Structural modification of gluten proteins in strong and weak wheat dough as affected by mixing temperature

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Structural modification of gluten proteins in strong and weak wheat dough as affected by mixing temperature

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Abstract

The effect of temperature (≥25 °C) on dough rheological properties and gluten functionality have been investigated for decades, but no study has addressed the effect of low temperature (< 30 °C) on gluten network attributes in flours with strong and weak dough characteristics. This study monitored changes in protein extractability in presence and absence of reducing agents, the content in readily accessible and SDS-accessible thiols, and the secondary structural features of proteins in doughs from commercial hard wheat flour (HWF) and soft wheat flour (SWF) mixed at 4, 15, and 30°C. SWF mixed at 4 and 15°C showed similar mixing properties as HWF mixed at 30°C (which is the standard temperature). The effect of mixing temperature is different at the molecular level between the two flours studied. Protein features of HWF did not change as mixing temperature decreased, with the only exception for an increase in SDS-accessible thiols. Decreasing mixing temperature for SWF caused an increase in SDS-protein solubility and SDS-accessible thiols, and an increase in β-turns structures at the expense of β-sheet structures. Thus, non-covalent interactions appear to drive protein network at low temperatures (4°C and 15°C) while covalent interactions dominate at standard mixing (30°C) in doughs from both flours.

Keywords: wheat dough, mixing temperature, protein conformation, protein solubility, thiols
Common wheat (*Triticum aestivum*) is the most versatile cereal since the flour from the grains can successfully be transformed into a large number of products - bread, cookies, biscuits, cakes - that are highly diverse in their palatability, structure and consumption occasions. The success of wheat is due to the capacity of its storage proteins to interact and develop the gluten network, which is the framework of all bakery products.

The functionality of gluten in defining product attributes differs based on ingredient formulation and processing conditions. However, regardless of the product formulation and processing condition, gluten formation is the key step for the preparation of cereal-based products. During mixing, the solvated proteins become flexible enough to undergo further structural modifications upon kneading. In most cases, kneading results in a rearrangement of the pattern of two major types of interactions: disulfide bridges and hydrophobic contacts between surface-exposed regions (Bonomi et al. 2014). It has recently been shown that gluten network formation is inherently different at the molecular level between soft and hard wheat flours. Development of a network in hard wheat dough appears to be driven more by disulfide linkages, whereas the network in soft wheat dough is governed primarily by hydrophobic interactions (Jazaeri et al. 2015).

Obtaining the highest number of interaction among proteins requires a control of mixing conditions, as over-kneading weakens the interactions among proteins and the strength of the gluten network. This occurs as a consequence of the conversion of relatively rigid elements of secondary structure (e.g. α-helices and β-sheets) into random coil structures that do not contribute to the strength of the overall gluten network (Bonomi et al. 2014; Robertson et al. 2007). Moreover, it has been suggested that during mixing, the size of protein aggregates decreases (Mecham et al. 1965; Tsen 1967) as a consequence of physical separation of the aggregates (Tsen 1967). This has been
suggested to involve breakdown of either non-covalent interactions (Tsen 1967) or of covalent bonds (Tanaka and Bushuk 1973; MacRitchie 1975; Graveland et al 1980; Danno and Hoseney 1982).

Baking performance of wheat flours strongly depends on the mixing conditions. Best results in terms of bread volume are obtained when the dough is mixed to optimum consistency at standard speed (63 rpm), instead of mixing the dough at high speed (1,250 rpm) with a fixed time (1 min) (Thanhaeuser et al 2014). In regards of mixing temperature, 29 °C and 30 °C are the AACC standard temperatures for straight dough bread-making (AACC 10-10.03) and for the farinograph test (AACC 54-21.02), respectively. Although these conditions are important in providing the right environment for enzymes that are important to the various biochemical reactions required to produce the desired end result, doughs mixed at 30 °C appear underdeveloped and give poor baking results (Kieffer et al. 1998). Thus, mixing at 22 °C is indicated as preferable by some Authors (Kieffer et al 1998; Thanhaeuser et al 2014). Doughs mixed at temperatures lower than 30 °C require longer mixing to achieve the same development stage as for conventional dough mixed at 30°C (Basaram and Gocmen 2003; Thanhaeuser et al 2014). Moreover dough production at low temperatures necessitates additional expense to maintain the temperature such as water jackets, pre-chilling of flour, and cooler ambient temperature. On the other hand, the dough is less sticky and the resulting bread shows higher loaf specific volume and better grain texture in comparison to conventional mixing conditions (Basaram and Gocmen 2003; Thanhaeuser et al 2014).

