Effect of the Dough Mixing Process on the Quality of Wheat and Buckwheat Proteins

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Abstract


The changes in the structure of cereal proteins during the mixing of flour into dough was described and evaluated. Wheat gliadins and glutenins (gluten proteins) have unique physical properties and play an important role in breadmaking. The effect of mixing time on the formation and the structure of the gluten network was determined using scanning electron microscopy (SEM). Buckwheat flour (gluten-free) was used to compare the development of structure during the mixing process.

Keywords: dough microstructure; farinograph; gluten; kneading; gluten; SEM

In general, wheat is the most widely used cereal for bread and bakery production processes throughout the whole world (DELCOUR & Hoseney 2003; KHAN & SHEWRY 2009; SHEWRY et al. 2009). Other cereals such as rice, corn, millet and sorghum are of considerable importance outside Europe; however, they do not reach the importance of wheat grain and do not possess the same baking quality. Wheat endosperm contains storage proteins with specific compositions, structures and properties that allow them to form complex viscoelastic structures (three-dimensional network) during dough mixing (GRUNDAS 2003; SHEWRY & LOOKHART 2003; WRIGLEY et al. 2006). During mixing, flour and other recipe components are hydrated and there is an input of mechanical energy (BUSHUK 1998). The gluten network retains fermentation gas, can determine the stability of gas cells during expansion, contributes to soft (sponge) and flexible (elastic) crumb and also influences the characteristic appearance of wheat-based bread and baked products (VAN VLIET et al. 1992; WRIGLEY et al. 2006).

Wheat gluten is formed by prolamins (gliadins) and glutelins (LÁSZTITY 1984; DELCOUR & HOSENEY 2003; SHEWRY 2003). The average molar mass of gliadins is about 30–80 kg/mol. The structure of gliadins is that of a single-chain macromolecule which is composed of helix (coil) and random bends (loop, unbonded regions). A large number of hydrogen bonds (trains) stabilise the helix, while intramolecular disulfide bonds (S-S) hold random bends together in gliadin macromolecules. Glutenins have a greater relative molar mass than the gliadins (typically from 70 kg/mol to 20 000 kg/mol, usually about 2000 kDa). Unlike gliadins, the structure of glutenins is formed of many differently long chains linked mainly by intermolecular (interchain) disulfide bonds. HAMER and VAN VLIET (2000) described a model of glutenin in dough based on the presence of large glutenin aggregates (the particle-gel model).

Wheat gliadins and glutenins are practically insoluble in water. Wheat gluten proteins can be isolated from dough by water-washing (a quantitative
A complex interplay between mechanical and chemical changes occurs in the glutenin protein network during mixing (Hamer & Van Vliet 2000). Mixing can be regarded as a series of breakdown-and-recovery processes. The protein concentration and the number of interactions then determine the extent and speed of the aggregation. S-S formation serves to stabilise the aggregated formed. It is clear from many studies that reactions involving sulphydryl (SH) and S-S groups take place during mixing. During mixing, disulfide bonds are formed by oxidation of sulphydryl groups of proteins; these are the strongest bonds forming the gluten structure. The number of these bonds is not high, but they significantly contribute to the structure of the gluten network. Mixing is a complex process that depends on mixer geometry and speed (Jongen et al. 2003). Duration of mixing, temperature of water, type and shape of mixer, shear stresses, and energy input are the important parameters of mixing.

Common buckwheat and Tartary buckwheat are the most famous pseudocereals and are grown mainly in Russia, China, Japan, Ukraine, and Poland (FAOSTAT 2017). Compared to wheat, buckwheat proteins have higher biological value, mainly because of their higher content of lysine and well-balanced composition of essential amino acids (Mota et al. 2016). The protein composition of buckwheat is characterised by a high proportion of albumins and globulins and a very low content of gliadins and glutelins. For this reason, buckwheat is a gluten-free product used for patients with gluten sensitivity (Cai et al. 2004; Khan et al. 2012). The lack of a protein network is due to the absence of gluten in buckwheat protein. Replacement of the protein network in buckwheat products can be achieved in different ways (protein cross-linking induced by alkaline conditions, starch network formed during extrusion, the addition of xanthan and HPMC as gluten substitutes, dairy or egg proteins, etc.) (Gallagher et al. 2003; Ahlborn et al. 2005; Guo et al. 2017).

