Effect of addition of protein fractions extracted from flours of different baking quality on gluten rheology

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Summary

The effect of change of the rheological properties of gluten with the addition of fractions with specific molecular weight was investigated. Fractions extracted from Hereward, Riband and Soissons flours were added to the dough prior to gluten extraction. Once extracted, the glutens were subjected to temperature sweeps and creep recovery rheological tests. In the temperature sweeps, Hereward fractions containing the larger polypeptides had a strengthening effect on the gluten, indicated by a decrease in tan δ and an increase in elastic creep recovery, while those fractions that comprised monomeric gliadins had a weakening effect. Adding total gluten also had a strengthening effect. For the biscuit-making flour Riband, the results were quite the reverse: all fractions appeared to strengthen the gluten network, while the addition of total gluten did not have a strengthening effect. For Soissons gluten, the addition of total gluten had a strengthening effect while adding any individual fraction weakened the gluten. The results were confirmed with creep-recovery tests.

Keywords

fractionation; rheology; gluten; creep recovery

Gluten is the major protein in wheat flour doughs, responsible for their unique viscoelastic behaviour during deformation. It is now widely accepted that gluten proteins are responsible for variations in baking quality, and in particular it is the very high molecular weight (VHMW) glutenin polymer fraction which is best related to differences in dough strength and baking quality amongst different wheat varieties [1–3]. Gluten proteins comprise a broad molecular weight distribution of polymers, classically divided into two groups based on their extractability in alcohols: gliadins and glutenins. The gliadins are single-chain polypeptides with molecular weight (MW) ranging from 2×10^4 to 7×10^4, whilst the glutenins are multiple-chain polymeric proteins in which individual polypeptides are thought to be linked by inter-chain disulphide and hydrogen bonds to give a wide molecular weight distribution (MWD) ranging from 10^5 up to 10^9 Da. Gluten has a bimodal MWD which roughly parallels the classical division based on solubility into gliadins and glutenins [4, 5].

Previous work has shown that certain combinations of high molecular weight (HMW) glutenins exert a strong influence on baking quality [6] and examination of near-isogenic wheat lines which contain varying proportions of HMW glutenins show a strong relationship between HMW glutenins, measures of dough strength and baking performance [1, 7, 8]. A strong correlation between shear modulus of hydrated gluten and the proportion of the largest HMW glutenins has also been observed [9]. Gluten viscoelasticity has long been associated with baking performance and dough strength, although the relationship is not always clear [10]. JANSSEN et al. [11] found that, at constant protein content, the gluten from a cultivar with good baking performance had higher shear modulus and lower loss tangent in small deforma-

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tion oscillatory shear measurements, and higher strain hardening in large deformation extensional tests than gluten from cultivars not associated with good baking performance. In a study of 20 varieties grown in one location, TRONSMO et al. [12] found that dynamic shear moduli were not well correlated with baking volume, but were closely correlated with measures of VHMW glutenins such as unextractable polymeric protein and sodiumdodecylsulfate (SDS)-insoluble proteins and bread form ratio (height/width). A strong relationship between loaf volume, bread quality score and the highest MW part of the MW distribution (1×10^6 to 1.5×10^7 Da) has been found [13], while it has been shown [14] that increasing glutenin-gliadin ratios in eight lines of wheat whilst maintaining constant protein content at 9% (by adjusting starch levels) increased measures of dough strength such as mixograph mixing time and R_{max}, elongational viscosity and strain hardening, but decreased dynamic shear moduli. Dynamic shear moduli of gluten fractions extracted from a poor and a good breadmaking variety of wheat have been measured [15], and it was found that HMW fractions exhibited a predominantly elastic character (shear modulus $G' >$ loss modulus G'', G' independent of frequency), whilst the LMW fractions showed viscous behaviour ($G'' > G'$, G' increasing with frequency). No relationship was found between baking volume and dynamic shear moduli or creep recovery, although such relationship was observed for unfractionated gluten by HAYTA and SCHOFIELD [16].

