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The role of gluten in a pound cake system: A model approach based on gluten–starch blends

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ABSTRACT

In order to evaluate the role of gluten in cake-making, gluten–starch (GS) blends with different ratios of gluten to starch were tested in a research pound cake formula. The viscosities of batters made from commercial GS blends in the otherwise standardised formula increased with their gluten content. High viscosities during heating provide the batters with the capacity to retain expanding air nuclei, and thereby led to desired product volumes. In line with the above, increasing gluten levels in the cake recipes led to a more extended oven spring period. Cakes with a starch content exceeding 92.5% in the GS blend suffered from substantial collapse during cooling. They had a coarse crumb with a solid gummy layer at the bottom. Image analysis showed statistical differences in numbers of cells per cm^2, cell to total area ratio and mean cell area ($p < 0.05$). Both density and mean cell area were related to gluten level. Moreover, mean cell area and cell to total area ratio were the highest for cakes with the lowest density and highest gluten levels. Relative sodium dodecyl sulfate (SDS, 2.0%) buffer (pH 6.8) extractabilities of protein from cakes baked with the different GS blends decreased with gluten content and were strongly correlated with the intensity of collapse. Taken together, the results teach that protein gives the cakes resistance to collapse, resulting in desirable volumes and an optimal grain structure with uniform cell distribution.

1. Introduction

Past research investigating the role of flour components on the baking properties of cake has focused either on the starch or the gluten components. Donelson and Wilson (1960a) have ascribed a greater importance of protein than of starch in the formation of cake structure. They produced cakes from reconstituted flours and found that, as the percentage of gluten in the recipe increases, cake volume reaches a maximum and then decreases. Donelson and Wilson (1960b) stated that, in cake, the gluten serves a function as a binder, rather than as a structural element such as in bread, where it makes up the basic framework. Most authors agree that, during cake mixing, full development of gluten is not required (Huebner, Bietz, Nelsen, Bains, & Finney, 1999; Willhoft, 1973). It is undisputed that, due to the high levels of fat and sugar, the development of a gluten network is limited in the cake batter. However, this does not imply that the gluten is functionally inert. Especially during baking, protein interactions may become important for cake structure. Howard, Hughes, and Strobel (1968), as a result of their study of cakes in which the flour component was replaced by pure starch, found it advisable to supplement the starch with polyvalent cations, soluble protein and surface-active lipids, to obtain a performance similar to that of flour. Differences in cake-baking performance and collapse of the structure have also been attributed to differences in starch characteristics. Miller and Trimbo (1965) showed the importance of early gelatinisation for cake quality. Howard et al. (1968) demonstrated the importance of intact granular starch to cake structure. Sollars and Rubenthaler (1971) compared different starches in cake baking. Baking performance varied with granule size, gelatinisation temperature, as well as the water requirement of the starch. Derby, Miller, Miller, and Trimbo (1975) found that differences in the degree of starch gelatinisation influence the product volume. For cake, a greater contact and interaction between starch and water resulted in higher volumes. According to Kim and Walker (1992), variations in cake-baking performance can be related to an optimum cake setting point by gelatinisation temperature control. Also, a sufficiently rigid starch gel has to be formed during baking to prevent collapse of the cake during cooling.

Most information in the literature on the quality-determining factors of cake flour has been obtained using different flour types (Kaldy, Rubenthaler, Kereliuk, Berhow, & Vandercook, 1991;

Abbreviations: ANOVA, analysis of variance; DSC, differential scanning calorimetry; GS, gluten–starch; RVA, Rapid Visco Analyser; SDS, sodium dodecyl sulfate; SE-HPLC, size-exclusion high performance liquid chromatography.

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Olewnik & Kulp, 1993). However, flour types seldom only vary in their ratio of gluten to starch, but also in other parameters such as protein properties, the level of damaged starch and other characteristics. Gluten–starch (GS) blends proved very useful for studying the functionality of gluten and starch in bread-making (Watanabe, Larsson, & Eliasson, 2002), the way they are affected by non-starch polysaccharides (Delcour, Vanhamel, & Hoseney, 1991), as well as for understanding dough rheological properties (Dreese, Faubion, & Hoseney, 1988a, 1988b; Miller & Hoseney, 1999; Petrofsky & Hoseney, 1995; Watanabe et al., 2002).

