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8 **Mechanism of gliadin-glutenin cross-linking during hydrothermal treatment**

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Abstract

The gluten proteins gliadin and glutenin are important for wheat flour functionality and they undergo changes during heat treatment involving sulfhydryl (SH) groups. To change the level of SH-groups during hydrothermal treatment, the oxidant potassium iodate (2.1 $\mu\text{mol/g}$ protein) and the reducing agent dithiothreitol (DTT, 6.1 $\mu\text{mol/g}$ protein) were added to 20% (w/w) gluten-in-water suspensions at room temperature, at 90 °C and after 15 min at 95°C and the viscosity was measured by the Rapid Visco Analyser (RVA). Protein extractabilities after hydrothermal treatment were determined with size-exclusion and reversed-phase HPLC. DTT decreased maximal RVA viscosity and the levels of extractable α - and γ -gliadin and this decrease was independent of the time of addition during hydrothermal treatment. In contrast, potassium iodate increased the levels of extractable α - and γ -gliadin. Its impact was less when added at later times during RVA analysis. A SH-blocking agent (N-ethylmaleimide, 8.0 $\mu\text{mol/g}$ protein) added at room temperature to the gluten suspension, decreased RVA viscosity at 95 °C and increased the extractabilities of glutenin and α - and γ -gliadin after hydrothermal treatment. Subsequent addition at 90 °C of a reducing agent (glutathione, 3.1 and 6.2 $\mu\text{mol/g}$ protein) recovered the control RVA profile and restored the control protein extractabilities after RVA analysis. This shows the importance of heat-induced gliadin-glutenin reactions for gluten viscosity and of the presence of free SH-groups for the polymerization of gluten proteins. A model explaining gliadin-glutenin polymerization through a sulfhydryl-disulfide exchange mechanism and demonstrating the effects of redox agents is put forward.

43 *Abbreviations:* db, dry basis; DTT, dithiothreitol, GSH, glutathione; HPLC, high
44 performance liquid chromatography; NEMI, N-ethylmaleimide; RP, reversed-phase; SDS,
45 sodium dodecyl sulfate; SE, size-exclusion; SH, sulfhydryl; SS, disulfide

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47

48 *Keywords:* Wheat gluten, Heat treatment, Gliadin-glutenin interaction, Protein
49 extractability, Redox agents, Thiol-disulfide interchange

1. Introduction

The storage proteins of wheat (consisting of monomeric gliadin and polymeric glutenin) are very important for wheat flour functionality. They have the unique rheological ability to form a dough matrix which determines bread quality. Gliadin represents a heterogeneous mixture of proteins containing α -, γ -, and ω -gliadins. All cysteine residues in α - and γ -type gliadins are involved in intra-chain disulfide (SS) bonds. In contrast, ω -gliadins lack cysteine residues. Glutenin consists of glutenin subunits (GS) of high molecular weight (HMW-GS) and low molecular weight (LMW-GS). The LMW-GS are classified as B-, C-, and D-types. LMW-GS form both intra-chain and inter-chain disulfide bonds among themselves and with HMW-GS leading to glutenin polymers (Veraverbeke & Delcour, 2002). Heat treatment of these proteins strongly influences the characteristics of baked products. The changes induced by heat eventually lead to large gluten protein aggregates with formation of gliadin-glutenin bonds through SS (cross-)linking in the process (Lagrain, Brijs, Veraverbeke & Delcour, 2005; Morel, Redl & Guilbert, 2002; Redl, Morel, Bonicel, Vergnes & Guilbert, 1999; Schofield, Bottomley, Timms & Booth, 1983; Singh & MacRitchie, 2004). Lagrain et al. (2005) applied a temperature profile and simultaneously measured rheological changes of a gluten-water suspension in the Rapid Visco Analyser (RVA). When the temperature was increased to 95 °C, mainly the glutenin extractability decreased. The polymerization of glutenin may involve oxidation (Singh et al., 2004) and sulfhydryl (SH)-SS exchange (Lagrain et al., 2005). Holding at 95 °C resulted in polymerization of both gliadin and glutenin and this caused a viscosity rise in the RVA profile (Lagrain et al., 2005). Oxidizing agents decreased RVA viscosities in the holding step and increased sodium dodecyl sulfate (SDS) protein extractabilities suggesting