The aim of the experiments described here was to observe the network of gluten proteins formed in wheat dough at different stages of the mixing process. The stages of dough mixing that were studied included the beginning (undeveloped dough), after the achievement of optimal consistency (optimally developed, standard dough) and after a decrease of consistency (overmixed dough) and were determined by the curve on a farinograph. Doughs were frozen immediately after mixing at each stage and were
subsequently observed using a scanning electron microscopy. In order to compare the protein structures in gluten (wheat) and gluten-free flours (or rather doughs) at different stages of mixing, the dough from buckwheat flour was prepared and measured in the same way as wheat.

MATERIAL AND METHODS

White wheat flour (T530) and white buckwheat flour (both Czech Republic) were used. Moisture content (ICC Standard No. 110/1), ash content (ICC Standard No. 104/1) (protein content using the Kjeldahl method (factor 5.7) (ICC Standard No. 105/2), protein quality using the Zeleny sedimentation test (ICC Standard No. 116/1) and solvent retention capacity profile (AACC 56-11) were determined in wheat and buckwheat flour. Doughs were prepared on a farinograph (Brabender) (ICC Standard No. 115/1; according to the constant flour weight procedure) and were sampled at different stages of the mixing of doughs.

The farinograph measures and records the consistency of dough during the mixing process from the beginning of mixing, to the achievement of optimal consistency, the phase of stability, up until overmixing (decreased consistency). Water absorption of the flour and mixing behaviour of the dough were determined.

The optimally developed wheat dough was obtained after 6 min of dough development, the undeveloped wheat dough after 2.5 min of dough development and the overmixed wheat dough after 22 min of mixing. The optimally developed buckwheat dough was obtained after 10 min of dough development, the undeveloped buckwheat dough after 6.5 minutes of dough development and overmixed buckwheat dough after 17 min of mixing. Farinograph mixed doughs (the complete weight of doughs was 300 g) were divided into four parts (the weight of each came to about 70 g). Dough parts were placed in a plastic bowl, transferred to the freezer and frozen immediately (final dough temperature of –18°C after 2 h).

The microstructures of prepared wheat and buckwheat doughs (optimally developed, undeveloped and overmixed doughs) were observed using scanning electron microscopy (SEM) (scanning electron microscope TESCAN VEGA3 LMU with a tungsten cathode and BSE detector). The information from the back-scattered electrons was recorded. Before SEM measuring, a thin plate of frozen dough (average length of approx. 5 mm, and average width of 2 mm) was cut out using a scalpel. The plate of frozen dough was fixed to stubs with carbon tape, covered with a layer of gold of 5 nm and placed into the microscope on an aluminium sample holder. Measurements were performed in Univac mode at a pressure of 10 Pa and acceleration voltage of 20 kV. The resulting micrographs showed the structural arrangement at different stages of dough mixing (optimally developed, undeveloped and overmixed dough).

RESULTS AND DISCUSSION

Determination of wheat and buckwheat flour quality. The values of moisture, ash, protein content, protein sedimentation quality (Zeleny test) and the solvent retention capacity profile (SRC) for wheat flour (T530) and buckwheat flour are presented in Table 1.

The results of the chemical analysis were expressed as the mean values of four repetitions. Standard error of the mean was obtained by correlation analysis (Microsoft Excel 2010) with a significance level of 0.01%.

Buckwheat flour had a higher content of ash. The content of protein in wheat flour was approximately twice as high as that of buckwheat flour.

Retention capacities were different for lactic acid SRC, with values of 133.3% and 88.2 for wheat and buckwheat flour, respectively. Lactic acid SRC is as-

<table>
<thead>
<tr>
<th>Flour</th>
<th>wheat</th>
<th>buckwheat</th>
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<tbody>
<tr>
<td>Moisture (%)</td>
<td>11.2 ± 0.01</td>
<td>12.0 ± 0.01</td>
</tr>
<tr>
<td>Ash (% d.b.)</td>
<td>0.59 ± 0.02</td>
<td>0.78 ± 0.02</td>
</tr>
<tr>
<td>Protein (% d.b.)</td>
<td>10.7 ± 1.02</td>
<td>5.2 ± 0.79</td>
</tr>
<tr>
<td>Zeleny test (ml)</td>
<td>41.1 ± 0.4</td>
<td>10.0 ± 0.7</td>
</tr>
<tr>
<td>Demi water SRC (%)</td>
<td>62.1 ± 0.7</td>
<td>87.0 ± 0.8</td>
</tr>
<tr>
<td>50% sucrose SRC (%)</td>
<td>108.4 ± 0.6</td>
<td>124.8 ± 0.8</td>
</tr>
<tr>
<td>5% sodium carbonate SRC (%)</td>
<td>84.3 ± 0.6</td>
<td>91.0 ± 0.7</td>
</tr>
<tr>
<td>5% lactic acid SRC (%)</td>
<td>133.3 ± 0.9</td>
<td>88.2 ± 1.0</td>
</tr>
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associated with a higher content of glutenin in wheat flour. The higher protein quality of wheat flour was compared with the values of the Zeleny test (47 ml).