In order to increase our current understanding of the role of gluten protein in cake-making, here we set out to use a model mixture of gluten and prime starch. This, we believed, would help us to not only control the protein content, but also reduce the complex interactions of other flour constituents, such as arabinoxylan and soluble proteins (including enzymes).

To determine pound cake quality, volume was taken into account. Because of the importance of physical cake batter properties for the characteristics of the resulting cakes (Shelke, Faubion, & Hoseney, 1990), viscosity measurements were performed during model baking. Furthermore, image analysis was used to study the influence of gluten on the crumb grain of the cake. To the best of our knowledge, only one (recent) report is available about the use of image analysis for the study of pound cake crumb (Sanchez-Pardo, Ortiz-Moreno, Mora-Escobedo, Chanona-Perez, & Necoechea-Mondragon, 2008). We related gluten concentrations to the changes in cake batter, properties during baking and final cake quality. Size-exclusion high performance liquid chromatography (SE-HPLC) was used to study the protein population in the resulting cakes.

2. Experimental

2.1. Materials

Wheat starch [10.9% moisture, 0.24% protein (N × 5.7), 2.0% damaged starch] and dry vital wheat gluten [6.62% moisture, 78.0% protein (N × 5.7), 0.5% arabinoxylan, 10.6% starch, 5.0% damaged starch] were also used by Pareyt, Wilderjans, Goesaert, Brijs, and Delcour (submitted for publication) and were provided by Tate and Lyle (Aalst, Belgium). Moisture contents were determined according to AACC Approved Method 44-19 (AACC, 1983). Protein contents were determined using an adaptation of the AOAC Official Method (AOAC, 1995), with an automated Dumas protein analysis system (EAS vario Max C/N, Elt, Gouda, The Netherlands), with 5.7 as the conversion factor. Starch and arabinoxylan levels were determined by gas chromatography, as described by Courtin and Delcour (1998). Starch damage was determined according to the Megazyme (Bray, Ireland) procedure (AACC Method 76-31). All contents were expressed on a dry matter basis.

Commercial sugar (700 μm average crystal size) was donated by Lotus Bakeries NV (Lembeke, Belgium). The margarine (19.0% moisture) was from Puratos NV (Groot-Bijgaarden, Belgium). Sodium bicarbonate (BICAR®) was obtained from Solvay Chemicals International NV (Brussels, Belgium) and sodium pyrophosphate from Acatris Food NV (Londerzeel, Belgium). Salt and eggs were commercial grade products. The protein contents of the eggs were determined as outlined above, starting from lyophilised eggs. In this case, we used a nitrogen to protein conversion factor of 6.25, as used by Johnson, Havel, and Hoseney (1979), and Raeker and Johnson (1995), to take into account the differences in amino acid composition between flour and egg protein.

Model flours were prepared by dry blending the wheat starch for 24 h with gluten, in ratios of 95% to 5%, 92.5% to 7.5%, 90% to 10%, 87.5% to 12.5% and 85% to 15%, based on dry weight starch and gluten, with a Chopin MR2L mixer (Villeneuve La Garenne, France). From the preliminary experiments, 24 h proved to be sufficient to enable perfect homogenisation and moisture equilibration. The thus-obtained GS blends were encoded as *Gx*, where *G* represents gluten and *x* refers to the percentage of gluten in the mixture.

Chemicals and reagents were from Sigma-Aldrich (Steinheim, Germany), unless specified otherwise, and of at least analytical grade.

2.2. Cake batter preparation

Pound cake was prepared using the GS blends. One hundred grams of margarine, 100 g of sugar, 100 g of fresh eggs, 1.0 g of salt and 2.0 g of baking powder (sodium bicarbonate and sodium pyrophosphate in a 4:3 ratio) were used per 100 g of GS blend (14.0% moisture). Deionised water was added to correct the moisture content of the GS blends.

First, margarine and sugar were mixed for 3 min at speed level 6 in a kitchen-aid electric KPM5 mixer (St. Joseph, MI). Then, fresh eggs as well as the additional water necessary to bring the moisture content were poured into the mixer of the GS blends to 14.0%. After 30 s the GS blends, salt and baking powder were added. After another 4 min of mixing, 250 g of batter was placed into baking forms (length 180 mm, width 76 mm, internal height 50 mm). Batter density was determined with a 100 ml container. The means of the duplicate readings are reported.