73 decreased gliadin-glutenin cross-linking. In contrast, reducing agents increased RVA
74 viscosity at lower temperatures and lowered SDS extractabilities. It was postulated that
75 addition of reducing agent facilitates gliadin-glutenin cross-linking during heating while
76 oxidants hinder gluten polymerization due to decreased levels of free SH-groups and less
77 flexibility of the glutenin chains (Lagrain, Brijs & Delcour, 2006). These observations were
78 ascribed to a SH-SS exchange mechanism (Lagrain et al., 2006) as originally proposed by
79 (Schofield et al., 1983). This was concluded from the observation that the total level of free
80 SH-groups remained constant irrespective of the temperature (Schofield et al., 1983).
81 Recently, an SH-SS exchange mechanism in gluten was also observed under high pressure
82 (Schurer, Kieffer, Wieser & Koehler, 2007) and in bread making (Lagrain, Thewissen,
83 Brijs & Delcour, 2007). The SH-SS exchange mechanism requires free SH-groups. Thus,
84 changes in the level of free SH during hydrothermal treatment should affect gliadin-
85 glutenin association.

86 To further increase insight into the mechanism of the covalent association between gliadin
87 and glutenin during hydrothermal treatment, the aim of the present work is to investigate
88 the importance of free SH-groups at certain temperatures. To this end, RVA viscosities and
89 protein extractabilities were measured in the presence of redox additives.

2. Experimental

2.1. Materials

All reagents were at least of analytical grade and from Sigma-Aldrich (Steinheim, Germany) unless otherwise specified. Vital wheat gluten [moisture content: 6.10%, crude protein content (N x 5.7): 83.5% on dry basis (db), starch content: 10.4% on db] was obtained by industrial gluten-starch separation of European spring wheat flour by Tate & Lyle Europe (Aalst, Belgium). The gluten powder passed through a 250 µm sieve.

2.2. Controlled heating and cooling

A Rapid Visco Analyser (RVA-4D, Newport Scientific, Sydney, Australia) was used to apply a temperature profile to 25.00 g of 20% (w/w) suspensions of control gluten in the presence or absence of additives (Lagrain et al., 2006). The RVA converts the current required to maintain constant mixing speed (160 rpm) of a paddle into a viscosity value in Poise (P, 0.1 kg m⁻¹ s⁻¹), the unit of dynamic viscosity. This viscosity value is further referred to as RVA viscosity. At the start of the RVA analysis, suspensions were homogenized by hand-shaking and mixing (900 rpm for 20 s). The temperature profile included a temperature increase from room temperature to 40 °C (in 1 min), a linear temperature increase to 95 °C in 14 min, a holding step (40 min at 95 °C), a cooling step (7 min) with a linear temperature decrease to 50 °C, and a final holding step at 50 °C (13 min). Potassium iodate (2.1 µmol/g protein) or dithiothreitol (6.1 µmol DTT/g protein) were added as aqueous solutions (200 µl) immediately before RVA analysis, after 13 min (at 90 °C) and after 30 min (after 15 min at 95 °C). N-ethylmaleimide (8.0 µmol NEMI/g protein) was added as aqueous solution (200 µl) immediately before RVA analysis. At 90

°C, 3.1 or 6.2 µmol glutathione (GSH)/g protein was added to the suspension with NEMI. After RVA analysis, the gluten suspensions were frozen in liquid nitrogen, freeze-dried and ground in a laboratory mill (IKA, Staufen, Germany). Three characteristic viscosity values were recorded. The initial viscosity, the highest viscosity (in cP) at the start of the RVA run; the minimal viscosity, the lowest viscosity before the holding step; and the maximal viscosity, the highest viscosity in the holding step at 95 °C (Lagrain et al., 2006). All RVA analyses were performed at least in triplicate. The standard deviations calculated for the initial, the minimal and the maximal viscosities were less than 10% of the respective mean values.