Studies in polymer physics have shown that molecular size, structure and molecular weight distributions of polymers are intimately linked to their rheological properties and ultimately to their performance in various end-use applications [17, 18]. Beyond a critical molecular weight, characteristic for each polymer, rheological properties such as viscosity, relaxation time and strain hardening start to increase rapidly with increasing MW. Above this critical MW, the polymers start to entangle, giving rise to the observed rapid increase in viscosity with MW. Entanglements can be viewed as physical constraints between segments of the polymer chain, rather like loose knots [10]. A relatively small variation in VHMW can give rise to a large increase in viscosity and strain hardening, and is likely to have a large effect on baking performance.

Hence, controlled addition of VHMW fractions to gluten is one way of investigating the effects of these fractions on gluten strength and viscoelasticity, in contrast to investigating naturally occurring variations in VHMW fractions amongst different wheat cultivars. The aim of this paper is to investigate the effect of addition of specific, well characterized [15] protein fractions of varying MW to gluten on small deformation shear rheological properties.

MATERIALS AND METHODS

Three flour varieties varying in bread-making quality were used: Hereward, which is a typical UK bread-making variety particularly suitable for the Chorleywood breadmaking process; Riband, which is a biscuit making flour; and Soissons, a French bread-making variety. The flours were provided by Weston Research Laboratories (Maidenhead, United Kingdom), were of 14% moisture (w/v) and were stored at 2 °C. All the chemicals used were provided by Sigma (Dorset, United Kingdom) and were of analytical grade. Analytical data for the flours are given in Tab. 1.

Tab. 1. Results of flour analysis.

	Hereward	Riband	Soissons
Protein[a] (N × 5.7) [%]	10.7	9.7	10.5
Water absorption[b] [%]	61.1	55.6	57.5
Starch damage[c] [%]	37	15	26
Hagberg falling No.	356	286	403
Ash [%]	0.54	0.62	0.58

a – protein determined by Dumas method, b – Brabender Farinograph (300 g flour) mixed to 600 BU, c – Farand method.

Gluten protein fractionation

The fractionation procedure proposed by GRAVELAND et al. [19] was used modified as described previously [15]. Briefly, 50 g of chloroform-defatted flour was mixed with 600 ml of water and stirred for 15 min. Subsequently, the mixture was centrifuged for 15 min at 1 500 *g*. The pellet of solid material was then mixed for 10 min (in a 50 g Simon pin mixer) with 5 g of starch and 2 ml of 0.5 M acetic acid. The resulting artificial dough was mixed with water (600 ml) and stirred by a magnetic stirrer for 1 h. During this period, pH was adjusted to 3.9 by adding dilute acetic acid and coagulates were broken down. The mixture was again centrifuged for 10 min at 500 *g* and the starch-containing pellet (designated R1) was removed and discarded. The supernatant was then centrifuged at 8 000 *g* for 20 min. The pellet obtained was designated gluten protein fraction R2. NaCl was then added to the supernatant at

a concentration of 1 mg·ml^{-1}. After stirring for 10 min, the mixture was centrifuged again and the pellet obtained was designated gluten protein fraction R3. The procedure was repeated and fractions R4–R6 were obtained. To obtain fraction R7, the procedure was repeated but NaCl was added at a concentration of 7 mg·ml^{-1}. All the centrifugations were carried out at 5 °C, while all extraction was carried out at room temperature. Finally, all the fractions were re-dissolved in 0.05 M acetic acid, dialysed (molecular weight cut-off of dialysis tubes used: 12 000 Da) overnight against water, and freeze dried. These fractions and their parent glutens have been previously characterized for the molecular weight composition and distribution by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) [15]; dynamic and multi-angle laser light scattering (MALLS and DLS); differential scanning calorimetry (DSC); small deformation rheology [20, 21]; and flow field flow fractionation (FlowFFF) [22].

Gluten extraction

Flour (10 g) and distilled water (5 ml) were placed in the chamber of the Glutomatic 2100 instrument (Perten Instruments, Segeltorp, Sweden) and the dough was mixed for 25 s. Subsequently, the dough was washed for 5 min using a total of 250 ml of water.

Creep recovery experiments

The creep tests were performed on a Stress Tech controlled stress rheometer (Rheologica, Lund, Sweden). A constant stress of 50 Pa was applied, ensuring that the sample was in the viscoelastic linear region, for 100 s and the recovery was observed for the next 200 s. The geometry and gap were: cone and plate (20 mm, 4°) and 2 mm, respectively. The parameters obtained were the creep compliance (J_c) and the recovery compliance (J_r).