2.3. Baking procedure

The cakes were baked in a rotary oven (National Manufacturing Company, Lincoln, NE) at 175 °C for 45 min. For each GS mixture, two batches of 6 cakes were baked. The change in centre height during baking was monitored with a camera (Canon Powershot S50, Canon, Machelen, Belgium) mounted on the oven door, taking photographs at 2 min intervals. A ruler was placed behind the baking form and allowed to monitor the height. Oven spring was defined as the difference in cake height at the start of baking and the maximum height reached during the process. Collapse during cooling was calculated by subtracting the height after cooling from the maximum height noted in the oven. After cooling for 2 h to room temperature, cake weights (g) and rapeseed displacement volumes (cm^3) were measured. Duplicate bakings of 6 loaves led to volume differences not exceeding 5%.

2.4. Cake batter viscosity

The Rapid Visco Analyser (RVA-4D, Newport Scientific, Sydney, Australia) was used to study the viscosity properties of cake batter during simulated baking. First, batter temperatures at the centre during conventional oven-baking were measured by thermocouples connected to a digital readout. The obtained temperature profile was then used to establish the heating profile to 20 g cake batter in the RVA (Fig. 1). The temperature profile included a holding step of 5 min at room temperature, a linear temperature increase from room temperature to 95 °C at 3.5 °C/min and a holding step of 10 min at 95 °C. The RVA converts the current required to maintain constant mixing speed (75 rpm) of a paddle into a viscosity value in poise (P; 0.1 $kg\,m^{-1}\,s^{-1}$), the unit of dynamic viscosity. All RVA analyses were performed at least in triplicate.

2.5. Texture analysis

After cooling the cakes to room temperature, each cake was cut into 4 slices with a thickness of 2.5 cm. Cake texture analysis was

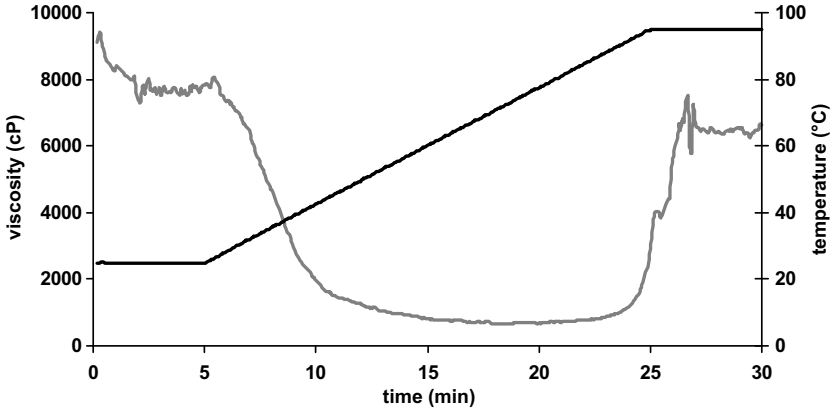

Fig. 1. Batter viscosity *versus* temperature plot for cake batter, measured with RVA.

performed on a TAXT2i texture analyser (Stable Micro Systems Ltd., Surrey, UK) with a 5 kg load cell. During the deformation test, a cylindrical probe (25 mm) compressed the cake slices while the force–time curve was recorded. The cake slices were tested under vertical compression at a constant speed, using test conditions as described by Megahey, McMinn, and Magee (2005). Firmness was defined as the force required for compressing the product by 25%, which is the peak force on the force–time curve. Springiness is derived from the ratio of the force required to hold the samples in compression to 75% of their original height for 60 s, to the peak force on the force–time curve. Texture analysis was carried out on 12 samples for each recipe.

