Correlations between gluten aggregation properties defined by the GlutoPeak test and content of quality-related protein fractions of winter wheat flour

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Abstract

The gluten aggregation properties of 19 wheat cultivars were investigated using a rapid small-scale (7 min, 8.5 g flour) technique (GlutoPeak test, GPT). Correlations between GPT indices and gliadin (GLIA), glutenin (GLUT), SDS-soluble protein (SDSS), and glutenin macropolymer (GMP) concentrations were established. Two groups of samples were distinguished based on the shape of their GPT curves. Flours of group 1 gave curves with a slower buildup in torque (measured as aggregation time) and a higher area under the curve at peak (maximum torque), compared to those of group 2. Group 1 was characterized by lower GLIA and higher GLUT and GMP contents than group 2. GLIA ($r = 0.70$, $p \leq 0.001$) and SDSS ($r = 0.59$, $p \leq 0.01$) concentrations were significantly correlated to peak torque. GLUT and GMP contents were correlated to the aggregation time (GLUT: $r = 0.72$, $p \leq 0.001$; GMP: $r = 0.77$, $p \leq 0.001$) and the area under the curve at peak (GLU: $r = 0.68$, $p \leq 0.01$; GMP: $r=0.78$, $p \leq 0.001$), confirming the importance of GMP for gluten strength. GPT could be an alternative to the labor-intensive quantitation of quality related protein fractions of wheat flour.

Keywords: GlutoPeak; Gliadin; Glutenin; Glutenin macropolymer

List of abbreviations: AGT, aggregation time; AU, arbitrary unit; BE, Brabender equivalents; EnMT, energy to maximum torque; DTT, dithiothreitol; GLIA, gliadin; GLUT, glutenin; GMP, glutenin macropolymer; GPT, GlutoPeak test; LOT, lift of time; LT120, loss of torque 120s after peak; MT, maximum torque; PMT, peak maximum time; PWG-GLIA, gliadin of the Prolamin Working Group; SDS, sodium dodecyl sulfate; SDSS, SDS-soluble protein; TRIS, tris(hydroxymethyl)-aminomethane

1. Introduction

The functionality of common wheat (*Triticum aestivum* L.) flour is mainly affected by the concentration and composition of gluten proteins and interactions with each other upon water addition and mixing to form dough. It has been already demonstrated that dough properties and bread volume are greatly affected by both gliadin (GLIA) and glutenin (GLUT) concentrations and their proportions in wheat flour (Cinco-Moroyoqui and MacRitchie 2008; Wieser and Kieffer, 2001). All wheat species and cultivars contain GLIA and GLUT that are organized into a viscoelastic network. However, it is also obvious that flours of different wheat cultivars yield products with completely different quality. Indeed, flour characteristics can greatly differ according to genotype and environmental conditions (Gupta et al., 1992; Hasniza et al., 2014). In addition to quantitative measures, it is generally accepted that wheat flour functionality is related to the presence or absence of specific protein types and subunits. One example is the composition of high-molecular-weight glutenin subunits (HMW-GS), which are the basis for a quality score system used until now (Payne et al., 1984).

The glutenin macropolymer (GMP) is a further quality-related gluten protein fraction. GMP is the part of GLUT with the highest molecular weight. It is also called gel protein because it is the gel-like layer that forms above the sedimented starch after suspending wheat flour in SDS solution and centrifugation (Moonen et al., 1982). Unextractable polymeric protein (Gupta et al., 1993) is related to GMP, because it also represents the fraction of insoluble GLUT. The concentration of GMP depends on the wheat cultivar and is correlated with the resistance to extension of wheat dough (MacRitchie and Lafiandra, 1997) and baking performance (Moonen et al., 1982; Pritchard, 1993). In a recent study, Thanhaeuser et al. (2014) showed that the

concentrations of GLIA, GLUT, and GMP can be adopted as suitable parameters to predict the baking performances of flour.

Along the decades several rheological approaches have been used for predicting dough properties prior to processing and bread quality (Banu et al., 2011; Dowell et al., 2008; Ktenioudaki et al., 2010; Mondal & Datta 2008; Olivier and Allen, 1992). Some of them (such as the Farinograph- or Mixograph-method) determine, for instance, the amount of mixing that dough requires or the amount of water that should be added to the flour to obtain dough of the desired consistency. Others simulate the rounding and molding in the baking process and measure the dough resistance to uniaxial (e.g. Extensograph, Kieffer-Rig) or biaxial extension (e.g. Alveograph).

Over the years, the needs along the value chain of wheat have changed. Breeders look for reliable methods to test the functional quality of wheat lines at early stages, with just a limited amount of sample. The milling industry needs fast and reliable methods for checking wheat quality right at the receiving station. Finally, the baking industry is looking for suitable methods that could predict end product quality for production and product development. The GlutoPeak test has been recently proposed for the evaluation of wheat flour quality. It measures the aggregation behavior of gluten upon addition of water and high-speed mixing (Kaur Chandi and Seetharaman, 2012). In a recent study on 120 commercial wheat flours, the GlutoPeak indices have been used for predicting the conventional parameters related to dough mixing stability, extensibility, and tenacity (Marti et al., 2015). The test has been also used as a valid screening tool for durum wheat quality (Marti et al., 2013, 2014).

To date, it is largely unknown how the quantitative composition of gluten proteins of wheat flour affects the results of the GlutoPeak test. Therefore, the aim of this study was to establish correlations between the concentrations of quality-related protein fractions such as GLIA, GLUT, and GMP and the gluten aggregation behavior of flours from a set of winter wheat cultivars.

2. Materials and Methods

2.1 Wheat Samples

Grains of 19 winter wheat cultivars and experimental lines showing high heterogeneity in terms of quality were grown at different locations in the US in 2013 and milled into white flour using the Miag Multomat Milling method. Moisture content of flour was measured by drying the sample at 180°C for 4 min in an infrared balance (MB 45, OHAUS, Parsippany, NJ). Protein content was determined by the Dumas combustion method according to AACC approved method 46-30 (N × 5.7). Ash content was measured according to the AACC approved method 08-01. Moisture contents ranged from 10.8 to 12.7%, and ash and protein contents varied from 0.39 to 0.57% and from 11.7 to 16.3% dry basis, respectively.

