This is the final draft post-refereeing.
The publisher's version can be found at <http://dx.doi.org/10.1016/j.jcs.2006.03.003>
Please cite this article as: Lagrain, B., Brijs, K., Delcour J.A. Impact of redox agents on the physico-chemistry of wheat gluten proteins during hydrothermal treatment, *Journal of Cereal Science* **2006**, 44, 49-53.

**Impact of redox agents on the physico-chemistry of wheat gluten proteins during
hydrothermal treatment**

Bert Lagrain*, Kristof Brijs and Jan A. Delcour

Laboratory of Food Chemistry, Katholieke Universiteit Leuven

Kasteelpark Arenberg 20, B-3001 Leuven, Belgium

*Corresponding author:

Bert Lagrain

Tel: + 32 (0) 16321634

Fax: + 32 (0) 16321997

E-mail address: bert.lagrain@biw.kuleuven.be

21 *Abbreviations:* db, dry basis; DTT, dithiothreitol; HPLC, high performance liquid
22 chromatography; HT, holding time; P, Poise; RVA, rapid visco analyser; SDS, sodium
23 dodecyl sulphate; SE, size exclusion; SH, sulphydryl.

24

25

26 *Keywords:* RVA, Wheat gluten, Heat treatment, Protein extractability, Oxidants,
27 Reducing agents

28

29 **Abstract**

30 The impact of the oxidants potassium bromate and potassium iodate and the reducing
31 agent dithiothreitol (DTT) on the rheological behaviour of 20% (w/v) gluten-in-water
32 suspensions during thermal treatment was monitored with the rapid visco analyser
33 (RVA). The suspensions were subjected to a linear temperature increase from 40 °C to
34 95 °C in 14 min, a holding step of 40 min at 95 °C, a cooling step (7 min) with a linear
35 temperature decrease to 50 °C, and a final holding step at 50 °C (13 min). Potassium
36 iodate (1.18 and 1.77 µmol/g protein) and potassium bromate (1.52 and 15.2 µmol/g
37 protein) decreased RVA viscosities in the holding step and increased sodium dodecyl
38 sulphate (SDS) protein extractabilities suggesting a greater heat resistance and decreased
39 gliadin-glutenin cross-linking. In contrast, DTT (1.65 and 3.30 µmol/g protein) made
40 RVA viscosity increase at lower temperatures and lowered SDS extractabilities. It is
41 postulated that low concentrations of reducing agent facilitate gliadin-glutenin cross-
42 linking during heating while oxidants hinder gluten polymerization due to decreased
43 levels of free sulphydryl groups and less flexibility of the glutenin chains.

44

1. Introduction

The storage proteins of wheat (consisting of monomeric gliadins and polymeric glutenins) are of great importance in bread making. During dough mixing, these flour proteins form a visco-elastic gluten network which is responsible for dough gas retention during proofing and contributes to the final bread structure. Gluten proteins polymerize during heat treatment. Sulphydryl (SH)-disulphide interchange reactions induced by heat affect all gluten proteins except the cysteine free ω -gliadins (Booth et al., 1980; Schofield et al., 1983) and result in the incorporation of gliadin monomers in the glutenin network (Morel et al., 2002; Redl et al., 1999; Singh and McRitchie, 2004). Lagrain et al. (2005) applied a temperature profile and simultaneously measured rheological changes in a gluten-water suspension using the Rapid Visco Analyser (RVA). Both gliadin and glutenin contributed to the initial viscosity in the RVA profile. When the temperature was increased to 95 °C, glutenin extractability decreased. Holding at 95 °C resulted in polymerization of both gliadin and glutenin. Above 80 °C, the RVA viscosity steadily increased with longer holding times while the extractability of gliadin and glutenin decreased. The formation of polymers through disulphide linking causes a viscosity rise in the RVA profile (Lagrain et al., 2005).

Most research on the effects of redox agents on gluten has been performed at ambient or proofing temperatures and little attention has been directed to understanding how rheological properties and gluten structure are affected by redox additives during hydrothermal treatment (Attenburrow et al, 1990; Hayta and Schofield, 2004). In addition, there do not appear to be any reports describing the combined impacts of redox agents and hydrothermal treatment on gliadin and glutenin separately. Indeed, although

68 redox agents affect the degree of cross-linking of the gluten network (Fitchett and
69 Frazier, 1986) and while it has been shown that dough proteins in the presence of
70 potassium iodate or bromate are less sensitive to heat denaturation (Veraverbeke et al.,
71 1999; Yamada and Preston, 1992), their separate impacts on either gliadin or glutenin
72 have, to the best of our knowledge, not been reported.