2.6. Image analysis of crumb grain

Six pound cakes were sliced at the centre with an electric slicer (Affettatrice Slicer 30N, Galesecca, Italy) yielding, for each cake, 3 slices with a thickness of 1.0 cm. This cutting method has been proved reproducible and accurate, based on the reports by Crowley, Grau, and Arendt (2000), Gonzales-Barron and Butler (2006), and Zghal, Scanlon, and Sapirstein (1999). For each recipe, 18 samples were placed on a flatbed scanner (HP Scanjet 3800, Hewlett–Packard Company, Beijing, China). One field of view with an area of 40×40 mm at the centre of the slice was evaluated for each image. Images had a resolution of 300 dots per inch (dpi) and were stored in bmp format of 473×472 pixels. The Otsu thresholding algorithm (Otsu, 1979) was used as an Image J plug-in for image segmentation (conversion to a binary image). This is one of the most commonly used techniques for optimal thresholding (Gonzales-Barron & Butler, 2006). Crumb cell detection was conducted on the binary images with the image processing toolbox of Matlab 6.1 (Mathworks, Natick, MA), as described by Lagrain, Boeckx, Wilderjans, Delcour, and Lauriks (2006). Three crumb grain features were selected for comparison: mean cell area, number of cells per cm^2 and cell to total area ratio.

2.7. Defatting of cake samples

Cake and batter samples were freeze-dried and ground in a laboratory mill (IKA, Staufen, Germany). Samples (1.0 g) were shaken with 10.0 ml hexane in a 30 ml test tube for 60 min. Hexane was removed and the extraction repeated. Finally, samples were dried under a stream of nitrogen.

2.8. Size-exclusion HPLC

Size-exclusion HPLC was conducted as described by Lagrain, Brijs, Veraverbeke, and Delcour (2005), using a LC-2010 system (Shimadzu, Kyoto, Japan) with automatic injection. Defatted batter and cake samples containing 1.0 mg of protein were extracted with 1.0 ml of a 0.05 M sodium phosphate buffer (pH 6.8) containing 2.0% sodium dodecyl sulfate (SDS, Acros Organics, Geel, Belgium). The extracts were loaded (60 μl) on a Biosep-SEC-S4000 column (Phenomenex, Torrance, CA). The elution solvent was acetonitrile/water (1:1, v/v) containing 0.05% (v/v) trifluoroacetic acid. The flow rate was 1.0 ml/min at a temperature of 30 °C (Veraverbeke, Larroque, Bekes, & Delcour, 2000) and eluted protein was detected at 214 nm. Total SDS-extractable protein of the cake and batter samples was expressed as a percentage of the peak area of the chromatogram obtained following the extraction of unheated batter with the SDS buffer in the presence of 1.0% dithiothreitol (Acros Organics, Geel, Belgium) and 2.0 M urea. Deviations were smaller than 2%.

2.9. Differential scanning calorimetry (DSC)

DSC was performed with a DSC Q1000 (TA Instruments, New Castle, DE). Simplified pound cake recipes were used to determine the temperature range of starch gelatinisation and egg denaturation during baking. Egg and flour were left out of the recipe, and a correction for moisture content was made. After mixing, the batter samples (*ca.* 8.0 mg) were accurately weighed into coated aluminum sample pans. The pans were sealed and equilibrated at 0 °C before heating (together with an empty reference pan) from 0 °C to 140 °C at 4 °C/min. Calibration was with indium and tin. The temperature ranges were determined with TA Q Series Advantage Universal Analysis software.

2.10. Statistical analysis

Statistical analysis was conducted using analysis of variance (ANOVA). Significant calculated mean values were compared using Student's *t*-test at $\alpha = 0.05$ level of significance.

3. Results and discussion

3.1. Batter viscosity

Table 1 shows batter characteristics for each mixture. The batter densities were the highest for G5 and G7.5, the batters with the highest starch content. This suggests that these batters had the lowest levels of incorporated air. Similar observations were made by Pareyt et al. (submitted for publication) for cookie doughs. Increased protein levels increased the initial viscosity of the batters (Table 1). The increase in viscosity is in line with wheat protein

Table 1
Batter characteristics of cake batters prepared with five different GS blends

Batter type	Batter density (g/cm^3)	Initial viscosity (cP)	Minimal viscosity (cP)	Temperature at minimal viscosity (°C)
G5	0.85 ± 0.01a	9.068 ± 70b	480 ± 23c	74.3 ± 0.24a
G7.5	0.87 ± 0.02a	9.051 ± 470b	552 ± 70c	74.6 ± 1.80a
G10	0.82 ± 0.01b	10.042 ± 706ab	739 ± 39b	75.9 ± 0.50a
G12.5	0.83 ± 0.02b	10.096 ± 458a	740 ± 36b	76.0 ± 1.41a
G15	0.81 ± 0.02b	10.224 ± 470a	1,002 ± 21a	76.4 ± 1.23a

Viscosity measurements were performed with the Rapid Visco Analyser (RVA). Values followed by the same letter in the same column are not significantly different ($p < 0.05$).

being the main cause of batter viscosity development during mixing (Loewe, 1993). A high batter viscosity prevents the loss of air and provides increased batter stability at room temperature.