2.2 Chemicals

The quality of all chemicals was analysis grade unless stated otherwise. Disodium hydrogenphosphate dihydrate, ethanol, hydrochloric acid (32%, w/w), potassium dihydrogenphosphate, 1-propanol, sodium chloride, sodium dodecyl sulfate (SDS), tris(hydroxymethyl)-aminomethane (TRIS), and urea were from VWR Merck (Darmstadt, Germany). Dithiothreitol (DTT) was from Serva (Heidelberg, Germany). Calcium Chloride

107 dihydrate was was from Sigma Aldrich (St. Louis, MO, US). Water was deionized by a water
108 purification system Arium 611VF (Sartorius, Goettingen, Germany).
109
110 *2.3 GlutoPeak test*
111 Gluten aggregation properties were measured using the GlutoPeak device (C.W. Brabender Inc.,
112 South Hackensack, NJ, USA), as reported by Kaur Chandi and Seetharaman (2012). An aliquot
113 of 8.5 g of flour was dispersed in 9.5 g of 0.5mol/L $CaCl_2$, scaling both water and flour weight
114 on a 14% flour moisture basis in order to keep the liquid-to-solid ratio constant (and equal to
115 1.26). Sample temperature was maintained at 34 °C by circulating water through the jacketed
116 sample cup. The paddle was set to rotate at 1,900 rpm and the test was carried out for 7 min. The
117 main indices automatically evaluated by the software provided with the instrument (Brabender
118 GlutoPeak v.1.1.0) are (Fig. 1): *i)* Lift Off Time (LOT, expressed in s), corresponding to the time
119 at which gluten aggregation starts; *ii)* the Peak Maximum Time (PMT, expressed in s),
120 corresponding to the time before torque falling off when gluten breaks down; *iii)* the Maximum
121 Torque (MT, expressed in Brabender Equivalents - BE), corresponding to the peak occurring due
122 to gluten aggregation. In addition, the following indices were calculated using Microsoft Excel
123 2010 (Microsoft, Redmond, VA): iv) Aggregation Time (AGT, expressed in s), corresponding to
124 the difference between PMT and LOT; v) Energy to Maximum Torque (EnMT; expressed in
125 arbitrary units - AU) corresponding to the area of the curve from the beginning of the test and the
126 maximum torque; vi) Loss of Torque 120 s after MT (LT120; %) corresponding to the decrease
127 in torque 2 min after peak, when the network was completely destroyed (Fig. 1). The EnMT
128 values were estimated as follows:
129

130 $$\sum_{n=0}^{PMT}[(x_n - x_{n-1}) * y_n]$$

131 where x is the time and y the torque. Measurements were performed in triplicate.

132

133 *2.4 Quantitation of Albumin and Globulin (ALGL), GLIA, and GLUT Fractions*

134 Flour (100 mg) was extracted sequentially with NaCl 0.4 mol/L/Na_2HPO_4/KH_2PO_4 0.067 mol/L
135 (pH 7.6) (2 × 1.0 mL) for 10 min at room temperature (22 °C) (ALGL), with 60% (v/v) ethanol
136 (3 × 0.5 mL) for 10 min at room temperature (GLIA), and with 50% (v/v) 1-propanol/urea 2
137 mol/L/TRIS-HCl 0.05 mol/L (pH 7.5)/1% (w/v) DTT (2 × 1.0 mL) for 30 min at 60°C under
138 nitrogen (GLUT) (Wieser et al., 1998). The suspensions were centrifuged for 20 min at 3750 × *g*
139 and RT (Heraeus Multifuge 3L-R; Thermo Fisher Scientific, Dreieich, Germany). The
140 corresponding supernatants (GLIA, GLUT, respectively) were combined, diluted to 2.0 mL with
141 the respective solvent, and filtered through a 0.45-µm membrane. Three separate extraction
142 experiments were carried out for each flour sample. Protein fractions were quantitated by RP-
143 HPLC as described recently (Thanhaeuser et al., 2014). Reference gliadin of the Prolamin
144 Working Group (PWG-GLIA) (van Eckert et al., 2006) dissolved in 60% (v/v) ethanol (2.5
145 mg/mL) was used for external calibration. Measurements were performed in triplicate. Data are
146 expressed in mg/g flour.

147

148 *2.5 Quantitation of SDS-Soluble and GMP Fractions*

149 Flour (100 mg) was extracted sequentially with 1% (w/v) SDS/NaH_2PO_4 0.05 mol/L (pH 6.9). (2
150 × 1.0 mL) for 30 min at room temperature (SDSS) and with 50% (v/v) 1-
151 propanol/Na_2HPO_4/KH_2PO_4 0.05 mol/L (pH 7.5)/1% (w/v) DTT under nitrogen (2 × 1.0 mL) for

30 min at 60°C under nitrogen (GMP). The suspensions were centrifuged for 25 min at 3750 × *g* and RT. The corresponding supernatants were combined, diluted to 5.0 mL (SDSS) and 2.0 mL (GMP) with the respective solvents, and filtered through a 0.45-µm membrane. Three separate extraction experiments were carried out for each flour sample. Protein fractions were quantitated by gel permeation (GP) HPLC as described recently (Thanhaeuser et al., 2014). PWG-GLIA dissolved in 60% (v/v) ethanol (2.5 mg/mL) was used for external calibration. From the GMP chromatograms the content of HMW- and low-molecular-weight (LMW-) GS were determined. Measurements were performed in triplicate. Data are expressed in mg/g flour.

2.6 Statistical analysis

Statistical evaluation of the data was carried out by a linear Pearson correlation and analysis of variance (ANOVA) with Fisher Least Significant Difference (LSD) post hoc test ($p \leq 0.05$) using Statgraphic Plus for Windows v. 5.1. (StatPoint Inc., Warrenton, VA, USA).