73 The aim of this study was to further increase insights in the impact of a hydrothermal
74 process on the properties of gluten proteins, and glutenin and gliadin in particular, by the
75 use of redox additives. Hydrothermal treatment was applied using the RVA (Lagrain et
76 al., 2005). The extractability of gliadin and glutenin by SDS, a good indicator of the
77 degree of cross-linking (Hayta and Schofield, 2004), was analyzed at different points
78 during the process using size-exclusion (SE)-high performance liquid chromatography
79 (HPLC).

80

81

2. Experimental

2.1. Materials

All reagents were of analytical grade and from Sigma-Aldrich (Steinheim, Germany) unless otherwise specified. Commercial wheat gluten [moisture content: 6.16%, crude protein content (N x 5.7): 78.9% on dry basis (db), starch content: 10.4% db] was from Amylum (Tate & Lyle, Aalst, Belgium) and was used without modification.

2.2. Controlled heating and cooling

The Rapid Visco Analyser (RVA-4D, Newport Scientific, Sydney, Australia) was used to apply temperature profiles to 25.00 g of 20% (w/v) suspensions of control gluten or gluten mixed with different additives. 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). The RVA was stopped at different points in the heating, holding and cooling phases of the profile and the gluten suspensions frozen in liquid nitrogen, freeze-dried and ground in a laboratory mill (IKA, Staufen, Germany). Potassium bromate [1.52 and 15.2 µmol/g protein (200 and 2000 ppm)], potassium iodate [1.18 and 1.77 µmol/g protein (200 and 300 ppm)], and dithiothreitol (DTT) [1.65 and 3.30 µmol/g protein (200 and 400 ppm)] were added as aqueous solutions immediately before RVA analysis.

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 (Fig. 1). 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 as described by Lagrain et al. (2005).

2.4. Protein content determination

Protein contents were determined as described by Lagrain et al. (2005).

2.5. Free sulphhydryl (SH) determination

Free SH groups were determined as described by Lagrain et al. (2005).

2.6. 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

The impact of different concentrations of DTT during hydrothermal treatment of a 20% (w/w) gluten-in-water suspension is shown in Fig. 2. As noted earlier (Lagrain et al., 2005), in the control profile, viscosity decreased in the heating step, increased when the gluten-water suspension was held at 95 °C, and decreased rapidly in the cooling phase. Addition of 3.30 µmol DTT/g protein (400 ppm) 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 (76.2 °C for 3.30 µmol DTT/g protein) than for the control (91.5 °C) (Fig. 2). The impact of the RVA run on the SDS extractability of the gluten proteins (mainly gliadin) increased with increasing concentrations of DTT (Table 2). In the heating step, SDS extractability of gluten proteins with DTT was higher (78.3% at 77 °C for 1.65 µmol DTT/g protein) than that of the control gluten (75.7%) (results not shown). However, when gluten suspensions with DTT were held at 95 °C, the extractability rapidly decreased and remained lower than that of the control (Table 3). Similar results (not shown) were obtained when cysteine or glutathione were used instead of DTT. Although no differences in free SH were observed between control gluten and gluten incubated with 1.65 µmol DTT/g protein (Table 4), the free SH content in the heating step of a gluten suspension with DTT (7.9 ± 0.3 µmol/g protein at 77 °C for 1.65 µmol DTT/g protein) was higher than that of a control suspension (5.3 ± 0.4 µmol/g protein) and remained higher throughout the RVA analysis (Table 4). These results on the impact of DTT during heating of gluten are generally in agreement with the previously described loss of gluten structure prior to heat setting by reduction of disulphide links, and the subsequent heat induced

reformation of network structure (Attenburrow et al. 1990) and the decreased extractability in 2.0% SDS (Hayta and Schofield, 2004).

