explain this phenomenon: either lipids are not involved in biscuit cohesiveness, or they are not accessible to the solvent. On the other hand, biscuit loses its structure in water. If biscuit cohesiveness was due to lipids, it would be maintained in water. Therefore, biscuit cohesiveness cannot be attributed to lipids. As for short dough, biscuit cannot be considered as a suspension of flour particles in a lipid matrix.

Starch Contribution. Bread and pasta samples retain their structures in water but baked samples appear swollen and less rigid than crude samples. Champenois et al. (1995) reported that starch solubility in water varies from 6 to 10% db and swelling from 1.2 to 6.0 g/g

of dry matter for bread dough and crumb, respectively. That agrees with our observations. However, even though starch solubility and swelling are higher, particularly in the baked product, the water-insoluble gluten network is strong enough to maintain the macroscopic structure of these samples in water.

Short dough and biscuit samples lose their structures in water. Starch solubility of these samples in water is lower than 1% (Table 2), and consequently starch is not involved in the cohesiveness of short dough and biscuit samples. Starch granules swelling might contribute to biscuit structure bursting, but short dough loses its structure even though no starch swelling occurs. Moreover, swelling values remain low compared to those reported for bread.

Sugars Contribution. Short dough in water loses its structure quickly whereas it remains intact in ethanol. At ambient temperature, 1 g of sucrose is solubilized by 170 mL of ethanol, while only 0.5 mL of water is necessary for a complete solubilization. In our experimental conditions, sugar solubilization is therefore limited in ethanol and complete in water. Samples destructuration can be therefore attributed to sugars solubilization in water. Consequently, sugars should be involved in short dough and biscuit cohesiveness.

CONCLUSION

Concerning short dough, the results presented here demonstrate that protein and starch are not involved in its cohesiveness and that lipids may be involved but are not solely responsible for it. Consequently, sugars are the remaining components that should be involved. In that pattern, dough structure could be presented as a suspension of proteins, starch-protein associations and isolated starch granules in a liquid continuous phase being based on an emulsion of lipids in a concentrated sugar solution (Figure 2b). Baltsavias et al. (1997) studied the rheological properties of short doughs in dynamic shear experiments at small deformation. They concluded that short doughs are bicontinuous systems: a fat phase and a nonfat phase made up of a saturated sucrose solution surrounding and bridging flour/starch particles.

As for dough, on the basis of the results presented here, cohesiveness of the biscuit structure cannot be attributed to only one of these components, starch, proteins, or lipids. Consequently, cohesiveness of the biscuit structure after baking could be attributed to sugars. They melt during the thermal treatment and could form bridges between starch−protein particles. The biscuit structure could therefore be defined as a composite matrix made of protein aggregates, lipids, and sugars, embedding starch granules (intacts or little damaged). The structure cohesiveness would be achieved by sugars that melt during baking and become glassy during cooling. They would form bridges between aggregates and lipids, and even a continuous phase of molten sugars embedding proteins, starch granules, and lipids (Figure 2c). This hypothesis is reinforced by calculation of volume fractions occupied by each anhydrous component from the biochemical composition of dough and the density of each component. In that case, starch would take up 50.3% of the total volume, sugars 25.3%, lipids 18.0%, and proteins only 6.4%.

Sugars are involved in the development of both color and texture of the biscuit during baking. Proteins are known to play a role during mixing by their spatial rearrangement and consequently, during baking, on product dimensions (Wade, 1988; Bloksma, 1990). Further studies are therefore needed to determine more accurately the role of these components on structure formation during baking.

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