3. Results and Discussion

3.1 Gluten Quality

Fig. 2 displays the gluten aggregation profile of winter wheat flours obtained by the GlutoPeak test. The parameters associated with the aggregation curves are reported in Table 1. During the test, the sample slurry is subjected to intense mechanical action, promoted by the speed of the rotating element, which allows the formation of the gluten network, and a strong increase of the torque curve is registered. Further mixing destroys the network, and the torque curve declines. Usually, hard wheat flours exhibit longer PMT and higher MT than flours of soft wheat cultivars (Lu and Seetharaman, 2014), while flours for wafers or batters show very much delayed peak

175 formation and much lower torque (data not shown). More recently, the area under the peak
176 (EnMT), which takes into consideration both the indices, has been found suitable for predicting
177 conventional parameters related to dough strength and extensibility (Marti et al., 2015). In
178 particular, wheat flours suitable for sponge-and-dough systems exhibited higher energy for
179 gluten aggregation than flours used for straight-dough systems.
180
181 Two different groups of samples can be distinguished based on the shape of the curves until the
182 MT. One group of samples (group 1; Fig. 2a) exhibited a long aggregation time (53 s < AGT <
183 95 s), indicating a slow increase in consistency. Moreover, the high values of the area under the
184 peak (26.7 AU < EnMT < 56.4 AU) indicated that gluten required long time and high energy for
185 aggregation. In addition, this group of samples were more stable during prolonged mixing,
186 showing a low loss of torque 2 min after peak (27% < LT120< 37%; median=33%). A second
187 group of samples (group 2; Fig. 2b) showed a rapid increase in consistency (39 s < AGT < 55 s),
188 low values of the area under the peak (13.7 AU<EnMT<26 AU), and high loss of torque (31% <
189 s LT120 < 41%; median=36%). This represented solely a tentative of grouping the samples
190 based on the shape of their GlutoPeak curves. Thus, the two groups of samples certainly
191 presented some overlapping, such as the case of sample M. Indeed, sample M was included in
192 the group 2 because it showed a GlutoPeak curve were similar to that one of the other samples of
193 the same group. However, sample M had high AGT and EnMT (29.6 AU) values, as the samples
194 reported in the same group.
195
196 Considering all the samples, two samples (D and F) exhibited a very unique gluten aggregation
197 profile that was characterized by low PMT (69 and 66 s, respectively), very high MT (39.3 and

198 43.3 BE, respectively), and a high LT120 (41% and 37%, respectively). On the other hand,
199 samples H and I showed high PMT (232 s), low MT (31 and 34 BE, respectively) and high
200 stability during mixing (LT120 about 27% and 30%, respectively). Generally, flours with poor
201 technological quality are characterized by a rapid buildup in consistency and a sharply defined
202 peak followed by a rapid breakdown, while strong flours have a much slower build up in dough
203 consistency and require more time to reach peak consistency (Goldstein et al., 2010).
204
205 *3.2 Concentrations of Protein Fractions*
206 The results of the quantitation of protein fractions are shown in Table 2. The GLIA content
207 varied from 55.5 to 98.3 mg/g flour of sample S and F, that showed the lowest and the highest
208 peak torque (MT) with the GlutoPeak test, respectively. It has been observed previously that
209 increasing protein content of wheat flour led to an increase of the torque (Kaur Chandi et al.,
210 2015, Marti et al., 2015). This study confirms the above observation, since the GLIA content
211 represents about the 60% of the total protein.
212
213 The GLUT content ranged from 29.5 (sample K) to 47.4 mg/g flour (sample E). Sample K was
214 characterized by the lowest EnMT value (13.7 AU), while sample E exhibited relatively high
215 EnMT (39.8 AU).
216
217 GLIA fractions are more subject to variation by external parameters such as nitrogen supply than
218 GLUT (Wieser et al., 1998), accounting for the wider variation in GLIA contents than GLUT
219 contents, in agreement with a recent study (Thanhaeuser et al., 2014). The polymeric GLUT are
220 mostly responsible for the elasticity of the dough, whereas the monomeric GLIA are the

221 extensibility-related proteins in the dough. Thus, the GLIA / GLUT ratio is related to the balance
222 of dough strength and extensibility of the sample. Sample C and Q exhibited the lowest values
223 (1.51 and 1.56, respectively) of the GLIA / GLUT ratio, while sample K showed the highest
224 index (2.82). These values can be expected for wheat flours (Thanhaeuser et al., 2014). A high
225 GLIA / GLUT ratio is often associated to low resistance to extension and high extensibility
226 (Wieser and Kieffer, 2001). It is the unique combination of dough viscosity and dough elasticity
227 that comprises the functional properties of dough. To investigate this relationship, two important
228 aspects should be considered (Wrigley et al., 2006). First, since dough properties are dependent
229 on protein content, the balance of GLIA / GLUT ratio can best be compared among samples with
230 similar protein contents. Moreover, at the same GLIA / GLUT ratio, the balance HMW-to-
231 LMW-GS in the polymeric fraction can significantly alter dough strength and extensibility. In
232 this study, the LMW / HMW ratio ranged from 1.42 (sample P) to 2.58 (sample B). The LMW /
233 HMW ratio seems to drive the gluten aggregation of these samples: sample P was characterized
234 by a very long AGT (85 s) and high EnMT (47.8 AU), whereas sample B exhibited a very short
235 AGT (39 s) and a very low EnMT (15.6 AU). Indeed, dough strength systematically decreased,
236 while extensibility increased as a result of decreasing the HMW / LMW ratio (Lawrence et al.,
237 1988). Moreover, according to Sapirstein and Fu (2000), the larger the GLUT aggregates, the
238 smaller the specific surface area and the longer the mixing required to achieve full development.
239
240 The sum of the GLIA and GLUT contents represented the gluten content. The gluten content
241 ranged from 89.3 (sample S) to 134.4 mg/g flour (sample O). Compared with the crude protein
242 content, gluten represented about 80% of the total protein (individual values not shown).
243

The concentrations of SDSS and GMP fractions are shown in Table 3. GMP refers to the fraction of GLUT that is unextractable in SDS solution (Weegels et al., 1996a). In the set of flours considered in the present study, the GMP concentrations ranged from 12.7 to 30.7 mg/g flour These GMP concentrations were in the range of wheat flours according to previous studies (Moonen et al., 1982; Thanhaeuser et al., 2014). The lowest and the highest GMP concentration were detected in samples K and O, respectively. Interestingly, these samples also showed the lowest and the highest EnMT value, respectively (Table 1), suggesting that the area under the peak could discriminate samples with different GMP content. Several studies have shown the importance of GMP in assessing wheat quality and predicting dough properties (Gupta et al., 1992, 1993; Moonen et al., 1982; Weegels et al., 1996b). In a recent study on 13 wheat cultivars Thanhaeuser et al. (2014) showed that GMP is a good predictor of the baking performance of wheat flour. The LMW / HMW ratio of GMP clearly showed that GMP is enriched in LMW-GS compared to GLUT. This points to a more important role of LMW-GS vs. HMW-GS in the formation of large polymers, which are related to dough strength.