In "baking" experiments in the RVA, batter viscosity was monitored during heating. Batter viscosity *versus* temperature plots (Fig. 1) illustrated a decrease in viscosity early in heating and a subsequent rapid increase at 85–95 °C, which was due to the combination of starch gelatinisation (temperature range 80–95 °C) and egg protein denaturation (temperature range 70–100 °C) (DSC results not shown).

As well as initial batter viscosities, minimal batter viscosities increased for the different GS blends with gluten content (Table 1). For the accompanying temperatures at minimal viscosity during baking, an increasing trend from 74 °C to 76 °C was reported, but the results are not statistically different (Table 1). This confirms that the viscosity increased earlier in the presence of higher starch contents.

For a related model system, Loewe (1993) found that viscosity is essential to a hydrated batter, as it helps suspending ingredients that are insoluble at ambient temperatures, preventing the formation of different layers with varying properties. He also reported that the absorption capacity of flour protein provides a viscous medium ensuring the even distribution of starch over the batter and aids in maintaining uniform dispersion of the ingredients for optimum performance.

3.2. Time-lapse photography and cake quality

Cake height increased faster when the recipe contained more gluten (Fig. 2). After 24 min (corresponding to a temperature of 91 °C at the cake centre), the expansion rate decreased for all the cakes. Cake structure may have been setting from this point onwards, because viscosity then increased sharply, as indicated in the RVA analyses (Table 1). As already mentioned above, this rapid viscosity increase is most likely due to the combination of starch gelatinisation and egg denaturation.

Mizukoshi, Maeda, and Amano (1980), for sponge cakes, reported that gas release, protein coagulation, starch gelatinisation and cessation of batter expansion all take place at the same temperature. They concluded that the structure formation of cakes during baking is related to the viscosity increase caused by the combined effects of starch gelatinisation and protein denaturation. In the present work, the temperatures at minimal viscosity and, hence, the onset of viscosity increase during heating, showed an increasing trend with gluten contents (Table 1). The structures of cakes with higher starch contents set earlier, and were thus unable to expand as long as the other cakes. This resulted in lower oven spring values, but the results were not statistically different (Table 2). During baking, the cakes with higher gluten content reached a higher maximum height. This was presumably not only due to later setting, but also due to higher batter viscosities during baking. Most of the air bubble sites for carbon dioxide gas and water vapour expansion were lost from the thin cake batters before the setting temperature was reached. Our results point out that minimal viscosity is related to the cake volume ($r^2 = 0.77$). This agrees with the results of Shelke et al. (1990), that high viscosities during heating allow the batter to retain expanding air nuclei, and thereby improve cake volume.

Table 2 lists the performance extremes of G5 and G7.5 of the GS blends in the recipes. These cakes had a very low volume and coarse grain structure with a dense, gummy layer at the bottom. It was reported earlier that such layers in cake have a greater density, a higher moisture content and more gelatinised starch than regular cake crumb (Miller, Trimbo, & Sandstedt, 1967). In the

Table 2
Characteristics of cakes prepared with five different types of GS blends

Cake type	Oven spring (%)	Collapse (%)	Cake density (g/cm^3)	Firmness (N)	Springiness
G5	25.8 ± 0.9a	10.98 ± 0.09a	0.346 ± 0.007a	3.60 ± 0.23a	0.505 ± 0.024a
G7.5	25.9 ± 0.7a	9.88 ± 0.06a	0.336 ± 0.020a	3.48 ± 0.60a	0.513 ± 0.018a
G10	25.4 ± 0.6a	8.70 ± 0.07b	0.309 ± 0.008b	3.05 ± 0.49b	0.499 ± 0.005a
G12.5	27.7 ± 1.2a	8.49 ± 0.32b	0.291 ± 0.003c	3.06 ± 0.24b	0.495 ± 0.008a
G15	28.1 ± 0.6a	7.83 ± 0.26b	0.294 ± 0.004c	3.06 ± 0.26b	0.486 ± 0.004a

Values followed by the same letter in the same column are not significantly different ($p < 0.05$).