Temperature sweep experiments

For the temperature sweep experiments, the initial temperature was 25 °C and the final 95 °C. The rate of temperature increase used was 1 °C·min^{-1} as this corresponds approximately to actual baking conditions. The strain used was 2%, the frequency 1 Hz, and the gap between the metal plates was 2 mm. Heating was applied through the lower plate and measurements were taken from the top plate [15]. Tan δ vs temperature was recorded for all samples. The tests were carried out following the creep-recovery tests, allowing 2 h for the glutens to relax from the stresses applied previously.

Addition of gluten protein fractions into gluten

Protein fractions were added into dough. Gluten was then isolated and the rheological tests described earlier were carried out. The additions were carried out as follows: to 2 g of flour, 0.02 g (1%) of protein fraction was added. Wheat starch (Sigma) was also added (0.17 g) to normalize the protein content. This was mixed for 30 s in a 2g-Mixograph (National Manufacturing, Lincoln, Nebraska, USA) with 1.1 ml of water, and then allowed to rest for 4 min. A further 0.1 ml of water was added and the mixture was mixed again for 30 s and left to rest for 5 min, before concluding the procedure with a further 90 s mixing.

The doughs obtained this way were washed out in the Glutomatic 2100 and the glutens were subjected to the rheological tests to which gluten from flour with no additions had been subjected. Reverse-phase HPLC was used in order to ensure that the added protein fraction did actually remain in the gluten and had not been washed away during the gluten extraction (results not shown).

RESULTS AND DISCUSSION

Temperature sweeps

Temperature sweeps were carried out as described previously [15] using glutens from the flours Hereward, Riband and Soissons to which protein fractions R2–R7, produced from their corresponding flours, were added to the flour prior to dough mixing and the gluten extraction. A control, for which the added protein was total gluten, was also used. The results are presented as a function of the variation of tan δ with increasing temperature (Fig. 1, 2 and 3).

The results indicate that the addition of both total gluten (increasing the flour protein content) and addition of individual protein fractions to flour prior to dough mixing and gluten washing out had an effect on the rheological properties of the extracted gluten.

For Hereward samples (Fig. 1), the results suggest that the addition of high molecular weight protein fractions (R2, R3, R4) strengthened the gluten network shown by increasing G' and decreasing tan δ, while the addition of fractions mainly comprising gliadin and low molecular weight glutenin polymers had a weakening effect shown by increasing tan δ when compared with the control to which total gluten was added. These suggestions are based on comparison of the tan δ values against the control gluten sample, where a decrease in tan δ is equated to an increase of the strength of the gluten network [20, 22].

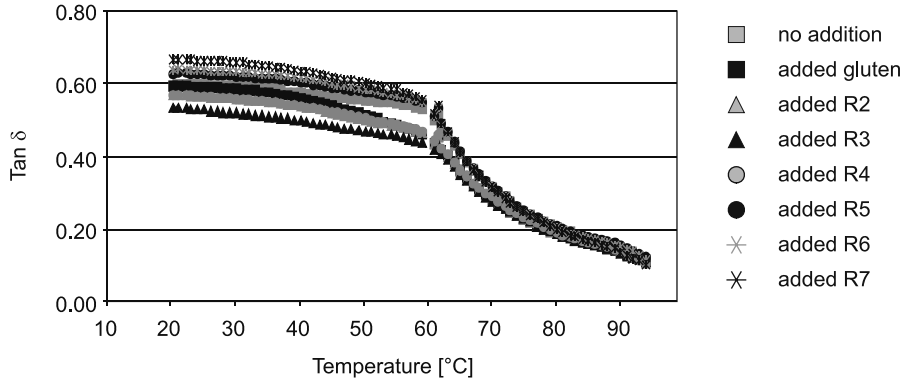

Fig. 1. Tan δ over a range of temperatures for gluten extracted from Hereward flour: Average of two measurements.