2.3. Size-exclusion HPLC

SE-HPLC was conducted using a LC-2010 system (Shimadzu, Kyoto, Japan) with automatic injection. Freeze-dried gluten samples (1.0 mg) were shaken with 1.0 ml of a 0.05 M sodium phosphate buffer (pH 6.8) containing 2.0% SDS for 60 min at room temperature. After centrifugation (5 min, 10 000 g), the supernatant (60 µl) was loaded on a Biosep-SEC-S4000 column (Phenomenex, Torrance, United States). The elution solvent was (1:1, v/v) acetonitrile (ACN)/water containing 0.05% (v/v) trifluoroacetic acid (TFA). The flow rate was 1.0 ml/min at a temperature of 30 °C (Dachkevitch & Autran, 1989) and eluted protein was detected at 214 nm.

The elution profiles were divided into two fractions using the lowest absorbance reading between the two peaks as the cutoff point. The first fraction corresponded to the level of SDS extractable glutenin, the second was assigned to the level of SDS extractable gliadin. Total SDS extractable protein, gliadin and glutenin were calculated from the peak areas and

expressed as percentage of the peak area of unheated gluten extracted with the SDS buffer in the presence of 1.0% DTT.

2.4. Reversed-phase HPLC

Samples (100.0 mg) were extracted three times with 3.0 ml 60% (v/v) ethanol (gliadin extract) and three times with 3.0 ml 0.05 M Tris/HCl buffer (pH 7.5) containing 50% propan-1-ol, 2.0 M urea and 1% (w/v) DTT and kept under nitrogen (reduced glutenin extract). The gliadin and glutenin extracts were loaded (80 µl) on a Nucleosil 300-5 C8 column (Machery-Nagel, Düren Germany). The elution system consisted of deionised water + 0.1% (v/v) TFA (A) and ACN + 0.1% TFA (v/v) (B). Proteins were eluted with a linear gradient from 24% B to 56% B in 50 min and detected at 214 nm.

α -Gliadin, γ -gliadin, ω -gliadin, B/C-LMW-GS, D-LMW-GS and HMW-GS were distinguished based on absorbance minima between specific peaks as outlined earlier by Wieser, Antes & Seilmeier (1998).

2.5. Statistical analysis of data

Significant differences ($P < 0.05$) in initial, minimal and maximal RVA viscosities were determined by the ANOVA procedure using the SAS package (SAS system for Windows V8, SAS Institute Inc., Cary, NC, USA) and were based on at least three individual measurements.

3. Results and Discussion

3.1 Addition of redox agents at different points during RVA analysis

The impact on RVA viscosity of the addition of 6.1 μmol DTT/g protein (800 ppm) at 0 min, at 13 min (90 °C) and at 30 min (after 15 min at 95 °C) during hydrothermal treatment of a 20% (w/w) gluten-in-water suspension is shown in Figure 1. The control gluten suspension showed a substantial initial RVA viscosity which decreased when the temperature was raised to 90 °C. In the holding step (95 °C), the RVA viscosity steadily increased. During cooling, the RVA viscosity decreased again and, in the final holding step at 50 °C, no viscosity changes were observed (Fig. 1). These effects were previously described by Lagrain et al. (2005). The interaction between gliadin and glutenin is responsible for the initial RVA viscosity (Lagrain et al., 2005). The decrease of RVA viscosity during heating can be ascribed to changes in physico-chemical properties of the gluten proteins, such as conformational changes and a loss of hydrogen bonds, which readily break on heating. The viscosity rise in the RVA profile at temperatures exceeding 90 °C was caused by formation of large glutenin polymers with the incorporation of gliadin through SS bonds impacting the rotation of the RVA paddle. The sudden decrease in apparent viscosity during cooling was due to the protein polymers aggregating tightly and sticking to the paddle caused by the loss of kinetic energy from heating (Lagrain et al., 2005). Addition of 6.1 μmol DTT/g protein before RVA analysis resulted in initial, minimal, and maximal viscosities that were all significantly lower ($P < 0.05$) than the control viscosities (Table 1). Minimal viscosity was reached at lower temperatures (Fig. 1). Addition of DTT at 13 or 30 min still resulted in a significantly lower maximal viscosity (Table 1). Heating gluten suspensions first decreased glutenin extractability and, during