The overall characterization of the protein fractions of winter wheat flours highlighted that most of the samples of group 1 are generally characterized by higher GLUT (38.8 - 47.4 mg/g flour), LMW-GS (25.0 - 31.0 mg/g flour), and GMP (22.4 - 30.7 mg/g flour) contents than most of the samples of group 2 (GLUT: 29.5 – 35.2 mg/g flour; LMW-GS: 19.3 – 23.7 mg/g flour; GMP: 12.3 – 21.9 mg/g flour). However, there are few exceptions (sample C, D, M and R) that would need further investigation. For example, although belonging to group 2, samples C and M had high values of LMW-GS (25.8 and 25.7 mg/g flour, respectively) and GLUT (38.8 and 41.2 mg/g flour). Sample M also showed high GMP (22.8 mg/g flour), whereas sample D had high

GLUT (38.9 mg/g flour). Finally, as regards group 1, sample R exhibited the lowest GMP content (19.6 mg/g flour) among the samples belonging to the same group.

3.3 Correlation of Quality Parameters with Gluten Aggregation Properties

The GlutoPeak parameters were correlated with the content of protein fractions. A linear Pearson correlation was used to describe relationships by means of correlation coefficients (r). The data are summarized in Table 4. Crude protein content was highly correlated with MT, confirming previous studies (Kaur Chandi et al., 2015, Marti et al., 2015), while no correlation was found with PMT. As expected, ALGL contents did not show any significant correlation with gluten aggregation (data not shown). GLIA were significantly correlated to peak torque ($r = 0.70$, $p \leq 0.001$). A high amount of GLIA will create a high-viscosity mass in the GlutoPeak device that results in detection of high torque (Melynk et al., 2012). The significant correlation between GLIA content and torque could be related to the fact that GLIA represent up to about 60% of the total protein (values not reported). Indeed, a significant correlation between the peak torque (GlutoPeak test) and the water absorption (Farinograph test) was recently demonstrated and likely related to the fact that both parameters are strongly associated with the protein content (Marti et al., 2014; 2015). Also gluten that was calculated as the sum of GLIA and GLUT was correlated to MT ($r = 0.75$, $p \leq 0.001$). The GLIA content showed high correlation with loss of torque 30 s after gluten aggregation occurs ($r = 0.65$, $p < 0.01$; data not shown), confirming that high levels of GLIA are inversely related with the gluten strength. The decrease in gluten strength caused by the addition of GLIA was demonstrated also in a dough system and during mixing under gentle conditions as those occurring in the Mixograph bowl and for prolonged time

289 (Fido et al., 1997). On the other hand, the amount of GLIA was not correlated with the loss of
290 torque during prolonged mixing (2 minutes after the maximum torque; LT120; data not shown).
291
292 The GLUT content is very highly significant correlated to the AGT ($r = 0.72$, $p \leq 0.001$), in
293 agreement with the increase in dough development time as the proportion of GLUT in a blend
294 increased (Uthayakumaran et al., 1999). More recently, using a fractionation/reconstitution
295 method, Melynk et al. (2012) demonstrated that a GLIA / GLUT ratio equal to 1 was optimal for
296 fast gluten aggregation. In our study, the GLIA / GLUT ratio ranged from 1.5 to 2.8, which can
297 be expected for wheat flour (Thanhaeuser et al., 2014). A comparison of the GLIA / GLUT ratio
298 with PMT showed that there was no relationship between these parameters ($r = -0.45$, $p > 0.05$;
299 data not shown). Conversely Melynk et al. (2012) showed a decrease in PMT as the GLIA /
300 GLUT ratio increased. Such differences could be related to the differences in gluten
301 characteristics, research plan and goals between the two studies. Indeed, Melynk et al. (2012)
302 adopted a fractionation/reconstitution study using pure GLIA and GLUT fractions in the absence
303 of starch and other flour components. In the present study, winter wheat cultivars from different
304 location (and with different composition) were used. According to Wrigley et al. (2006), the
305 balance of GLIA / GLUT ratio can best be compared among samples with similar protein
306 content. As an example, sample D, F, H, and I had a similar protein content (14.9, 15.1, 15.0, and
307 15.2%, respectively; data not shown) but a different GLIA / GLUT ratio (2.01, 2.79, 1.60, and
308 1.92, respectively). For these samples, PMT and GLIA / GLUT ratio was inversely correlated (r
309 $= -0.76$; $p \leq 0.001$; data not shown), confirming the hypothesis that high amounts of GLIA present
310 may act as a barrier to gluten network formation, increasing the time needed for GLUT to
311 associate (Melnyk et al., 2012).

GLUT ($r = 0.68$, $p=0.002$) and gluten ($r = 0.71$, $p\leq0.001$) contents are significantly correlated to EnMT, confirming previous studies reporting the importance of GLUT in increasing dough strength (Uthayakumaran et al., 1999; Wieser and Kieffer, 2001). The area under the peak corresponds to the energy required for gluten aggregation (EnMT) and it is related to dough strength, as recently reported Marti et al. (2014). This value might also reflect the transfer of energy during mixing (data not shown) and thus resistance to breakdown.