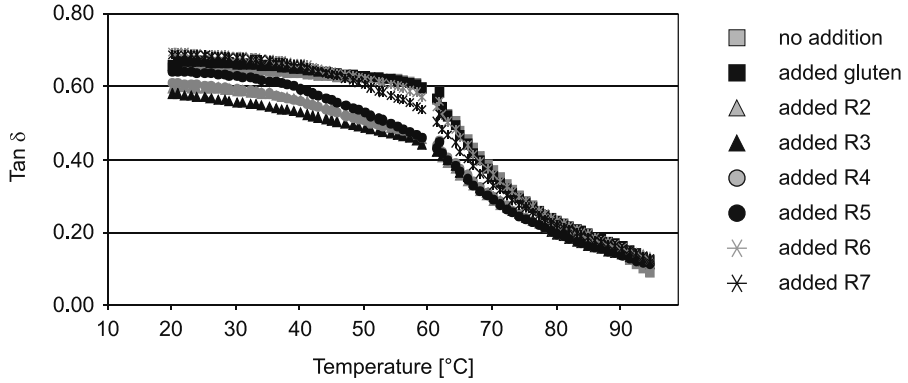

Fig. 2. Tan δ over a range of temperatures for gluten extracted from Riband flour: Average of two measurements.

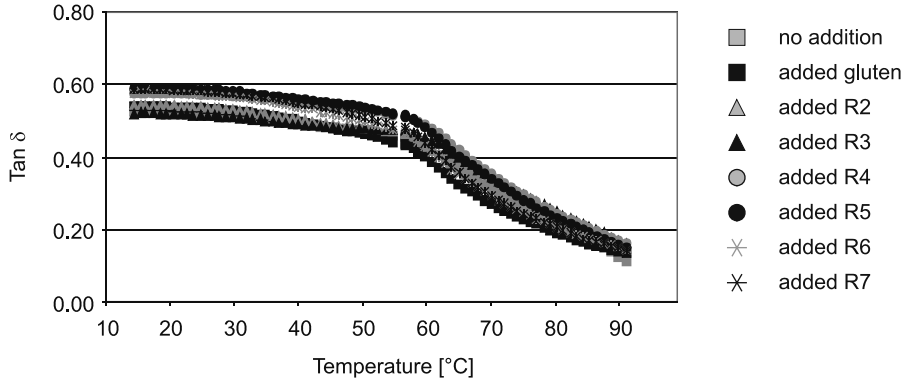

Fig. 3. Tan δ over a range of temperatures for gluten extracted from Soissons flour: Average of two measurements.

For gluten from Riband flour, however, the results were markedly different (Fig. 2). The addition of total gluten did not have a strengthening effect on the gluten network. All the gluten protein fractions, including the LMW fractions, when added to gluten resulted in strengthening of the gluten, as shown by decreasing tan δ.

Whilst such a strengthening effect may have been expected for addition of the fractions containing high molecular weight glutenin polymers, the strengthening effect on Riband caused by fractions containing low molecular weight polymers and/or gliadin was unexpected. The explanation for this unexpected observation is not clear. It is

recommended that further experimentation be carried out in order to assess whether this is common in weak flours and if so, to establish a molecular weight cut off point at which this phenomenon occurs.

For gluten from Soissons flour, results (Fig. 3) were the opposite than those for Riband. Adding any of the protein fractions weakened the gluten network, while adding total gluten had a strengthening effect. All samples were more elastic than the corresponding samples from both Hereward and Riband, particularly at the lower temperatures, and that was in agreement with previous findings [23].

Creep recovery

The samples used in these tests were the same as those used subsequently for the temperature sweeps. The tests were carried out in succession allowing 2 h for the gluten to relax from the stresses applied previously.

Fig. 4 shows that for Hereward gluten, the addition of protein fractions containing high molecular weight glutenin polymers (fractions R2–R4) resulted in gluten networks with greater instant recoveries and lower viscous elements, indicating strengthening of the networks. The creep recovery experiments therefore confirm the observations from the temperature sweeps. As before, fractions R5–R7 containing low molecular weight glutenin polymers and gliadin, had a different effect, resulting in weakening of the gluten network, as indicated by the lower instant recoveries and the higher values for the viscous element. The addition to the flour of total gluten prior to the gluten extraction had a slight strengthening effect on the