holding at 95 °C, also gliadin extractability (Lagrain et al., 2005). The effect of the addition of 6.1 μ mol DTT/g protein on protein SDS extractability after RVA analysis was the same for the addition after 0, 13 or 30 min (Table 2). Addition of DTT decreased both gliadin and glutenin SDS extractabilities of control gluten after hydrothermal treatment. The decrease in gliadin extractability could be attributed to a decrease in α - and γ -gliadin extractabilities as calculated from RP-HPLC data (Fig. 2). The levels of α -gliadin and γ -gliadin decreased drastically after RVA analysis of control gluten, to respectively 13% and 6% of their original values, whereas that of ω -gliadin only slightly decreased to 73% of its original extractability. Addition of DTT did not affect ω -gliadin extractability after RVA analysis, but α - and γ -gliadin became nearly unextractable in 60% ethanol (Fig. 2), irrespective whether DTT was added at 0, 13 or 30 min during RVA analysis. The decrease in extractable gliadin levels after hydrothermal treatment (Fig. 2a) was accompanied by a significant increase in the apparent levels of the glutenin subunits (Fig. 2b), suggesting that a major portion of gliadins, unextractable in 60% ethanol after heat treatment, became extractable in the glutenin fraction. This resulted mainly in an apparent increased proportion of B/C-LMW-GS. The total amount of extractable protein (gliadin + glutenin) after complete reduction lowered when holding gluten at 95°C for 15 min and longer in the RVA (Lagrain et al., 2005) and this effect was enhanced with the addition of DTT, suggesting a greater heat sensitivity of gluten proteins in the presence of a reducing agent. While adding 2.1 μ mol potassium iodate/g protein at the start of RVA analysis resulted in almost no viscosity increase in the holding step, the addition at 13 min or at 30 min led to a maximal viscosity that did not differ significantly from the control viscosity (Figure 3, Table 1). Potassium iodate increased the protein extractabilities at the end of the RVA run

compared to the control after hydrothermal treatment, but to a lesser extent than added at later points during hydrothermal treatment. The effect could be ascribed to both higher glutenin and gliadin extractabilities (Table 2). More specifically, the addition of potassium iodate mainly resulted in increased α - and γ -gliadin extractabilities after RVA analysis accompanied by apparent decreases in LMW-GS compared to the control after hydrothermal treatment (Figure 4). The sum of the levels of extractable gliadin and glutenin after reduction was higher in the presence of potassium iodate after RVA treatment, but it decreased when iodate was added later in the process approaching the levels of control total extractability (results not shown).

Reducing agents, such as DTT, facilitate gliadin-glutenin cross-linking during heating. This effect depends on the available concentration of SH-groups in the system (Lagrain et al., 2006). Therefore, DTT addition had the strongest effect on the solubility because of the highest concentration of SH-groups. In the control gluten suspension, the SH-concentration was lower, and, hence, the extent of SH-SS interchange was lower than when DTT was added. Moreover the effect of DTT was independent of the time of addition during hydrothermal treatment. Thus, the introduction of a certain level of free SH-groups at any time during hydrothermal treatment results in proportionally more gliadin-glutenin cross-linking and less extractability of the proteins even after total reduction. In contrast, oxidants lower the level of free SH and decrease glutenin flexibility. This hinders SH-SS exchange reactions between gliadin and glutenin that normally occur at temperatures of at least 90 °C (Lagrain et al., 2006). As such, oxidizing agents terminate gluten polymerization at that specific point of their addition during hydrothermal treatment with, depending on their concentration, little if any further reaction afterwards. Since ω -gliadins do not contain SH-

groups, they are not involved in SH-SS exchange reactions and their extractability is not influenced by redox agents.