The SDSS content and the MT were weakly correlated ($r = 0.59$, $p<0.05$), probably because this fraction contained more than 85% of the crude protein, which is also correlated with the peak torque (Marti et al., 2015). GMP has been referred to the SDS-unextractable fraction (Don et al., 2003). Several studies showed strong correlation between GMP content and dough strength (Gupta et al., 1993; Weegels et al., 1996b). More recently, Thanhaeuser et al. (2014) showed that the GMP content as well as the GLIA and GLUT contents were suitable predictors of the baking performance of German wheat flours. In the present study, the GMP content was highly correlated with the aggregation time ($r = 0.77$, $p\leq0.001$) and strongly with the area under the peak ($r = 0.78$, $p\leq0.001$), confirming that the amount of GMP strongly contribute to the technological performance of wheat flour (Thanhaeuser et al., 2014). The enrichment of LMW-GS in GMP compared to GLUT (Tables 2 and 3) was reflected in the correlation to the GlutoPeak data (Table 4). Whereas in GLUT the correlations of the HMW- and LMW-GS contents with quality parameters were comparable, the correlation of the content of LMW-GS in GMP to GlutoPeak parameters was always higher than for the HMW-GS content. This is in agreement with the role of LMW-GS that, on average, contain more cysteine residues than

HMW-GS and, thus, are more subject to polymerization and thiol-disulfide interchange reactions, which are the key of gluten formation (Wrigley et al., 2006). However, our results also suggest that a suitable mixture of HMW- and LMW-GS is required to enable the formation of large polymers that are required for gluten strength.

None of the quality-related protein fractions considered in this work were significantly correlated (data not shown) with the LT120 parameter, which represents the loss of torque due to prolonged mixing (2 min) at very high speed (1900 rpm). The suitability of this GlutoPeak index in predicting bread-making performance remains to be investigated.

4. Conclusions

Three main conclusions can be drawn from this study of flours from 19 winter cultivars. First, the rapid small-scale GlutoPeak test used in this study is able to clearly distinguish wheat flours according to their gluten aggregation behavior. The results are available within less than 7 min, and only a small amount of flour (8.5g) is required. Time and sample size are two important criteria for the evaluation of technological quality in the wheat production chain. Second, more indices, in addition to peak torque and peak maximum time, should be taken into consideration as quality parameters of wheat flour: the area under the curve (related to flour strength) and the aggregation time (related to the increase in consistency and thus to gluten aggregation time). Last but not least, parameters obtained by means of the GlutoPeak test are correlated with compositional data of flour such as the concentrations of quality-related gluten protein fractions but are more easily accessible compared to the labor-intensive quantitation of protein fractions.

Further studies are necessary to correlate the GlutoPeak indices with the breadmaking performance.

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Table 1

Aggregation time (AGT), peak maximum time (PMT), maximum torque (MT), energy to maximum torque (EnMT), and loss of torque 120 s after peak (LT120) determined by GlutoPeak test of wheat flours[(a)].

FLOUR	AGT (s)	PMT (s)	MT (BE)	EnMT (AU)	LT120 (%)
A	$44^{b,c}$	123^{d}	32.2^{e}	22.6^{e}	$33.6^{c,d}$
B	39^{a}	81^{b}	$32.7^{e,f}$	$15.6^{a,b}$	$25.2^{e,f}$
C	48^{d}	211^{j}	$30.1^{a,b}$	23.3^{e}	$35.7^{e,f,g}$
D	$45^{c,d}$	69^{a}	39.3^{i}	23.6^{e}	40.6^{j}
E	72^{g}	108^{c}	41.5^{j}	39.8^{i}	$33.3^{c,d}$
F	$44^{b,c}$	66^{a}	43.3^{k}	26.0^{f}	$37.0^{g,h,i}$
G	78^{h}	158^{f}	36.0^{h}	39.1^{i}	$32.7^{c,d}$
H	63^{f}	233^{l}	$31.1^{c,d}$	29.5^{g}	27.2^{a}
I	95^{j}	232^{l}	34.2^{g}	52.9^{k}	29.8^{b}
J	$44^{c,d}$	141^{e}	$30.5^{b,c}$	$17.0^{b,c}$	$37.3^{h,i}$
K	39^{a}	108^{c}	30.3^{b}	13.7^{a}	$36.1^{f,g,h}$
L	$41^{a,b}$	137^{e}	33.1^{f}	$18.8^{c,d}$	37.6^{i}
M	55^{e}	168^{g}	31.6^{d}	23.3^{e}	30.9^{b}
N	79^{h}	181^{h}	39.7^{i}	49.6^{j}	$35.4^{e,f}$
O	85^{i}	216^{k}	41.4^{j}	56.4^{l}	$36.6^{f,g,h,i}$
P	81^{h}	159^{f}	41.9^{j}	47.8^{j}	$35.8^{e,f,g}$
Q	53^{e}	187^{i}	$30.5^{b,c}$	25.7^{f}	29.6^{b}
R	61^{f}	121^{d}	36.5^{h}	35.2^{h}	32.4^{c}
S	$46^{c,d}$	161^{f}	29.4^{a}	20.1^{d}	$35.0^{d,e}$

[(a)] Mean value of triplicate determinations. Values associated with different letters are significantly different (one-way ANOVA, LSD test, $p \leq 0.05$).

Table 2

Contents of albumins and globulins (ALGL), gliadins (GLIA), glutenins (GLUT), low-molecular-weight glutenin subunits (LMW-GS), high-molecular-weight glutenin subunits (HMW-GS), and gluten, as well as the LMW / HMW and GLIA / GLUT ratios determined by modified Osborne fractionation of wheat flours[1].