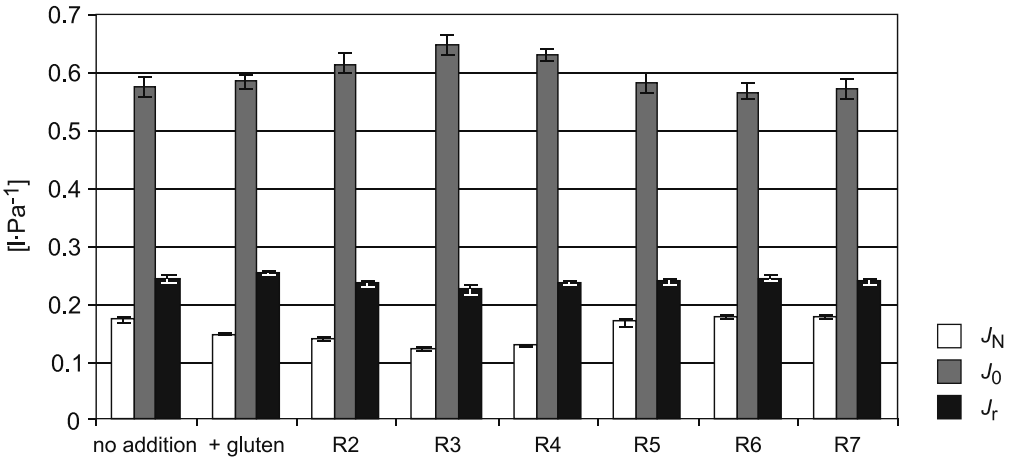

Fig. 4. Newtonian compliance (viscous element J_N), instant recovery (elastic element J_0) and time dependent compliance (slow recovery J_r) of Hereward glutens with various additions. Average of two measurements.

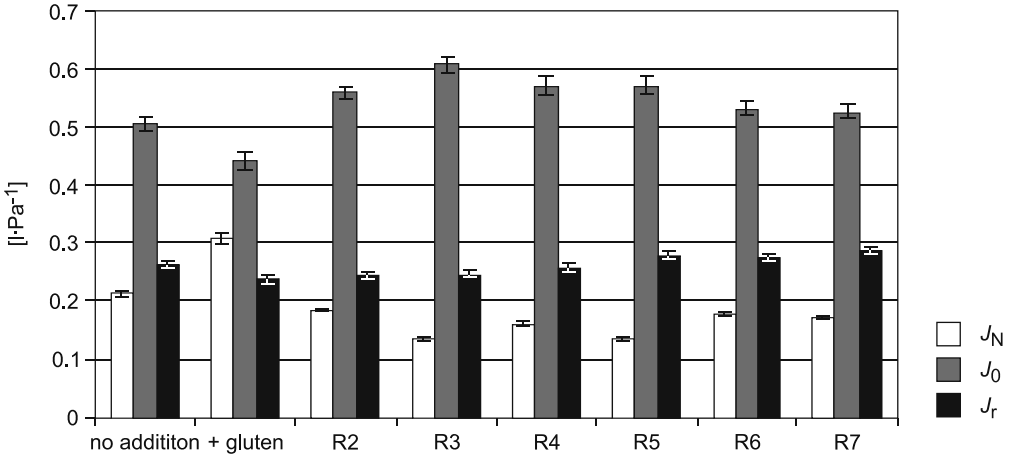

Fig. 5. Newtonian compliance (viscous element J_N), instant recovery (elastic element J_0) and time dependent compliance (slow recovery J_r) of Riband glutens with various additions. Average of two measurements.