Neither potassium bromate (Fig. 3) nor potassium iodate (Fig. 4) had a drastic impact on the initial and minimal viscosity values. However the minimal viscosity was reached at higher temperatures (94.0 °C for 1.18 µmol potassium iodate/g protein and 15.2 µmol potassium bromate/g protein; Table 1). The effect of oxidants was more pronounced in the holding phase at 95 °C where slower viscosity increases were recorded. The gluten-water suspensions with 1.52 µmol potassium bromate/g protein had higher maximal viscosities than the control (Fig. 3, Table 1). With higher concentrations of oxidant, the viscosity increase in the holding step was delayed and was lower than in the control profile. Oxidants increased the protein extractabilities at the end of the RVA run. The effect could be ascribed to higher gliadin extractabilities (Table 2). Whereas the oxidants decreased the SDS extractabilities slightly after 30 min at 40 °C, after 19 min at 95 °C, 1.18 µmol potassium iodate/g of protein resulted in an SDS extractability which was twice that for the heated control sample. At the end of the RVA run, the extractability was almost three times higher. These effects were also observed in the presence of potassium bromate, but to a lesser extent (Table 3). Both oxidants strongly reduced the level of free SH groups of gluten proteins after 30 min at 40 °C (Table 4). The level of free SH of gluten proteins in the presence of oxidants remained lower than that of the control suspension during the holding step at 95 °C. At the end of the RVA analysis, there was no difference in the levels of free SH between gluten suspensions with or without oxidant (Table 4).

It has been suggested that oxidants generate bonds between readily accessible thiol groups, and so reduce unfolding of the proteins during heating and decrease interchain disulphide

170 bonding in gluten polymers (Attenburrow et al, 1990; Hayta and Schofield, 2004; Nagao et
171 al., 1981).

172 The RVA profiles of gluten-water suspensions supplemented with 1.77 μmol potassium
173 iodate/g protein and 15.2 μmol potassium bromate/g protein were comparable to those of
174 gluten suspensions heated with an SH-blocking agent (100 μmol N-ethylmaleimide/g
175 protein) and to those of control gluten suspensions held at temperatures lower than 95 °C
176 (Lagrain et al., 2005). In addition, protein extractabilities, mainly gliadins, were higher in
177 the presence of oxidants, whereas the free SH content remained lower. The slow RVA
178 viscosity increase in the holding step in the presence of an oxidant indicated lower heat
179 sensitivity as previously observed (Veraverbeke et al., 1999). Oxidants lowered the level of
180 free thiol groups prior to heating (Table 4) and decreased glutenin flexibility. This may
181 hinder sulphhydryl-disulphide exchange reactions between gliadin and glutenin that
182 normally occur at temperatures of at least 90 °C (Lagrain et al., 2005; Schofield et al.,
183 1983). In agreement with their oxidation potential (Wieser, 2003) the effects were more
184 pronounced for the fast acting oxidant potassium iodate than for the rather more slowly
185 acting oxidant, potassium bromate. In contrast, DTT increased the RVA viscosity at lower
186 temperatures and, although the initial and minimal viscosities were lower due to the action
187 of the reducing agent, the profile almost completely recovered in the holding phase at 95
188 °C. Protein extractabilities in the holding step and at the end of the RVA run were also
189 lower at low concentrations of DTT. This reductant must have increased the heat sensitivity
190 of the gluten proteins and most likely favoured the association of the proteins during
191 thermal treatment through disulphide bonding by initially increasing the molecular
192 flexibility of the glutenin polymers by increasing the levels of free SH-groups that at higher

193 temperatures were able to participate in sulphhydryl-disulphide interchange reactions leading
194 to an increase in cross-linking of gliadin and glutenin above 75 °C.
195

4. Conclusions

DTT initially lowered RVA viscosity, but still resulted in an RVA viscosity increase at lower temperatures and lower SDS extractabilities than the control suspension. In contrast, potassium iodate and potassium bromate lowered RVA viscosities and increased protein extractabilities in the holding step, suggesting a greater heat resistance of the gluten proteins and less gliadin-glutenin cross-linking. Redox additives may affect the capacity of gluten proteins to associate during heating through sulphydryl-disulphide exchange reactions by altering the level of free thiol groups which can affect the flexibility of glutenin chains and initiate the polymerization reactions. The control of gluten polymerization, and in particular the degree of gliadin-glutenin cross-linking, during processes involving heating, such as bread making and extrusion, may well provide a key to process optimization. However, further research is necessary to validate the proposed model of hydrothermal treatment of gluten proteins under real processing conditions.

211 **Acknowledgements**

212 Financial support was obtained from the Institute for the Promotion of Innovation through
213 Science and Technology in Flanders (IWT-Vlaanderen, Brussels, Belgium). Sarah Clarysse
214 is thanked for excellent technical assistance.