FLOUR	ALGL (mg/g flour)	GLIA (mg/g flour)	GLUT (mg/g flour)				GLIA / GLUT	GLUTEN[2] (mg/g flour)
			Total	HMW-GS	LMW-GS	LMW / HMW		
A	$19.2^{e,f,g}$	$71.1^{e,f}$	30.3^{a}	9.1^{a}	20.4^{a}	2.25	2.35	101.4
B	15.7^{b}	$63.7^{b,c,d}$	33.6^{b}	$9.2^{a,b}$	23.7^{c}	2.58	1.89	97.3
C	16.3^{c}	$58.6^{a,b}$	$38.8^{e,f}$	$12.5^{e,f}$	$25.8^{d,e}$	2.06	1.51	97.4
D	17.6^{i}	$78.0^{g,h}$	$38.9^{e,f}$	14.2^{h}	23.5^{c}	1.66	2.01	116.9
E	19.3^{k}	$81.7^{h,i}$	47.4^{j}	15.9^{j}	30.1^{i}	1.89	1.72	129.1
F	$17.5^{g,h,i}$	98.3^{l}	$35.2^{b,c}$	11.3^{c}	23.4^{c}	2.06	2.79	133.5
G	18.3^{j}	$86.9^{i,j,k}$	$44.6^{h,i}$	15.0^{i}	28.4^{h}	1.89	1.95	131.5
H	$17.1^{e,f,g,h}$	$74.6^{f,g}$	$46.7^{i,j}$	14.8^{i}	31.0^{i}	2.09	1.60	121.3
I	$19.0^{j,k}$	89.0^{k}	$39.6^{f,g}$	11.5^{c}	$27.3^{f,g}$	2.38	2.25	128.6
J	$17.3^{f,g,h,i}$	$73.0^{e,f,g}$	$36.0^{c,d}$	$12.9^{f,g}$	$22.7^{b,c}$	1.77	2.03	109.0
K	$17.1^{e,f}$	$83.1^{h,i,j}$	29.5^{a}	9.8^{b}	19.3^{a}	1.98	2.82	112.6
L	$16.7^{c,d}$	$65.9^{c,d}$	$37.3^{d,e}$	13.2^{g}	23.6^{c}	1.79	1.77	103.2
M	$16.7^{c,d}$	$71.6^{e,f}$	41.2^{g}	$14.5^{h,i}$	$25.7^{d,e}$	1.78	1.74	112.8
N	$16.9^{e,f,g}$	$83.1^{h,i,j}$	$40.6^{f,g}$	14.9^{i}	25.0^{d}	1.68	2.05	123.7
O	$16.7^{c,d,e}$	$88.3^{j,k}$	$46.1^{h,i,j}$	17.3^{k}	$28.0^{f,g}$	1.62	1.92	134.4
P	16.1^{c}	80.7^{h}	44.4^{h}	18.0^{l}	$25.5^{d,e}$	1.42	1.82	125.1
Q	15.6^{b}	$62.8^{b,c}$	$40.3^{f,g}$	$12.2^{d,e}$	$27.6^{f,g}$	2.26	1.56	103.1
R	$17.4^{h,i}$	$68.2^{d,e}$	$38.8^{e,f}$	$11.6^{c,d}$	$26.7^{e,f}$	2.31	1.76	107.0
S	15.1^{a}	55.5^{a}	33.8^{b}	11.3^{c}	22.0^{b}	1.94	1.64	89.3

(1) Mean value of triplicate determinations. Values associated with different letters are significantly different (one-way ANOVA, LSD test, $p \leq 0.05$).

(2) Sum of GLIA and GLUT.

Table 3

Contents of SDS-soluble proteins (SDSS), glutenin macropolymer (GMP), low-molecular-weight glutenin subunits (LMW-GS), high-molecular-weight glutenin subunits (HMW-GS), and the LMW / HMW ratio of GMP determined by SDS fractionation of wheat flours[(1)]

FLOUR	SDSS (mg/g flour)	GMP (mg/g flour)			LMW /HMW
		Total	HMW-GS	LMW-GS	
A	86.8^{a}	$14.2^{b,c}$	3.2^{a}	$9.3^{b,c}$	2.91
B	$99.8^{c,d}$	$12.7^{a,b}$	3.1^{a}	$8.3^{a,b}$	2.68
C	88.5^{a}	$20.8^{f,g,h}$	6.3^{d}	$13.6^{f,g,h}$	2.16
D	$116.7^{h,i}$	$21.9^{g,h,i}$	6.6^{d}	$14.3^{g,h,i}$	2.17
E	124.7^{j}	27.0^{k}	8.0^{f}	17.7^{k}	2.21
F	113.3^{g}	$20.6^{f,g}$	$5.3^{b,c}$	$13.5^{f,g}$	2.55
G	124.1^{j}	$22.4^{h,i,j}$	6.5^{d}	$14.7^{h,i,j}$	2.26
H	110.8^{g}	28.0^{k}	8.1^{f}	18.3^{k}	2.26
I	$102.4^{d,e}$	$23.5^{i,j}$	$5.1^{b,c}$	$15.4^{i,j}$	3.02
J	106.3^{f}	$19.1^{e,f}$	5.4^{c}	$12.5^{e,f}$	2.31
K	96.4^{b}	12.3^{a}	2.9^{a}	8.0^{a}	2.76
L	100.1^{c}	$18.4^{d,e}$	5.4^{c}	$12.0^{d,e}$	2.22
M	$113.9^{g,h}$	$22.8^{h,i,j}$	7.3^{e}	$14.9^{h,i,j}$	2.04
N	$97.2^{b,c}$	24.5^{j}	6.6^{d}	16.0^{j}	2.42
O	$116.9^{e,f}$	30.7^{l}	8.4^{f}	20.1^{l}	2.39
P	116.9^{i}	30.0^{l}	10.5^{g}	19.7^{l}	1.88
Q	94.6^{b}	$22.4^{h,i,j}$	6.1^{d}	$14.7^{h,i,j}$	2.41
R	109.7^{g}	$19.6^{e,f}$	$5.1^{b,c}$	$12.9^{e,f}$	2.53
S	90.1^{a}	$16.3^{c,d}$	4.8^{b}	$10.7^{c,d}$	2.23

(1) Mean value of triplicate determinations. Values associated with different letters are significantly different (one-way ANOVA, LSD test, $p \leq 0.05$).

Table 4

Correlation coefficients (r) and level of significance (p) between concentration of gliadins (GLIA), glutenins (GLUT), high-molecular-weight glutenin subunits (HMW-GS), low-molecular-weight glutenin subunits (LMW-GS), gluten, SDS-soluble proteins (SDSS), glutenin macropolymer (GMP), and the parameters determined by the GlutoPeak test (aggregation time (AGT), peak maximum time (PMT), maximum torque (MT), energy to maximum torque (EnMT)).