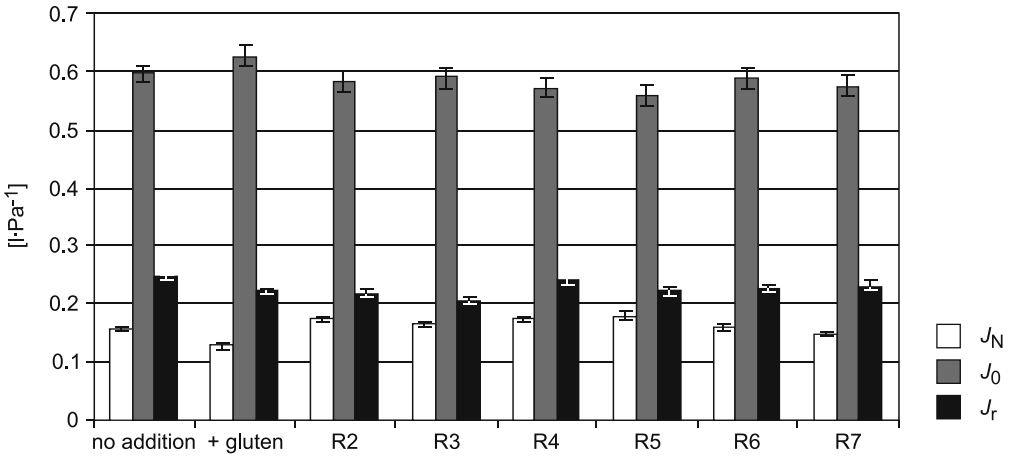

Fig. 6. Newtonian compliance (viscous element J_N), instant recovery (elastic element J_0) and time dependent compliance (slow recovery J_r) of Soissons glutens with various additions. Average of two measurements.

polymer, as indicated by a decrease in the viscous element and a small increase in the instant recovery. Riband gluten (Fig. 5) again demonstrated different responses to the addition of the various protein fractions. As with the temperature sweeps, the addition of total gluten to the flour prior to the extraction of the gluten resulted in weakening of the gluten, as indicated by an increase in the viscous element and a decrease in the instant recovery values. The addition of all the protein fractions, regardless of the size of the polymers, resulted in an increase of the instant recovery (the elastic element) of the gluten and a decrease in its viscous character. Soissons gluten samples (Fig. 6) also exhibited similar responses to those observed for the temperature sweeps. The addition of all fractions led to weakening of the network, while adding total gluten resulted in strengthening of the samples as indicated with the increased instant recovery.

The reason why all fractions from Riband had a strengthening effect and those from Soissons a weakening one, in both temperature sweeps and creep tests, is not especially clear but it is thought to be related with the molecular weight distribution of the proteins from glutens from those cultivars as determined previously [15, 23].

CONCLUSION

Gluten protein fractions extracted from Hereward, Riband and Soissons flours had a very different effect to the strength of the corresponding glutens when added back prior to gluten extraction, as determined by small deformation shear rheological tests. For Hereward, polymeric protein fractions strengthened the gluten, while fractions comprising monomeric gliadins had a weakening effect. For Riband, all fractions effectively strengthened the gluten while for Soissons, all fractions weakened the gluten. Addition of total gluten had a strengthening effect for the two bread-making flours, while for the biscuit–making Riband flour, the effect was the opposite.

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REFERENCES

1. Gupta, R. B. – Khan, K. – MacRitchie, F.: Biochemical basis of flour properties in bread wheats. I. Effects of variation in the quantity and size distribution of polymeric protein. Journal of Cereal Science, *18*, 1993, pp. 23–41.
2. MacRitchie, F. – Lafiandra, D.: Structure-function relationships of wheat proteins. In: Damodaran, S. (ed.): Food proteins and their applications. New York : Marcel Dekker, 1997, pp. 293–324.
3. Weegels, P. L. – Hamer, R. J. – Schofield, J. D.: Critical review: functional properties of wheat glutenin. Journal of Cereal Science, *23*, 1996, pp. 1–18.
4. Aussenac, T. – Carceller, J. L.: Size characterisation of glutenin polymers by HPSEC-MALLS. Journal of Cereal Science, *33*, 2001, pp. 131–142.
5. Arfvidsson, K. – Wahlund, G. – Eliasson, A. C.: Direct molecular weight determination in the evaluation of dissolution methods for unreduced glutenin.