215

References

- Attenburrow, G., Barnes, D.J., Davies, A.P., Ingman, S.J., 1990. Rheological properties of wheat gluten. *Journal of Cereal Science* 12, 1-14.
- Booth, M.R., Bottomly, R.C., Ellis, J.R.S., Malloch, G., Schofield J.D., Timms, M.F., 1980. The effect of heat on gluten-physico-chemical properties and baking quality. *Annales de Technologie Agricole* 1, 399-408.
- Fitchett, C.S., Frazier, P.J., 1986. Action of oxidants and other improvers, In: Blanshard, J.M.V., Frazier, P.J. and Galliard, T. (Eds.), *Chemistry and Physics of Baking*. The Royal Society of Chemistry, London, pp. 179-198.
- Hayta, M., Schofield, J.D., 2004. Heat and additive induced biochemical transitions in gluten from good and poor breadmaking quality wheats. *Journal of Cereal Science* 40, 245-256.
- Lagrain, B., Brijs, K., Veraverbeke, W.S., Delcour, J.A., (2005). The impact of heating and cooling on the physico-chemical properties of wheat gluten-water suspensions. *Journal of Cereal Science* 42, 327-333.
- Morel, M.-H., Redl, A., Guilbert, S., 2002. Mechanism of heat and shear mediated aggregation of wheat gluten upon mixing. *Biomacromolecules* 3, 488-497.
- Nagao, S., Endo, S., Tanaka, K., 1981. Scanning electron microscopy studies of wheat protein fractions from doughs mixed with oxidants at high temperature. *Journal of the Science of Food and Agriculture* 32, 235-242.
- Redl, A., Morel, M.-H., Bonicel, J., Vergnes, B. Guilbert, S., 1999. Extrusion of wheat gluten plasticized with glycerol: Influence of process conditions on flow behavior, rheological properties, and molecular size distribution. *Cereal Chemistry* 76, 361-370.

239 Schofield, J.D., Bottomley, R.C., Timms, M.F., Booth, M.R., 1983. The effect of heat on
240 wheat gluten and the involvement of sulfhydryl-disulfide interchange reactions. *Journal of*
241 *Cereal Science* 1, 241-253.

242 Singh, H., MacRitchie F., 2004. Changes in proteins induced by heating gluten dispersions
243 at high temperature. *Journal of Cereal Science* 39, 297-301.

244 Veraverbeke, W.S., Courtin, C.M., Verbruggen, I.M., Delcour, J.A., 1999. Factors
245 governing levels and composition of the sodium dodecyl sulphate-unextractable glutenin
246 polymers during straight dough breadmaking. *Journal of Cereal Science* 29, 129-138.

247 Wieser, H., 2003. The use of redox agents, In: Cauvain (Ed.), *Bread making, Improving*
248 *quality*. Woodhead Publishing Limited, Cambridge, pp. 424-446.

249 Yamada, Y., Preston, K.R., 1992. Effects of individual oxidants on oven rise and bread
250 properties of Canadian short process bread. *Journal of Cereal Science* 15, 237-251.

251

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 different concentrations of DTT, KBrO₃, KIO₃.

Sample	Initial Viscosity (cP)	Minimal Viscosity			Maximal Viscosity (cP)
		Visc	Time	T (°C)	
		(cP)	(min)		
Control	1531a	672b	13.2b	91.2b	2071b
C + 1.65 µmol DTT/g protein	1279b	680b	12.8c	89.0c	2020bc
C + 3.30 µmol DTT/g protein	1200b	533c	9.6d	76.2d	1523d
C + 1.18 µmol KIO ₃ /g protein	1373ab	661b	13.9a	94.0a	1795c
C + 1.77 µmol KIO ₃ /g protein	1561a	759a	13.8a	94.0a	1100e
C + 1.52 µmol KBrO ₃ /g protein	1306b	650b	13.3ab	91.9b	2383a
C + 15.2 µmol KBrO ₃ /g protein	1319b	648b	13.8a	94.0a	1266e

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

Relative standard deviations were less than 10%

258 Table 2. Extractabilities of gluten proteins in 2.0% SDS before or after RVA analysis in the
 259 presence of different concentrations of DTT, KBrO₃, KIO₃.