3.2 Combination of NEMI and glutathione

The gluten proteins contained about 8.0 μmol free SH per g protein (Lagrain et al., 2005). Addition of an equimolar level of the SH-blocking agent NEMI to the gluten suspension resulted in an RVA profile with no viscosity increase in the heating and holding phases (Fig. 5). Potassium iodate and NEMI had similar effects on RVA viscosity (Fig 3 and 5). Subsequent addition of increasing concentrations of reducing agent with one SH-group (GSH) at minimal RVA viscosity (after 13 min and at 90 °C) increased RVA viscosity at 95 °C, and 6.2 μmol GSH/g protein restored the control RVA profile (Fig. 5). NEMI increased gliadin and, to a lesser extent, glutenin extractabilities after heat treatment of the control, suggesting less gliadin-glutenin reactions. Subsequent addition of increasing GSH concentrations decreased protein extractabilities to approximate those of a control suspension at 6.2 μmol GSH/g protein. This was reflected in decreased α - and γ -gliadin extractabilities and apparent increases in LMW-GS extractabilities after RVA analysis over the control extractability (Fig. 6).

The addition of 8.0 μmol NEMI/g protein may alkylate and, as such, block the free SH-groups that were measured in unheated gluten proteins. Apparently this did not suffice to completely prevent further glutenin polymerization, since glutenin extractability decreased even in the presence of such a concentration of NEMI (Table 3). Addition of higher concentrations of NEMI (100 μmol /g protein) had similar effects on RVA viscosity (Lagrain et al., 2005), but completely inhibited the effect of hydrothermal treatment on

protein extractability with no change in gliadin and glutenin extractabilities after heating (results not shown). The fact that low concentrations of NEMI could not totally inhibit further glutenin linking during heating, indicates the possible exposure of previously inaccessible free SH-groups by conformational changes. Such groups may induce glutenin linking mainly through oxidation. This then would result in almost no free SH-groups at 95 °C preventing gliadin-glutenin cross-linking.

The addition of GSH at 90 °C after SH blocking probably restored the ability of gliadin to link to glutenin through a SH-SS exchange mechanism. However, in comparison to the control, the alkylated SH-groups were no longer accessible for reduction and, therefore, the protein extractability after addition of GSH approximated, but did not completely reach the control extractabilities.

3.3 Model of gliadin-glutenin cross-linking

As a whole, our results combined with earlier studies (Guerrieri, Alberti, Lavelli & Cerletti, 1996; Singh et al., 2004) lead to a view on gliadin-glutenin reactions and the impact of oxidants and reducing agents therein that we visualize in Figure 7. Glutenin is presented as a large molecule containing free SH-groups, while gliadin is a smaller molecule with only intramolecular SS bonds and, hence, no free SH-moieties. Heating to 90 °C first resulted in conformational changes exposing previously unavailable areas (Guerrieri et al., 1996) possibly containing free SH-groups and, next, polymerization of glutenin with oxidation of most but not all SH-groups (Lagrain et al., 2005; Singh et al., 2004). At 90 °C still some free SH-groups can be measured (Lagrain et al., 2006; Lagrain et al., 2005). At temperatures exceeding 90 °C, these free SH-groups of glutenin can induce a covalent

linkage with gliadin through a heat induced SH-SS exchange mechanism. This exchange reaction is catalyzed by SH-groups and readily occurs in (other) proteins at higher temperatures. The free SH-group carries out nucleophilic attack on the sulfur atom of a disulfide (Volkin & Klibanov, 1987). It is probable that conformational changes at 90 °C in the gluten proteins (Guerrieri et al., 1996) are necessary for gliadin-glutenin cross-linking. Addition of a reducing agent, such as DTT, increases the level of free SH-groups. Before heating this first leads to depolymerization of protein-protein disulfides with the formation of proteins with free SH-groups. At higher temperatures, these free SH-groups increase the level of gliadin-glutenin cross-linking. These observations were also made for gluten under high pressure in the presence of cysteine supporting the hypothesis of an ionic mechanism for SH-SS exchange reactions in gluten proteins such as stated by Schurer et al. (2007). In contrast, the presence of NEMI or an oxidant such as potassium iodate decreases the level of free SH-groups (Lagrain et al., 2006) leaving little if any free SH-groups in glutenin and hence little if any gliadin-glutenin cross-linking at temperatures exceeding 90 °C. However, subsequent addition of a reducing agent (*e.g.* GSH) containing at least one free SH-group, leads to incorporation of this group in glutenin and, as such, gliadin-glutenin cross-linking becomes possible again (Fig. 7). The subsequent addition of reducing agent at 90 °C can restore the control situation at this temperature. This then will lead to a similar level of gliadin-glutenin cross-linking with the recovery of the control RVA profile (Fig. 5) and the control SDS extractability (Table 3).