Generally, sucrose SRC is related to the characteristics of pentosans. Pentosans (non-starch polysaccharides, especially arabinoxylans) are the important part of dietary fibre and the soluble pentosans serve as water-binding components. They play the most important role in absorbing and retaining water in dough. The amount of pentosans in buckwheat is higher than in wheat (Berghofer & Schoenlechner 2009; Drobot et al. 2014; Wang & Zhang 2015). The higher retention capacity in sucrose solution (sucrose SRC) for buckwheat flour indicated a higher content of water-soluble pentosans in this flour.

Moreover, buckwheat flour showed higher values of water SRC and sucrose SRC than those of wheat flour. Water and sucrose SRC are affected by a higher degree of swelling and by water-soluble buckwheat flour components (soluble fibre, soluble proteins, etc.).

Evaluation of the farinograph measurement – wheat dough. Firstly, the amount of water required to achieve a dough consistency of 500 F.U. was determined. The water absorption for the wheat flour (absorption re-calculated at a dough consistency of 500 F.U. and at a flour humidity of 14%) was 178 ml. The measurement was then repeated \( n = 3 \) and the farinograph curve of the optimally developed dough was recorded. The farinograph curves of undeveloped and overmixed doughs were recorded in further replicates. Doughs were sampled, frozen and then used for microscopy. The farinograms of optimally developed, undeveloped and overmixed wheat doughs are shown in Figures 1–3, respectively.

Don et al. (2005) studied the effects of undermilling, optimal mixing and overmilling for three different wheat varieties. They observed significant changes in the ratio of low and high molar mass glutenin subunits upon dough overmilling. Overmixing broke the fragments into even smaller structures. Discrete clumps of gluten protein are present in the
early stages of mixing but later become extended and form a continuous network that gives dough its viscoelastic properties (Moss 1974). Using a set of hard red spring wheat, KHAN et al. (1989) found a positive correlation between the quantity of glutenin and mixing time, whereas the quantity of gliadin was correlated negatively with this parameter. GUPTA et al. (1993) noted that not only the quantity of the glutenin network but also its size was important. BEASLEY et al. (2002) concluded that variations in mixing time could be best explained by variations in acid-extractable polymeric protein.

**Evaluation of the farinograph measurement – buckwheat dough.** The rheological behaviour of buckwheat flour differed from that of wheat flour because of the absence of gluten-forming proteins. The water absorption for the buckwheat flour (absorption re-calculated at a consistency of 300 F.U. and at a flour humidity of 14%) was 151 ml. It was not possible to achieve a consistency of 500 F.U. when measuring buckwheat dough. In several titration assays, the maximum consistency of buckwheat dough was 300 F.U. The time of the addition of water from the farinograph burette was more important during the preparation of buckwheat dough. If the water required to achieve a consistency of 300 F.U. was added within 20 s as for wheat dough, the buckwheat dough did not achieve the desired consistency. For this reason, the volume of water was added gradually to the buckwheat dough over the course of 2 min, resulting in a constant course of the farinograph curve. The sampling of buckwheat dough for SEM measurements was similar to the procedure for wheat dough. The farinograms of optimally developed, undeveloped and overmixed buckwheat doughs are shown in Figures 4–6, respectively.

**Evaluation of SEM measurements – wheat dough.** The resulting protein gel was observed in undeveloped dough using scanning electron (SEM) microscopy. Separate grains of wheat A-starch (large granules) and B-starch (small granules) were not yet associated with the developing protein gel (network) (the section marked 1 is shown in Figure 7). As well as grains of A-starch and B-starch which have been associated with developing protein gel (network) was observed (the section marked 2 is shown in Figure 7A).