Sample	SDS extractable protein (%)	SDS extractable gliadin (%)	SDS extractable glutenin (%)
Control (before RVA run)	82.4 (0.1)	50.8 (0.4)	31.7 (0.3)
Control (C, after RVA run)	16.8 (0.1)	15.7 (0.1)	1.1 (0.1)
C + 1.65 µmol DTT/g protein	13.6 (0.2)	12.8 (0.3)	0.9 (0.1)
C + 3.30 µmol DTT/g protein	11.1 (0.0)	10.3 (0.0)	0.8 (0.0)
C + 1.18 µmol KIO ₃ /g protein	37.0 (1.2)	34.0 (1.4)	3.0 (0.2)
C + 1.77 µmol KIO ₃ /g protein	47.9 (2.4)	42.3 (2.1)	5.7 (0.3)
C + 1.52 µmol KBrO ₃ /g protein	28.1 (1.1)	26.2 (1.1)	1.9 (0.1)
C + 15.2 µmol KBrO ₃ /g protein	56.5 (0.4)	46.2 (0.4)	10.3 (0.0)

260 Standard deviations are shown in brackets.

261 Table 3. Extractabilities of gluten proteins in 2.0% SDS with addition of DTT, KBrO₃,
 262 KIO₃ during RVA analysis.

Total SDS extractability (%)				
RVA Temperature (°C)	Control	1,65 µmol	1.18 µmol	1.52 µmol
		DTT/g protein	KIO ₃ /g protein	KBrO ₃ /g protein
40 ¹	82.4 (0.1)	82.5 (2.6)	77.2 (0.3)	79.2 (1.2)
95 ²	24.8 (1.0)	19.0 (1.8)	50.9 (2.8)	40.3 (0.8)
50 ³	18.9 (0.5)	14.4 (0.0)	37.0 (1.2)	30.9 (0.4)
End	16.8 (0.1)	13.6 (0.2)	37.0 (1.2)	28.1 (1.1)

263 ¹After 30 min at this temperature

264 ²After 19 min at this temperature

265 ³In the cooling step

266 Standard deviations are shown in brackets.

267

268 Table 4. Free SH content ($\mu\text{mol SH/g protein}$) of gluten proteins with addition of DTT,
 269 KBrO_3 , KIO_3 during RVA analysis as determined by the reaction with DTNB in 2% (w/v)
 270 SDS, 3.0 M urea, 1.0 mM EDTA, 0.05 M NaH_2PO_3 .

RVA Temperature ($^{\circ}\text{C}$)	Control	1,65 μmol DTT/g protein	1.18 μmol $\text{KIO}_3/\text{g protein}$	1.52 μmol $\text{KBrO}_3/\text{g protein}$
40 ¹	7.6 (0.2)	7.5 (0.0)	3.5 (0.2)	4.7 (0.0)
95 ²	2.1 (0.1)	2.6 (0.1)	1.6 (0.1)	1.6 (0.2)
50 ³	2.5 (0.1)	2.7 (0.1)	2.3 (0.2)	2.2 (0.1)
End	2.6 (0.5)	3.1 (0.7)	2.5 (0.4)	2.5 (0.6)

271 ¹After 30 min at this temperature

272 ²After 19 min at this temperature

273 ³In the cooling step

274 Standard deviations are shown in brackets.

275 **Fig. captions**

276 Fig. 1. RVA profile (40 min HT at 95 °C) of a gluten-water suspension with indication of
277 characteristic viscosity values. Initial viscosity (A), minimal viscosity (B), maximal
278 viscosity (C).

279 Fig. 2. RVA profiles (40 min HT at 95 °C) of gluten-water suspensions with DTT. Control
280 viscosity profile (—), 1.65 μmol DTT/g of protein (—), 3.30 μmol DTT/g protein
281 (—), temperature (—).

282 Fig. 3. RVA profiles (40 min HT at 95 °C) of gluten-water suspensions with potassium
283 bromate. Control viscosity profile (—), 1.52 μmol KBrO_3/g protein(—), 15.2 μmol
284 KBrO_3/g protein(—), temperature (—).

285 Fig. 4. RVA profiles (40 min HT at 95 °C) of gluten-water suspensions with potassium
286 iodate. Control viscosity profile (—), 1.18 μmol KIO_3/g protein (—), 1.77 μmol
287 KIO_3/g protein (—), temperature (—).

288

289

290 **Fig.s**

291 Fig. 1

292

293 Fig. 2

294

295 Fig. 3

296

297

298

299 Fig. 4

300

301