4. Conclusions

We can conclude that, at any time during hydrothermal treatment, free SH-groups initiate gliadin-glutenin reactions at higher temperatures. The extent of these reactions depends on the available concentration of SH-groups in the system. Removing such groups by addition of an oxidant or a SH blocking agent strongly reduces the extent to which gliadin becomes unextractable. By subsequently increasing the level of free SH-groups by adding a reducing agent, the capacity of gliadin to link to glutenin is restored. These findings can contribute to more insight in the impact of redox agents in cereal based processes such as bread, pasta, and cookie production.

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Fig. captions

Fig. 1. RVA profiles (40 min at 95 °C) of gluten-water suspensions with addition of DTT (6.1 $\mu\text{mol/g}$ gluten) at 0, 13 and 30 min. Control viscosity profile (-----), DTT at 0 min (——), DTT at 13 min (——), DTT at 30 min (——), temperature (-----). Arrows indicate moments of addition.

Fig. 2. Areas in RP-HPLC chromatogram representing gluten extractability with 60 % ethanol and 0.05 M Tris/HCl buffer (pH 7.5) with 50% propan-1-ol, 2.0 M urea and 1% (w/v) DTT after RVA analysis with addition of DTT (6.1 $\mu\text{mol/g}$ gluten) at 0, 13 and 30 min during RVA analysis. Fig. 2a shows the gliadin fraction with ω -gliadin (grey), α -gliadin (black) and γ -gliadin (white). Fig. 2b shows the reduced glutenin fraction with the apparent amounts of D-LMW-GS (grey), HMW-GS (white) and B/C-LMW-GS (black); AU, arbitrary units; C, control gluten suspension; HTT, hydrothermal treatment.

Fig. 3. RVA profiles (40 min at 95 °C) of gluten-water suspensions with addition of potassium iodate (2.1 $\mu\text{mol/g}$ gluten) at 0, 13 and 30 min. Control viscosity profile (-----), potassium iodate at 0 min (——), potassium iodate at 13 min (——), potassium iodate at 30 min (——), temperature (-----). Arrows indicate moments of addition.

Fig. 4. Areas in RP-HPLC chromatogram representing gluten extractability after RVA analysis with addition of potassium iodate (2.1 $\mu\text{mol/g}$ gluten) at 0, 13 and 30 min during RVA analysis. Fig. 4a shows the gliadin fraction with ω -gliadin (grey), α -gliadin (black) and γ -gliadin (white). Fig. 4b shows the reduced glutenin fraction with the apparent amounts of D-LMW-GS (grey), HMW-GS (white) and B/C-LMW-GS (black); AU, arbitrary units; C, control gluten suspension; HTT, hydrothermal treatment.

Fig. 5. RVA profiles (40 min at 95 °C) of gluten-water suspensions with addition of NEMI (8.0 $\mu\text{mol/g}$ gluten) at 0 min and GSH at 13 min. Control viscosity profile (-----), 8.0 μmol NEMI/g of protein (—), NEMI + 3.1 μmol GSH/g protein at 13 min (—), NEMI + 6.2 μmol GSH/g protein at 13 min (———), temperature (————). Arrow indicates moment of addition.

Fig. 6. Areas in RP-HPLC chromatogram representing gluten extractability after RVA analysis with addition of NEMI (8.0 $\mu\text{mol/g}$ gluten) at 0 min and GSH at 13 min during RVA analysis. Fig. 6a shows the gliadin fraction with ω -gliadin (grey), α -gliadin (black) and γ -gliadin (white). Fig. 6b shows the reduced glutenin fraction with the apparent amounts of D-LMW-GS (grey), HMW-GS (white) and B/C-LMW-GS (black); AU, arbitrary units; C, control gluten suspension; GSH1, 3.1 μmol GSH/g protein at 13 min; GSH2, 6.2 μmol GSH/g protein at 13 min; HTT, hydrothermal treatment.