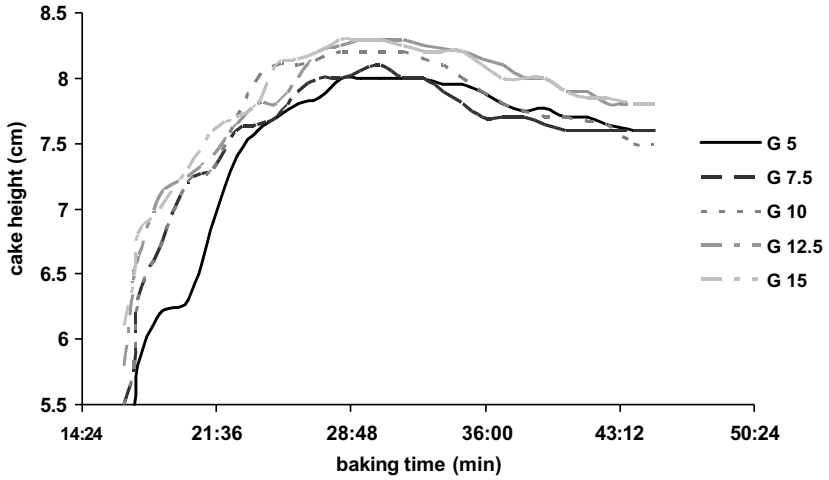

Fig. 2. Cake height *versus* baking time for cakes baked with five different GS blends.

present case, only the cakes with the highest starch content showed a gummy layer, which correlates with the ability of gluten to absorb water and to form a stable structure that keeps the starch granules dispersed in the batter.

Miller et al. (1967) found that layer cakes with a gummy layer collapse. They observed that, during baking, first a gummy layer develops at the bottom, and that, thereafter, a band of air cells forms along the top of the batter. According to the authors, this weak structure is the reason for collapse of the baked cakes upon cooling (Table 2). For cakes not having a gummy layer, gluten also increased cake volume and suppressed collapse. Taken together, it can be stated that G5 and G7.5 gave the lowest volume and the highest firmness, while G12.5 and G15 gave the best results (Table 2).

3.3. Image analysis

Fig. 3 shows examples of images from each cake type. A visual assessment already indicated a clear distinction between the crumbs of G5 and G7.5 and those of the other cakes. In line with baking test results, substantial differences were found between the crumb grain features of the different cake types. ANOVA showed the differences in image characteristics to be significant. Crumbs of cakes baked with G5 and G7.5 had smaller pores and a non-homogeneous appearance. Gluten improved the grain structure of the cakes, giving a uniform cell distribution as well as higher volumes. The mean cell area increased from 0.16 to 0.24 mm^2 with higher gluten content (Table 3) and was inversely related to the cake density ($r^2 = 0.87$). In line with the above, the mean cell area was significantly lower and the number of cells per cm^2 significantly higher for G5 and G7.5. This can be ascribed to the higher collapse, resulting in lower cake volumes. Table 3 shows no clear trend for the cell to total area ratio. This parameter is determined by the combination of mean cell area and number of cells per cm^2 and thus can be the same for either a crumb with lots of small cells (e.g., G7.5) or one with few large cells (e.g., G12.5 and G15).

Other image analysis studies on the crumb structure of bakery products allow us to conclude that the three-dimensional structure of a food is the result of the organisation and interaction of its protein and starch components. To date, image analysis in cereal research has been used to quantify bread crumb grain features (Crowley et al., 2000; Lagrain et al., 2006; Sapirstein, Roller, & Bushuk, 1994; Zghal et al., 1999) and to compare crumb microstructure of pound cakes (Sanchez-Pardo et al., 2008). The cell to total area ratio (Table 3) is consistent with values reported for pound cakes which ranged from 34% to 46%, depending on the position in the cake (Sanchez-Pardo et al., 2008). The values of mean cell area are somewhat lower than those found in the same paper, which can be due to the difference in recipe and resulting crumb structure of the cakes.