To the best of our knowledge, this is the first study to evaluate the effects of mixing temperatures (4, 15, and 30 °C) on protein structural characteristics of hard and soft wheat flour doughs. The objective of this research was to evaluate protein solubility, thiols content, protein conformation, and farinograph characteristics of hard and soft wheat flours at various mixing temperatures. Studying such molecular parameters could lead to a more complete understanding of
the role of mixing temperature on the formation of the gluten network in functionally contrasting flours.

MATERIALS AND METHODS

Flours. Commercial soft (SWF, proteins: 9.0±0.1 g/100g<sub>d.b.</sub>) and hard (HWF, proteins: 13.04±0.06 g/100g<sub>d.b.</sub>) wheat flours, for biscuit- and bread-making respectively, were kindly provided by Horizon Milling LLC (Mankato, MN, USA). Protein content (N × 5.7) was determined according to AACC approved method (AACC 46-30.01).

Chemicals. All chemicals were of analytical grade, unless otherwise stated. Deuterium oxide (D<sub>2</sub>O), dithiothreitol (DTT), disodium hydrogen phosphate, sodium dihydrogen phosphate, and 5,5’ dithiobis (2-nitrobenzoate) (DTNB) were from Sigma Aldrich, (St Louis, MO, USA). Sodium chloride (NaCl) was from Fisher Scientific (Fair Lawn, NJ, USA), and sodium dodecyl sulfate (SDS) from Life Technologies (Grand Island, NY, USA). A RC-DC (reducing compatible and detergent compatible) Protein Assay for determining protein concentration was from Bio-Rad (Hercules, CA, USA).

Dough Preparation. Dough samples from both strong (HWF) and weak (SWF) flours were prepared at three temperatures (4, 15, and 30 °C) in a Farinograph-AT (C.W. Brabender Inc., Hackensack, NJ, USA) equipped with a 50 g mixing bowl. All the samples were prepared at the optimal water absorption, which is the amount of water to add to 100g of flour to attain a consistency of 500±20 Farinograph Unit (FU). The mixing bowl was kept at the desired temperature by means of a temperature controlled water bath attached to the farinograph. Mixing water was
delivered at the desired temperature. Dough samples for analyses were collected at dough development time, which is the time from first addition of water to the point of maximum consistency range. Each dough sample was prepared in duplicate. Samples were collected with minimal additional physical manipulation. The fresh dough was used as is for protein conformation spectroscopic studies. For protein solubility, and for measuring readily accessible and SDS-accessible thiols, the samples were immediately transferred to liquid nitrogen and lyophilized. The freeze-dried samples were then ground using a pestle and mortar to a powder (particle size < 0.5 mm).

**Protein Solubility.** Protein solubility in the freeze-dried dough was determined following the method of Jazaeri et al (2015) with little modification. Soluble proteins were extracted at 25°C in 0.05 M sodium phosphate buffer of pH 7.0 with 0.1 M NaCl and 1% SDS (w/v) in presence or absence of 10 mM DTT. A 1 ml volume of the buffer was added to 10 mg of sample and mixed on shaker for 60 min at 25°C. After centrifugation at 10,000×g for 5 min, the amount of protein in the supernatant (100 µl) was determined spectrophotometrically using the RC-DC Protein Assay, which is based on the Lowry assay (Lowry et al 1951). Bovine serum albumin was used as standard and results (average of four determinations) were expressed as mg soluble protein/g protein.

**Readily Accessible and SDS-Accessible Thiols.** Readily accessible thiols (SH) were determined following the method of Iametti et al (2006). An aliquot (100 mg) of sample was suspended in 5 mL of buffer (0.05M sodium phosphate, 0.1 M NaCl, pH 7.0) containing 0.5 mM DTNB. The suspension was incubated at 25°C for 60 min and centrifuged at 10,000 g for 3 min. The supernatant was subsequently filtered using Fisher Scientific filter paper (particle retention in the range of 5 – 10 µm; Pittsburgh, PA, USA), and the absorbance was read at 412 nm. SDS-accessible thiols were
determined by the same method but in presence of 1% SDS (w/v) in the suspension buffer. The amounts of readily accessible and SDS-accessible thiol groups were calculated using the extinction coefficient of 14150 M$^{-1}$ cm$^{-1}$ (Eyer et al 2003) and the average of four determinations was reported.