Fig. 7. Model for gliadin-glutenin cross-linking through SH-SS exchange reactions during hydrothermal treatment. (I.1) In the absence of additives heating to 90 °C leads to conformational changes exposing previously unavailable free SH-groups and polymerization of glutenin with oxidation of SH-groups. (I.2) Glutenin can link to gliadin at temperatures exceeding 90 °C through a SH-SS exchange reaction and the generated free SH-group can react further with either gliadin or glutenin. (II.1) Reducing agents first depolymerize glutenin and increase the level of free SH-groups and, hence, (II.2) increase gliadin-glutenin cross-linking. (III.1) Addition of an oxidizing agent decreases the level of free SH and, hence, hinders glutenin linking and gliadin-glutenin cross-linking above 90 °C. (III.2) Subsequent addition of a SH containing agent can introduce new free SH-groups in the gluten proteins and again induce gliadin-glutenin covalent cross-linking.

396 **Fig.s**

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Tables

Table 1. Characteristic RVA viscosities (means of at least 3 observations) of gluten-water suspensions (20% w/v) during hydrothermal treatment in the presence of 6.10 μmol DTT/g protein or 2.1 μmol KIO_3 /g protein added at 0 min, 13 min or 30 min during RVA analysis.

Sample	Initial	Minimal	Maximal
	Viscosity (cP)	Viscosity (cP)	Viscosity (cP)
Control (C)	1405a	662a	2587a
C + DTT at 0 min	1228b	485b	2271b
C + DTT at 13 min	1424a	605a	1864b
C + DTT at 30 min	1416a	689a	2098b
C + KIO_3 at 0 min	1216a	682a	1010c
C + KIO_3 at 13 min	1498a	650a	2383a
C + KIO_3 at 30 min	1428a	705a	2384e
Control (C)	1405a	662a	2587a

Values in the same column followed by a different letter differ significantly ($P < 0.05$).

Relative standard deviations were less than 10%

421 Table 2. Extractabilities of gluten proteins in 2.0% SDS containing buffer before or after
 422 hydrothermal treatment (HTT) with 6.1 μmol DTT/g protein or 2.1 μmol KIO_3 /g protein
 423 added at 0 min, 13 min or 30 min during RVA analysis.

Sample	SDS	SDS	SDS
	extractable	extractable	extractable
	protein (%)	glutenin (%)	gliadin (%)
Control (before HTT)	80.9 (1.4)	25.8 (0.4)	55.2 (1.0)
Control (C, after HTT)	20.4 (0.3)	0.8 (0.0)	19.6 (0.3)
C + DTT at 0 min	10.0 (1.0)	0.3 (0.0)	9.7 (0.9)
C + DTT at 13 min	11.7 (0.9)	0.4 (0.0)	11.3 (0.9)
C + DTT at 30 min	11.7 (0.0)	0.2 (0.0)	11.5 (0.0)
C + KIO_3 at 0 min	51.0 (0.1)	4.9 (0.0)	46.1 (0.1)
C + KIO_3 at 13 min	47.8 (0.1)	4.3 (0.0)	43.5 (0.1)
C + KIO_3 at 30 min	24.2 (0.3)	0.8 (0.0)	23.4 (0.3)

424 Standard deviations are shown in brackets.

425

426 Table 3. Extractabilities of gluten proteins in 2.0% SDS containing buffer before or after
 427 hydrothermal treatment (HTT) with 8.0 μmol NEMI/g protein at 0 min and GSH at 13 min.

Sample	SDS	SDS	SDS
	extractable	extractable	extractable
	protein (%)	glutenin (%)	gliadin (%)
Control (before HTT)	80.9 (1.4)	25.8 (0.4)	55.2 (1.0)
Control (C, after HTT)	20.4 (0.3)	0.8 (0.0)	19.6 (0.3)
C + NEMI (0 min)	58.6 (0.3)	5.4 (0.0)	53.2 (0.3)
C + NEMI (0 min) + GSH ^b (13 min)	44.8 (0.1)	2.7 (0.0)	42.1 (0.1)
C + NEMI (0 min) + GSH ^c (13 min)	26.3 (1.2)	0.9 (0.1)	25.3 (1.5)

428 ^a 3.1 μmol / g protein

429 ^b 6.2 μmol / g protein

430 Standard deviations are shown in brackets.

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