3.4. Protein extractabilities

In the present case, the protein component of the cake batters and cakes originates not only from gluten but also from the eggs used. Indeed, whereas egg protein content determined by Dumas analysis was 16.8%, and that of the gluten used was 78.0%, the relative ratios between egg and gluten proteins ranged from 4.1 to 1.4 for the cakes baked with the G5 to G15 blends, respectively.

Fig. 4 shows the relative protein extractabilities in SDS buffer for cakes and batters, prepared with the different GS blends, calculated from SE-HPLC data. For all the cakes, a major decrease in protein extractability during baking was observed. For the different cake types, protein extractability decreased with increasing gluten:egg ratios from 25% to 11%, as a percentage of the total protein present (Fig. 4). Protein interactions and reactions in cake crumb thus seem to be more effective at higher gluten concentrations.

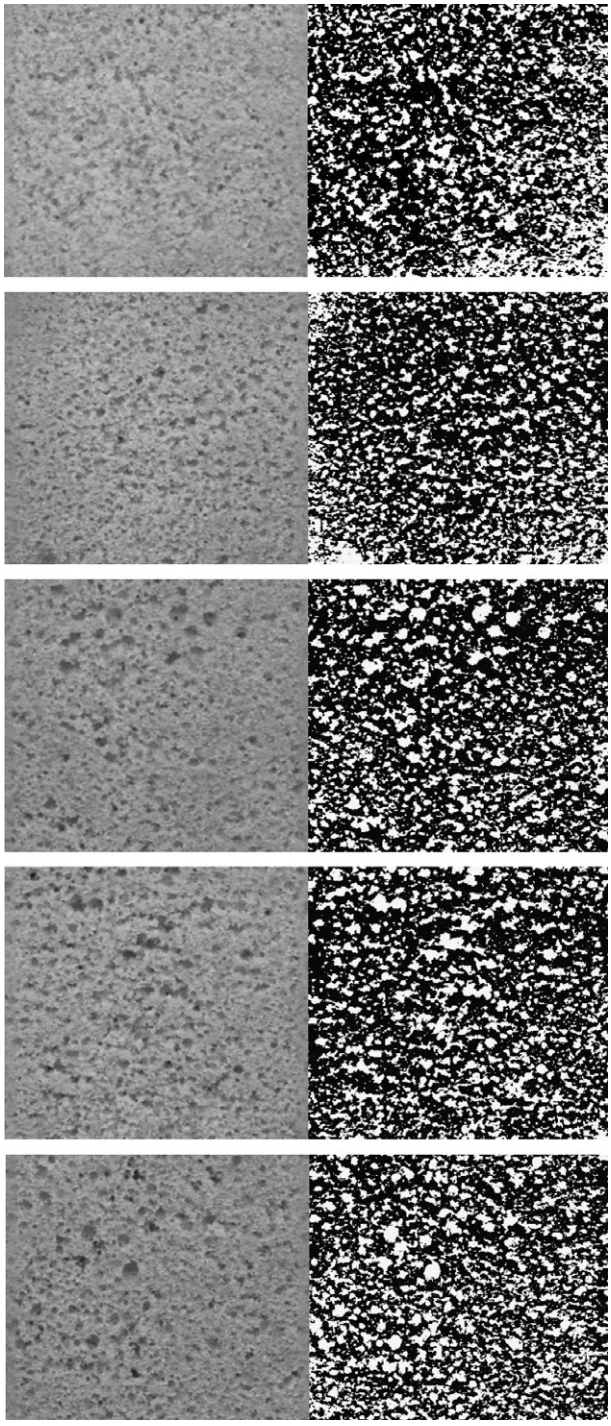

Fig. 3. Left: Gray-level images of 40 × 40 mm field of view of cakes baked with G5, G7.5, G10, G12.5, G15, respectively, from top to bottom. Right: Binary images of cakes baked with G5, G7.5, G10, G12.5, G15, respectively, from top to bottom, segmented with the Otsu Thresholding algorithm.

This is in line with the lower protein extractabilities found in gluten–starch-based cookies with higher gluten contents (Pareyt et al., submitted for publication), which indicates a more efficient aggregation of proteins when less starch is present.