State Water and Protein Conformation. The infrared spectra of dough samples were recorded using an Attenuated Total Reflectance (ATR) Fourier Transfer Infrared (FTIR) spectrophotometer (Bruker Tensor 37, Bruker Optics, Inc., Billerica, MA, USA). The ATR-FTIR spectrophotometer was equipped with a horizontal multi-reflectance zinc selenide (ZnSe) crystal accessory. Spectra were collected in the 4000-600 cm$^{-1}$ infrared spectral range at room temperature. Each spectrum was an average of 32 scans at 4 cm$^{-1}$ resolution. Background spectrum of the empty trough sampling plate was collected before each sample. A minimum of 4 spectra per sample was used for spectral analysis. Spectra were collected within 10 minutes of sample preparation in order to limit molecular and structural changes as much as possible. The sample was pressed firmly onto the crystal to eliminate air and to achieve the best possible contact. Spectral analysis was performed by using OPUS software v. 7.0 according to Bock and Damodaran (2013). Reference H$_2$O-D$_2$O mixtures matched to the moisture content of the dough samples (~45 for all the samples except for dough HWF at 4°C, whose moisture was ~50%, Table I) were collected and vector-normalized. The difference of the vector-normalized spectra obtained in the 3000 – 3800 cm$^{-1}$ region were analyzed for changes in state of water structure in dough compared with the reference state in the H$_2$O-D$_2$O mixture following the approach used by Bock and Damodaran (2013). The reference H$_2$O-D$_2$O mixtures were also used for subtraction of water contributions in the amide I region (1600 – 1700 cm$^{-1}$) of the vector-normalized spectra. The quantitative estimation of protein secondary structure in the amide I region of dough was based on second derivative spectra using a five-point Savitsky-Golay function as described by Bock and Damodaran (2013). The spectral regions were assigned as
149 1620-1644, 1644-1652, 1652-1660 and 1660-1685 cm\(^{-1}\) for \(\beta\)-sheets, unordered, \(\alpha\)-helix, and \(\beta\)-turn structures respectively. The second derivative area for each secondary structural region was divided by the total area of the amide I region.

Statistical Analysis. Dough samples were prepared in duplicate. For each subsample, protein solubility, readily and SDS-accessible thiols, and ATR-FTIR analyses were carried out in duplicate. Analysis of variance (ANOVA) was performed utilizing Statgraphics XV version 15.1.02 (StatPoint Inc., Warrenton, VA, USA). Mixing temperature and/or type of secondary structures were used as factor. When a factor effect was found significant (\(p \leq 0.05\)), significant differences among the respective means were determined using Fisher’s Least Significant Difference (LSD) test.

RESULTS AND DISCUSSION

Mixing Properties of Flours. Mixing properties of SWF and HWF flours as affected by mixing temperature (4, 15, and 30 °C) are shown in Fig. 1A and 1B, respectively. The farinograph indices obtained from the curves are summarized in Table I. At 30 °C – which is the standard temperature according to the AACC official method - the HWF exhibited higher water absorption (64.7% vs 55.5%), longer dough development time (2.13 min vs 1.03 min) and greater stability (15 min vs 1.1 min) than the SWF. Regardless of the type of flour, as the temperature decreased from 30 to 4 °C, the optimal water absorption increased (Table I). As expected, a decrease in temperature increased dough consistency, and thus, higher amounts of water were required to obtain the desired dough consistency (500±20 FU). Decreasing mixing temperature also resulted in an increase in dough development time and dough stability (Table I).

In general, long dough development time is undesirable because it means longer processing time and increased energy requirements for dough mixing. On the other hand, low temperatures
increased dough stability and thus improves dough handling. Our results on mixing properties are similar to those obtained by Basaran and Gocmen (2003) who investigated the role of mixing temperature (17, 23 and 30 °C) on dough mixing and bread characteristics. Interestingly, at low temperature (4 °C and 15 °C), SWF showed a mixing profile very similar to that of HWF at 30 °C.