In undeveloped wheat dough, there were undeveloped structures, parts of which (a discontinuous layer of gel) made up a protein gel which covered the surface of starch grains. Starch grains were partially associated with the protein gel; on the other side separate single starch grains were observed. In this case, protein and starch were not properly associated and connected, and a contiguous layer of gluten protein was not observed on the starch grain surface.
A detailed view of these two parts (non-associated and associated parts) can be seen in Figure 7B. A swollen protein gel, in which starch grains were suspended, formed a continuous phase in optimally developed wheat dough (Figure 8A). The non-associated parts (described in undeveloped dough) were not present in optimally developed dough. Filaments or strands formed by gluten proteins could be observed in some places in optimally developed wheat dough (Figure 9; the section marked 1 in Figure 9B). Water bound in the protein gel was released during the overmixing of wheat dough. Gel concentration was decreased in the dough which led to the loss of the continuous gluten structure. The longer mixing

Figure 7. SEM micrograph of undeveloped wheat dough (A) and detail of the developing gel (B)

Figure 8. SEM micrograph of optimally developed wheat dough (A) and detail of the protein filament (strand) (B)
time of wheat dough caused cracking (breakdown) of protein filaments and destruction of the continuous structure of dough. This structure loosened and a larger amount of separated protein filaments was observed (Figure 8B).

Disintegration of the continuous protein (gluten) gel was observed in overmixed wheat dough (Figure 9A). It was possible to capture the cracking of protein gel filaments due to the overmixing of dough (Figure 9B).

With continued processing, the overall size of the glutenin protein aggregates in the system decreased, which could be the result of a loss of polymerization sites. This can lead to a breakdown of the protein matrix (Shewry 2003).

Figure 9. SEM micrograph of overmixed wheat dough (A) and detail of crackling protein filaments (B)

Figure 10. SEM micrograph of undeveloped of buckwheat dough (A) and detail of the developing gel (B)
Evaluation of SEM measurements – buckwheat dough. In contrast to wheat dough, dough from buckwheat flour does not contain gluten. The buckwheat dough was mixed under the same conditions as wheat dough to determine the structures formed in gluten-free flour. As for wheat dough, a developing gel was observed in undeveloped buckwheat dough (Figure 10). The shapes and sizes of buckwheat starch granules are different from those of wheat. Buckwheat starch granules are polygonal and have smaller diameters than wheat starch granules. Buckwheat starch has a higher swelling power than wheat starch, probably as a consequence of

Figure 11. SEM micrograph of optimally developed buckwheat dough (A) and detail of hydrated buckwheat starch and proteins (B)

Figure 12. SEM micrograph of overmixed of buckwheat dough (A) and detail of more hydrated buckwheat polysaccharides and proteins (B)
the weaker but more extensive bonding forces in the granule structure (Mazza & Oomah 2005).

Unlike for wheat dough, the continuous protein phase with suspended starch grains was not present in optimally developed buckwheat dough. A fine coating was observed around starch grains (Figure 11A). However, the coating was not continuous, and it did not form filaments (strands) like gluten proteins in wheat dough (Figure 8B).

Disintegration of the coating on the surface of starch grains was observed in overmixed buckwheat dough (Figure 12). However, no other significant changes occurred in the overmixed buckwheat dough structure. Compared to changes in the structure of overmixed wheat dough, the range of the changes was very small in overmixed buckwheat dough.

**CONCLUSION**

Mixing time significantly affected the structures of the three-dimensional gluten networks in wheat and buckwheat doughs, which consequently can influence dough baking quality. Differences were found between the optimally developed dough from gluten-containing and gluten-free flour.

Pronounced differences were observed between undeveloped, optimally developed and overmixed wheat dough. Gluten filaments and a continuous phase of gel were observed in all volumes of optimally developed wheat dough. In undeveloped wheat dough, it was possible to distinguish the sections in which starch grains were not yet suspended in the gel and sections where the starch grains were already suspended in the developing gel. There were the cracks in the continuous dough structure in overmixed wheat dough. A cracking of gluten filaments was captured in SEM analysis.

Generally, it was possible to observe a continuous phase of gluten protein in wheat dough, while in buckwheat dough only parts of the starch grains were covered by the gel layer. This gel layer could be formed by hydrated components of buckwheat dietary fibre (soluble pentosans and other polysaccharides). No significant differences were observed between the structures of undeveloped, optimally developed and overmixed buckwheat doughs.

This analysis of the behaviour of buckwheat dough and its comparison with the behaviour of wheat dough under similar conditions was carried out to better understand the changes in the internal structure of buckwheat dough during mixing. As buckwheat products are growing in popularity among people with coeliac disease and those suffering from allergies to wheat gluten, as well as among the general public, the study of the behaviour of buckwheat doughs during baking processes is of considerable practical significance.

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