			AGT	PMT	MT	EnMT
PROTEIN		r [(a)]	0.64	0.03	0.71	0.61
		p [(b)]	0.003	n.s.	≤0.001	0.006
GLIA		r	0.51	-0.09	0.70	0.55
		p	0.026	n.s.	≤0.001	0.014
GLUT	TOTAL	r	0.72	0.48	0.45	0.68
		p	≤0.001	0.039	n.s.	0.002
	HMW-GS	r	0.64	0.35	0.55	0.64
		p	0.003	n.s.	0.014	0.003
	LMW-GS	r	0.66	0.52	0.27	0.59
		p	0.002	0.02	n.s.	0.008
GLUTEN[(c)]		r	0.70	0.12	0.75	0.71
		p	≤0.001	n.s.	≤0.001	≤0.001
SDSS		r	0.36	-0.24	0.59	0.31
		p	n.s.	n.s.	0.008	n.s.
GMP	TOTAL	r	0.77	0.52	0.57	0.78
		p	≤0.001	0.02	0.011	≤0.001
	HMW-GS	r	0.61	0.41	0.50	0.60
		p	0.006	n.s.	0.028	0.007
	LMW-GS	r	0.77	0.52	0.57	0.77
		p	≤0.001	0.02	0.011	≤0.001

[(a)] $r \leq \pm 0.54$, no correlation; $\pm 0.54 < r \leq \pm 0.66$, weak correlation; $\pm 0.66 < r \leq \pm 0.78$, medium correlation; $\pm 0.78 < r \leq \pm 1$, strong correlation.

[(b)] $p > 0.05$, not significant (n.s.); $p \leq 0.05$, significant; $p \leq 0.01$, highly significant; $p \leq 0.001$, very highly significant.

[(c)] Sum of GLIA and GLUT.

FIGURE CAPTIONS

Fig. 1

Example of GlutoPeak curve of a winter wheat flour. The indices of importance are highlighted: lift of time (LOT), aggregation time (AGT), peak maximum time (PMT), maximum torque (MT), energy to MT (EnMT), and loss of torque 120s after peak (LT120).

Fig. 2

GlutoPeak curves of 19 winter wheat flour samples A to S. (a) Flours characterized by a slow buildup in consistency (longer aggregation time) and a long time to achieve peak consistency (group 1). (b) Flours that exhibited a rapid buildup in consistency (shorter aggregation time) followed by a rapid break down (group 2).