Protein Solubility. Protein solubility gives insight on the type of protein interactions occurring in flour or dough. The extent of contribution of hydrophobic and covalent interactions to protein network stabilization can be determined by adding detergents (e.g SDS) or reducing agent (e.g. DTT) respectively to the extraction buffer (Iametti et al 2006, 2012; Lagrain et al 2007; Bonomi et al 2012). Protein solubility of SWF and HWF dough as affected by mixing temperature is shown in Fig. 2. When the flours were mixed into dough to the point of maximum dough development at the standard temperature (30 °C), a decrease in SDS–protein solubility was observed for both SWF (from 693.87 ± 72.2 mg/g protein, in flour and dough, respectively) and HWF (from 811.33 ± 4.0 mg/g protein, in flour and dough, respectively), in agreement with previous studies (Jazaeri et al 2015; Hayta and Schofield 2004). The formation of a developed gluten network decreases the amount of SDS-soluble proteins due to the increased protein-protein interaction. At 30 °C, the two dough samples did not show statistically significance difference (p≤0.05) in SDS-protein solubility (HWF: 509.8 ±86.33 mg/g protein; SWF: 425.02 ±126.23 mg/g protein). Jazaeri et al (2015) and Kuktaite et al (2004) found dough from weak flours to have higher SDS-protein solubility than dough from strong flours at optimal mixing time. Similarly, Hayta and Schofield (2004) found extracted gluten from strong flour to have significantly lower SDS solubility than that from poor bread making flour. Differences in extraction methods, type of flour (cultivar, growing season, and location) and milling conditions could account for differences in protein solubility.
The amount of SDS-extractable proteins increased as mixing temperature decreased for SWF dough (Fig. 2A). However, no significant (p≤0.05) differences were observed between SDS-solubility in SWF dough at 15 and 30 or 4°C. The increased solubility at lower temperatures could be due to protein depolymerization as a result of the long mixing time, which could lead to lower molecular weight development through protein disaggregation (Weegels et al 1997).

At low temperature (4 and 15 °C), addition of DTT to the SDS solution used for protein extraction did not result in a significant increase (p≤0.05) in protein solubility, indicating that SWF dough samples were stabilized by hydrophobic interactions. This could be due to breakdown of disulfide linkages as a result of the long mixing times (Weegels et al 1997) or to lack of formation of disulfide linkages from sulphydryls because of the low temperature (Hayta and Schofield 2004). On the other hand, the significant (p≤0.05) increase in the amount of soluble proteins in presence of DTT in the dough at 30°C indicates that this temperature promoted the formation of covalent interactions. Moreover, at low temperature, the amount of proteins remaining insoluble in SDS+DTT was significantly (p≤0.05) higher compared to dough mixed at 30 °C. Dough mixing at temperature ≤ 15°C seems to promote the formation of macromolecular aggregates that do not solubilize easily under the conditions used in this study, regardless of the presence of DTT. Indeed, low temperatures were associated with an increase in the amount of unextractable fraction, which has been found to be strongly correlated with dough strength and bread quality (Weegels et al 1996; Don et al 2003).

As for dough from HWF, there was not a clear trend in protein solubility changes with mixing temperature (Fig. 2 B). A significant (p≤0.05) increase in SDS-extractable proteins was observed when the mixing temperature was decreased from 30 to 15° C. However, SDS-protein solubility of dough samples mixed at 4 °C was comparable to that of the dough samples mixed at 15 °C and 30 °C. The SDS-DTT protein solubility of dough followed the trend observed for SWF. The
30°C mixed dough sample showed significant difference (p≤0.05) between SDS-protein solubility and SDS-DTT solubility, an indication that covalent interaction is the gluten network stabilizing force in the dough. However, at low temperatures (4 and 15 °C), there was no significant difference (p≤0.05) between SDS and SDS-DTT protein solubility, suggesting that hydrophobic interactions are the dominant force in the gluten network. Reasons given for similar observations in SWF would suffice in explaining the observations in HWF dough. That is, dough mixing at temperatures ≤ 15°C promoted the formation of insoluble macromolecular aggregates under the conditions of the study. This is evidenced in the decreasing SDS-DTT protein solubility for the mixed doughs at 30, 15, and 4°C which were 961.02±12.84, 529.01±65.34 and 404.51±166.67mg/g protein respectively.