A similar decrease in protein extractability, due to protein aggregation and cross-linking, was already demonstrated for bread (Borneo & Khan, 1999; Lagrain, Thewissen, Brijs, & Delcour, 2007; Veraverbeke, Courtin, Verbruggen, & Delcour, 1999) and cookie baking (Pareyt et al., submitted for publication; Pomeranz,

Table 3
Digital image analysis of crumb grain characteristics of cakes prepared with five different types of GS blends

Cake type	Mean cell area (mm^2)	Cells/cm^2	Cell to total area ratio
G5	0.161 ± 0.016d	187.7 ± 6.8a	0.34 ± 0.02b
G7.5	0.176 ± 0.012c	187.6 ± 9.5a	0.36 ± 0.03a
G10	0.202 ± 0.017b	135.6 ± 9.4b	0.39 ± 0.03a
G12.5	0.239 ± 0.010a	138.2 ± 6.3b	0.36 ± 0.01a
G15	0.243 ± 0.006a	138.4 ± 6.2b	0.37 ± 0.01a

Values followed by the same letter in the same column are not significantly different ($p < 0.05$).

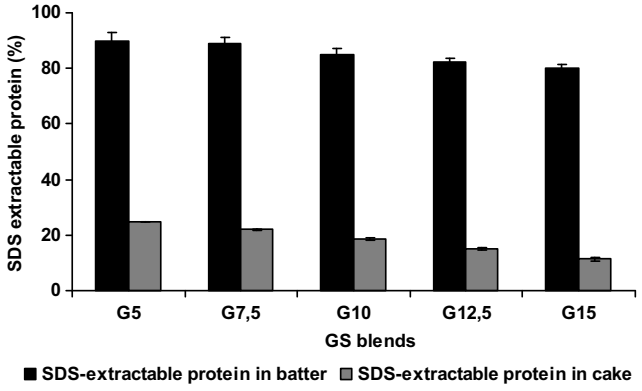

Fig. 4. Extractabilities (%) of protein in 2.0% SDS buffer, for cakes baked with different GS blends, calculated from SE-HPLC data, expressed as a percentage of total protein in the batter.

Lookhart, Rubenthaler, & Albers, 1989). Also, when heated above 70 °C, both egg white and yolk proteins become involved in disulfide bond bridges (Kiosseoglou, 2004). Therefore, the final cake structure can be regarded as a mixed gel network system based on gluten development, but significantly modulated by the heat-setting ability of the egg proteins (Kiosseoglou & Paraskevopoulou, 2006). In this study, because the egg content remained constant for the different cake types, the results are solely attributed to differences in gluten protein contents.

The varying protein aggregation properties may account for differences in cell wall characteristics. A strong correlation was found between protein extractability and collapse data for cakes made with the five different GS blends ($r^2 = 0.92$). These results suggest that protein provides the cell walls with structural material and a higher resistance to collapse. Bread loaves shrink upon cooling when the crumb does not show evidence of cracks in the gas cell membranes. When gas discontinues, the loaves shrink, due to negative internal pressure created by cooling (Kusunose, Fujii, & Matsumoto, 1999). Possibly, this also holds for cakes. Indeed, changes in the protein fraction can be expected to result in stiff cell walls, causing cracks in some cell walls and resulting in a gas continuous crumb. Table 2 shows a decreasing trend in springiness values for the cakes with higher gluten contents, which indicates higher rigidity of the cell walls and possibly gas continuous cake structures with little collapse (Table 2).

4. Conclusions

This study on pound cakes, prepared with five different GS blends starting from non-chlorinated starch, generated the following conclusions. First, increasing levels of gluten improved cake quality *inter alia* by providing the cake batter with sufficient viscosity at mixing and the early baking stages. The absorption capacity of flour protein provides viscosity and gives the batter a capacity to retain expanding air nuclei and to ensure the uniform dispersion of the ingredients for optimum performance.

Moreover, gluten increased cake volume and, at the same time, suppressed collapse. SE-HPLC results showed that, during pound cake baking, protein extractability decreased substantially and for the resulting cakes, relative protein extractability decreased with increasing gluten concentrations. Protein interactions and reactions in cake crumbs provided the cell walls with a resistance to collapse.

Finally, gluten improved the grain structure of the cakes, giving a uniform cell distribution as well as better volumes.

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