Journal of Cereal Science, *39*, 2004, pp. 1–8.
6. Payne, P. I. – Nightingale, M. A. – Krattiger, A. F. – Holt, L. M.: The relationship between HMW glutenin subunit composition and the bread-making quality of British-grown wheat cultivars. Journal of the Science of Food and Agriculture, *40*, 1987, pp. 51–65.
7. Lawrence, G. J. – MacRitchie, F. – Wrigley, C. W.: Dough and baking quality of wheat lines deficient in glutenin subunits controlled by the *Glu-A1*, *Glu-B1* and *Glu-D1* loci. Journal of Cereal Science, *7*, 1988, pp. 109–112.
8. Uthayakumaran, S. – Newberry, M. – Keentok, M. – Stoddard, F. L. – Békés, F.: Fundamental rheology of bread dough with modified protein content and glutenin:gliadin ratios. Cereal Chemistry, *7*, 2000, pp. 744–749.
9. Cornec, M. – Popineau,Y. – Lefebvre, J.: Characterisation of gluten subfractions by SE-HPLC and dynamic rheological analysis in shear. Journal of Cereal Science, *19*, 1994, pp. 131–139.
10. Dobraszczyk, B. J. – Morgenstern, M. P.: Review: rheology and the breadmaking process. Journal of Cereal Science, *38*, 2003, pp. 229–245.
11. Janssen, A. M. – van Vliet, T. – Vereijken, J. M.: Rheological behaviour of wheat glutens at small and large deformations. Comparison of two glutens differing in bread making potential. Journal of Cereal Science**,** *23*, 1996, pp. 19–31.
12. Tronsmo, K. M. – Magnus, E. M. – Faergestad, E. M. – Schofield, J. D.: Relationships between gluten rheological properties and hearth loaf characteristics. Cereal Chemistry, *80*, 2003, pp. 575–586.
13. Lemelin, E. – Branlard, G. – Salvo, L. – Lein, V. – Aussenac, T. – Dayde, J.: Breadmaking stability of wheat flours: relation between mixing properties and molecular weight distribution of polymeric proteins. Journal of Cereal Science*, 42*, 2005, pp. 317–326.
14. Uthayakumaran, S. – Beasley, H. L. – Stoddard, F. L. – Keentok, M. – Phan-Thien, N. – Tanner, R. I. – Békés, F.: Synergistic and additive effects of three high molecular weight glutenin subunit loci. I. effects on wheat dough rheology. Cereal Chemistry**,** *79***,** 2002, pp. 294–300.
15. Stathopoulos, C. E. – Tsiami, A. A. – Dobraszczyk, B. D. – Schofield, J. D.: Effect of heat on rheology of gluten fractions from flours with different bread-making quality. Journal of Cereal Science, *43*, 2006, pp. 322–330.
16. Hayta, M. – Schofield, J. D.: Dynamic rheological behaviour of wheat glutens during heating. Journal of the Science of Food and Agriculture, *85*, 2005, pp. 1992–1998.
17. Doi, M. – Edwards, S. F.: The theory of polymer dynamics, Oxford : Oxford University Press, 1986. 408 pp. ISBN 0-1985-2033-6.
18. Kurzbeck, S. – Oster, F. – Münstedt, H. – Nguyen, T. Q. – Gensler, R.: Rheological properties of two polypropylenes with different molecular structure. Journal of Rheology, *43*, 1999, pp. 359–374.
19. Graveland, A. – Henderson, M. H. – Paques, M. – Zandbelt, P. A.: Composition and structure of gluten proteins. In: Schofield, J. D. (Ed.): Wheat structure, biochemistry and functionality. London : Royal Society of Chemistry, 2000, pp. 90–99.
20. Tsiami, A. A. – Bot, A. – Agterof, W. G. M. – Groot, R. D.: Rheological properties of glutenin subfractions in relation to their molecular weight. Journal of Cereal Science, *26*, 1997, pp. 15–27.
21. Tsiami, A. A. – Li, W. L. – Pyle, D. L. – Schofield, J. D.: Molecular characterisation of gluten and barley protein. In: Lafiandra, D. – Masci, S. – D'Ovidio, R. (Eds): The gluten proteins. Cambridge : Royal Society of Chemistry, 2004, pp. 331–334.
22. Stathopoulos, C. E. – Tsiami, A. A. – Schofield, J. D.: Rheological and size characterisation of gluten proteins from cultivars varying in baking quality. In: Gooding, M. J. – Barton, S. A. – Smith, G. P. (Eds): Wheat quality. Series Aspects of Applied Biology, No. 64. Warwick : The Association of Applied Biologists, 2001, pp. 181–183.
23. Stathopoulos, C. E. – Tsiami, A. A. – Schofield, J. D. – Dobraszczyk, B. J.: Effect of heat on rheology, surface hydrophobicity and molecular weight distribution of glutens extracted from flours with different bread-making quality. Journal of Cereal Science, *47,* 2008, pp. 134–143.

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