Readily Accessible and SDS-Accessible Thiols. The content of readily accessible and SDS-accessible thiols in dough from SWF and HWF are shown in Fig. 3A and Fig. 3B, respectively. When doughs were mixed at 30 °C (standard temperature), the readily accessible thiols in both SWF (7.02 ±0.1 μmol/g protein) and HWF (7.05±1.8 μmol/g protein) doughs were comparable to free thiols levels in extracted gluten reported by others (Koehler 2003a,b; Gomez et al. 2011). However, SWF dough showed significantly (p≤0.05) higher SDS-accessible thiols than HWF (Fig. 3A and 3B), in agreement with Jazaeri et al (2015). In general, in the presence of SDS, the thiol content showed a marked increase. Indeed, thiols buried within protein structures (or a protein aggregate) may become available to suitable reagents only upon protein denaturation by physical or chemical agents (Iametti et al 2013).

Mixing temperature did not affect the levels of readily accessible thiols in SWF doughs (Fig 3A). As for SDS-accessible thiols, significant (p≤0.05) differences were measured among samples, but without a clear trend. Indeed, SDS-accessible thiols in the dough mixed at 30 °C were higher than those of the one mixed at 15 °C, but lower than when mixing at 4 °C. The marked SDS-
dependent increase in accessible thiols observed for SWF dough mixed at 4°C indicates that disrupting of hydrophobic interactions and the consequent destabilization of the structure resulted in the exposure of a considerable amount of thiol groups (Iametti et al 2006). These results confirm the protein solubility data (Fig. 2A) that at low temperature SWF dough was characterized by hydrophobic interactions. The low protein solubility in presence of DTT and the high amount of unextractable proteins (Fig. 2A) at 4°C suggest the formation of intermolecular disulfide bonds that promote formation of large and insoluble aggregates (Lagrain et al 2007), where accessibility to the polar disulfide reducing agent DTT may be difficult even when SDS is present, at least when solubility studies are carried out at room temperature as it was the case here.

HWF dough samples exhibited a different trend in thiol changes as mixing temperature decreased (Fig. 3B) compared to SWF. Unlike SWF, the readily accessible thiols in HWF dough responded to temperature changes. While the 15°C dough showed lower levels of readily accessible thiols than at 30°C, the 4°C dough had a slightly higher but insignificant level than at 30°C (7.54 ± 0.9 vs 7.05± 1.8 µmol/g protein). With respect to SDS-accessible thiols, their levels in the dough increased as mixing temperature decreased. This indicates maximum formation of disulfide bonds at the standard mixing temperature of 30°C, in agreement with changes in protein solubility as due to the addition of disulfide-reducing agents (Fig. 2B). In addition, the increased levels of SDS-accessible thiols in the dough mixed at 4°C and 15°C may be due to the long mixing time (Table I) that possibly resulted in cleavage of disulfide bonds or exposure of thiols that were buried in inaccessible portions of protein aggregates, but were made accessible by the detergent treatment (Iametti et al 2006).
State of water in dough. The changes in the structural and energy states of water as affected by mixing temperature were characterized as reported by Bock et al (2013). The difference spectra obtained by subtracting the H$_2$O-D$_2$O reference spectrum (45-50% H$_2$O-D$_2$O, according to the dough moisture in Table I) is shown in Fig. 4. Dough samples exhibited two absorption bands; one centered at around 3580 cm$^{-1}$ and the other at around 3160 cm$^{-1}$, and a negative trough at 3400 cm$^{-1}$, regardless the mixing temperature. Based on previous studies, the positive peak in the difference spectrum at 3600 cm$^{-1}$ can be assigned to OH stretch vibration of monomeric non-hydrogen-bonded water molecules and some hydrogen-bonded water dimmers, whereas the peak at around 3160 cm$^{-1}$ can be assigned to water populations hydrogen-bonded to gluten network. The negative trough area represents fraction of water that has been transmuted from small hydrogen-bonded cluster states (3400 cm$^{-1}$) to other structural and energy states represented by the absorption bands at 3160 cm$^{-1}$ and 3580 cm$^{-1}$ (Bock et al 2013).