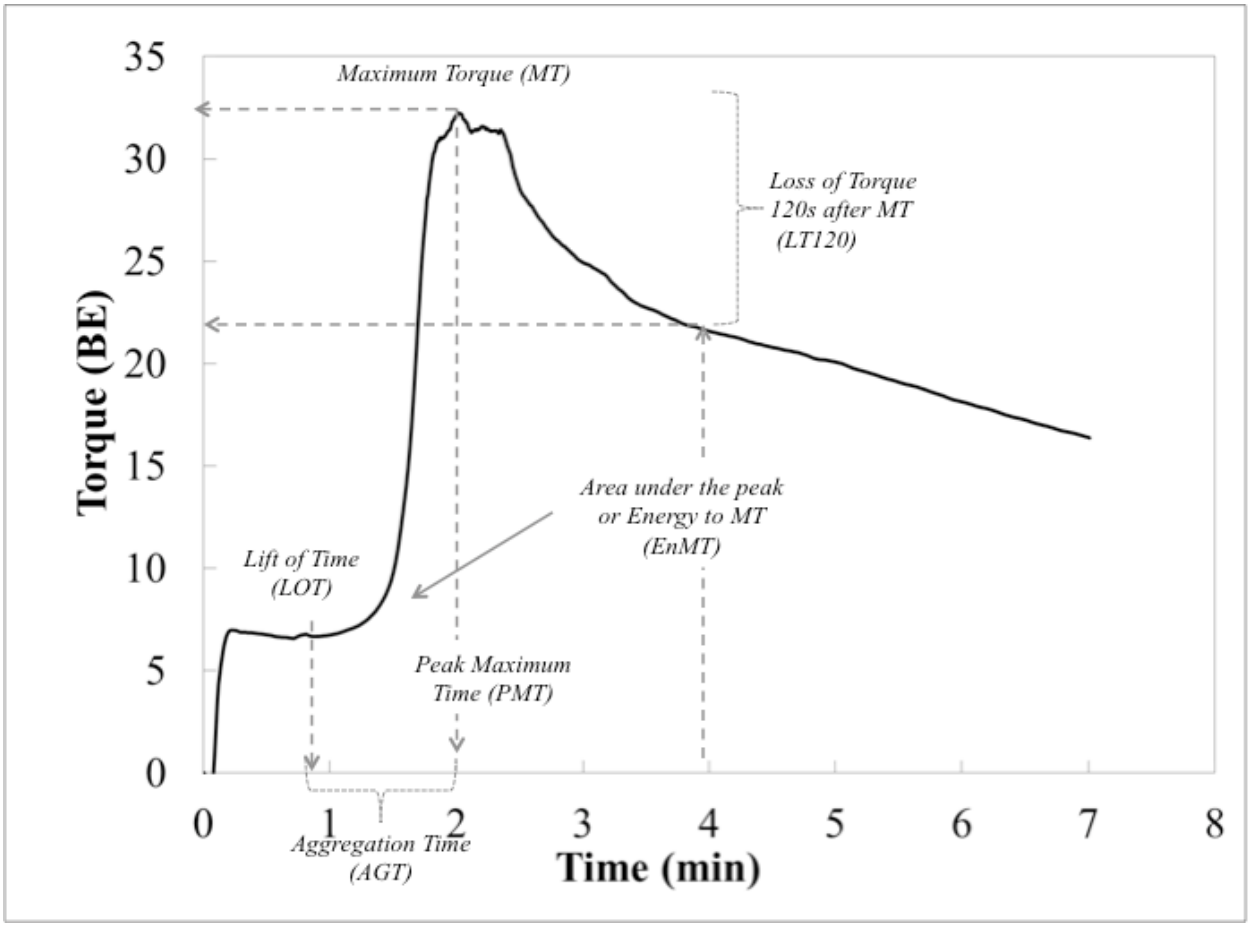
Maximum Torque (MT)
Loss of Torque
120s after MT
(LT120)
Area under the peak
or Energy to MT
(EnMT)
Lift of Time
(LOT)
Peak Maximum
Time (PMT)
Aggregation Time
(AGT)
Torque (BE)
Time (min)
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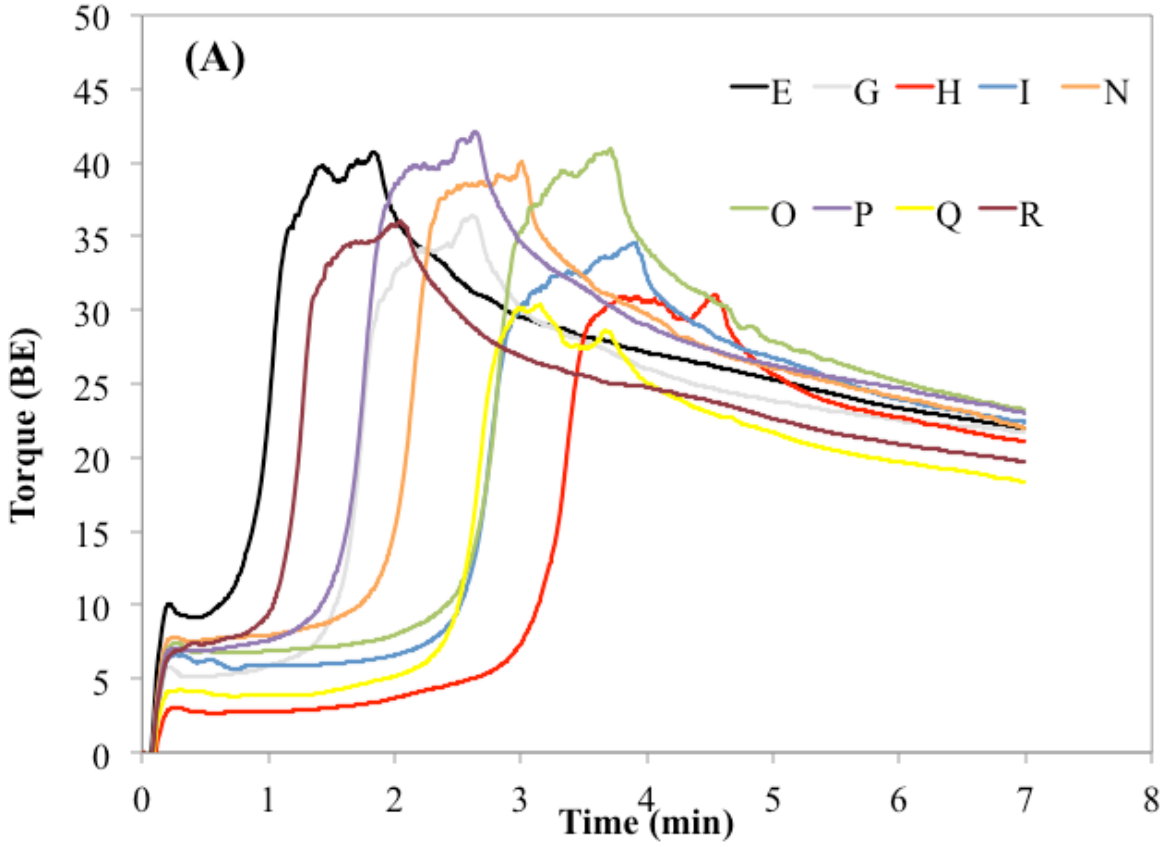
Figure 1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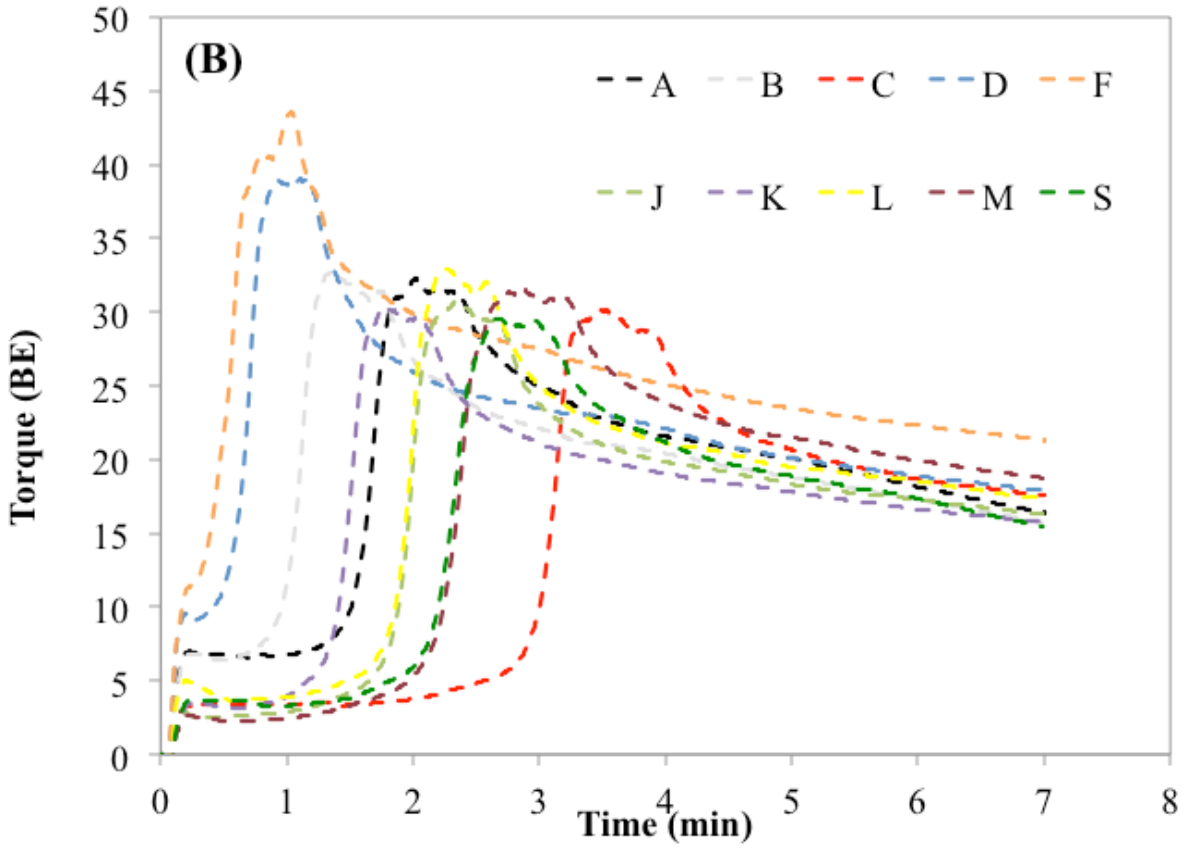
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Figure 2