In SWF doughs, mixing temperature seemed not to affect the monomeric non-hydrogen-bonded water molecules, since the related peak was centered at around 3580 cm$^{-1}$, regardless the mixing temperature. On the other hand, the peak related to water populations hydrogen-bonded to the dough slightly shifted from 3163 to 3158 cm$^{-1}$ as mixing temperature decreased from 30˚C to 4˚C, suggesting that the hydrogen bonds of this structured water subpopulation was likely more rigid and more structured in the sample at 4˚C compared to the control dough (30˚C).

In the case of HWF, the OH stretch peak shifted to higher frequencies (from 3566 to 3585 cm$^{-1}$) as the mixing temperature decreased from 30˚C to 4˚C, suggesting a decrease in the number of hydrogen bonds per water molecule or increased distortion of the bonds in HWF dough mixed at low temperature. Typically, water strongly bonded via hydrogen bonding to functional groups in polymer networks requires less energy (low frequency) for OH stretch vibration. The stronger the hydrogen bond strength, the greater is the shift of OH stretch absorption to a lower frequency (Bock et al 2013). Intensity of the OH stretch
peak followed the order 15°C > 30°C > 4°C, indicating that the monomeric water molecules increased when dough was mixed at 15°C but decreased when the dough was mixed at 4°C. This could interact with thiol groups through hydrogen bonding and affect their availability for detection and may be part of the reason behind the least amount of readily accessible thiols found in the dough mixed at 15°C (Fig. 3b). Although the intensity of the 3160 cm⁻¹ band varied with mixing temperature (4°C), the peak position of the band did not change, indicating that the energy state of this population of water was not affected by the moisture content of dough, as confirmed by the protein conformation data (see Fig. 5). Unexpectedly, despite a decrease in the intensity of the structured water peak, the intensity of the free water peak did not decrease but rather increased at low mixing temperature.

It has been reported that even if hydrogen bonds are weak, they play a key role in determining the physical properties of dough (Tkachuk and Hlynka, 1968), as demonstrated by the increase in stability when dough was mixed at low temperature (Fig 1). The results were in agreement with the protein aggregate formation, since the increase in water structure can be responsible for increased non-covalent interactions (Fig. 2). In addition, it has been reported that structured water occurs at the hydrophobic patches on the protein surface (Zelent et al, 2009), in agreement with protein aggregation in Fig. 2. Investigating the impact of dough moisture on the state of water, Bock et al (2013) also highlighted a shift in frequency of the structured water peak, as the moisture content of the dough was increased. However, in the humidity range of 40-45%, which was very similar to the dough moisture in our study (Table I), the impact of dough moisture on the state of water was much greater than what was detected in Fig 4a, suggesting that the mixing temperature affected the way the flour interacts with water.

**Protein Conformation.** The secondary structure contents of protein in dough samples are shown in Fig. 5. In both SWF and HWF dough mixed at 30°C, the structures were in the order β-sheets > β-
turns > random > α-helix, in agreement with previous studies (Jazaeri et al 2015; Bock and Damodaran 2013; Pézolet et al 1992; Li et al 2006). In weak doughs, high levels of β-sheets structures have been associated with protein hydrophobicity (Jazaeri et al 2015). In strong doughs, formation of β-sheets seems to be facilitated by disulfide linkages (Jazaeri et al 2015), which is in agreement with the protein solubility data in presence and absence of reducing agent (Fig 2b) and with the thiols content (Fig 3b).

Dough mixing temperature affected protein conformation differently in the two flours (Fig. 5). In SWF dough, β-sheet structures significantly (p≤0.05) decreased with a gain in β-turn structures, as temperature decreased (Fig. 5A). However, random and α-helix structures were not significantly (p≤0.05) affected by changes in mixing temperature. It has been reported that any change in wheat dough that causes a greater hydration of dough is responsible for an increase in β-turn content at the cost of decrease in β-sheet content up to 45% moisture content (Bock et al 2013). On the other hand, β-turn structures subsequently dropped at 50% moisture content (Bock et al 2013). In the present study, as mixing temperature decreased, the moisture content of the dough increased from 42.1 to 46.6% (Table 1) and the β-turn structures – regions where there are groups of polymer solvent interactions (Belton 1999) increased, whereas the β-sheet structures – regions where there are groups of polymer surface interactions (Belton 1999) decreased. Decreasing β-sheet structures with temperature might suggest a weakening of hydrophobic interactions, as mixing temperature decreased.

In SWF dough, the β-sheet to turn ratio decreased as the mixing temperature decreased (1.55, 2.36, and 3.19 for 4, 15, and 30°C, respectively; data not shown), suggesting the formation of a more hydrated system, consistent with the slightly decreasing shift in peak frequency for water population strongly bonded to dough (Fig. 4A). Interestingly, the β-sheet to turn ratio for SWF dough at 4°C
was comparable with those of HWF dough at 30°C (1.62; data not shown) which also showed the least amount of free water (Fig. 4B).

In HWF dough, a decrease in mixing temperature caused only a slight increase in β-sheet structure, whereas β-turn, α-helix and random structures were not affected by temperature changes (Fig 5B). This may indicate that gluten proteins in HWF dough are not susceptible to change in their conformation at the low temperatures, unlike those in SWF. This is supported by the fact that the SDS protein solubility did not show a consistent trend as mixing temperature decreased from 30°C to 4°C (Fig. 2B). These results are in agreement with those of Bock et al (2013) who found that weak flour dough was unable to maintain its secondary structural distribution upon bran addition compared to the strong flour dough.

Protein conformation may therefore be of little relevance in protein stabilization in HWF dough when mixed at lower temperatures (4°C and 15°C). On the other hand, given the increase in SDS-accessible thiols as mixing temperature decreased (Fig. 3B) and the corresponding increase in SDS protein solubility (Fig 2B), it is possible that the longer mixing times associated with low temperature (Table I) enhanced cleavage of intermolecular disulfide linkages in high molecular weight glutenin subunits; these events have been reported to occur at higher levels in HWF (Payne et al 1981, 1987).

**CONCLUSIONS**

From the results of this study on the effect of mixing temperatures on protein structural characteristics of SWF and HWF flour doughs, three main conclusions can be drawn. First, at mixing temperature lower than 30 °C, dough from SWF flour showed a similar rheology as dough from HWF flour mixed at standard temperature. Second, network formation in dough samples mixed at 4 °C and 15 °C appear to be driven more by hydrophobic interactions, whereas the network
formed at 30 °C is mainly characterized by covalent interactions. Finally, the effect of low mixing temperature is different at the molecular level between the HWF and SWF flours considered in this study. In particular, in HWF flour mixing temperature strongly affected only the SDS-accessible thiols content, whereas, in SWF flour mixing temperature resulted in changes in all the molecular parameters considered in this study.

LITERATURE CITED


FIGURE LEGENDS

Fig. 1. Mixing profiles of soft (A) and hard (B) wheat dough prepared at 4, 15 and 30 °C.

Fig. 2. Protein solubility of soft (A) and hard (B) wheat dough mixed at 4, 15 and 30 °C. Values with the same letters are not significantly different (p≤0.05).

Fig. 3. Readily accessible and SDS-accessible thiols of soft (A) and hard (B) wheat dough prepared at 4, 15 and 30 °C mixing temperature. Values with the same letters are not significantly different (p≤0.05).

Fig. 4. ATR-FTIR difference spectra of soft (A) and hard (B) wheat dough prepared at various mixing temperatures in the OH stretch region.

Fig. 5. Distribution of protein secondary structure forms in the dough from soft (a) and hard (b) wheat prepared at various mixing temperatures. A separate ANOVA was run for each structure. Symbols associated with different letters are significantly different (one-way ANOVA, LSD test, p≤0.05).
Table I.

Mixing properties of soft and hard wheat flours at various mixing temperatures

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<th>Hard wheat flour (HWF)</th>
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Fig. 1
For Peer Review

![Graph showing soluble protein (mg/g of protein) at different mixing temperatures: 4°C, 15°C, and 30°C. The graph compares two conditions: 1% SDS and 1% SDS + 10 mM DTT. Bars labeled with letters (a, b, c) indicate significant differences at the 0.05 level.](A)
Fig. 2.
Fig. 3.
Fig. 4.
Fig. 5.