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Hydrated gluten modified by a transglutaminase

C. Larré, G. Deshayes, J. Lefebvre* and Y. Popineau

A microbial transglutaminase (TGase), was used to add covalent bonds in glutes obtained from three near-isogenic lines differing in their high Mr glutenin subunits content. After TGase treatment, 29–45% of the total proteins remained soluble instead of 70–90% for the untreated glutes, due to the formation of large insoluble polymers as shown by SDS-PAGE. The enzymatic treatment was effective in making gels with high elastic modulus from all tested

glutens: G' was increased from 10 to 50 times and G'' from 2 to 4 times. The dynamic moduli of the reaction products were less frequency dependent than those of the non treated gluten. Despite the low lysine content of the gluten, the formation of permanent connections reinforced the network structure and modified the viscoelasticity properties of gluten.

1 Introduction

Viscoelasticity of dough is related to glutenin polymer content and size of gluten. These polymers composed by subunits linked by interchains SS bonds associate in a transient network through low energy intermolecular interactions. The use of near-isogenic lines differing only in high Mr subunits composition has permitted to show the influence of these subunits on wheat bread-making quality [1]. Moreover, the proportion of large-size glutenin content was related to the viscoelastic properties of gluten [2].

Transglutaminase is known to catalyse the formation of an isopeptidic bond between protein-bound glutamyl and lysine residues. Sakamoto et al. [3] reported that microbial transglutaminase can be used to polymerise soluble proteins through intermolecular covalent cross-links. Gliadins, in solution, were shown to be substrate and some polymers were obtained despite their low content in lysine [4].

In the present work, our objective was to examine the potentiality of doughy gluten to be substrate for microbial transglutaminase. Three near isogenic lines of gluten differing in their high Mr subunits and in their rheological behaviours were used. The viscoelastic properties of the reaction products were investigated by dynamic assays in shear to study the effect of the addition of covalent cross-links to the various gluten networks.

2 Materials and methods

Analyses were performed on three near-isogenic lines (NIL) of the wheat cultivar Sicco provided by P. I. Payne. Their high Mr glutenin subunits (HMW-GS) composition was: NIL 5 + 10: 1/7 + 9/5 + 10; NIL 2 + 12: 1/7 + 9/2 + 12; NIL double null: null/7 + 9/null [1]. Glutens were extracted according to Cornec et al. [5]. Transglutaminase (EC 2.3.2.13) derived from *Streptovorticillum* sp No 8112 was kindly provided by Ajinomoto Co., Inc (Japan).

2.1 Transglutaminase reaction

Freeze-dried gluten (300 mg) was mixed with 600 µl of 5% transglutaminase solution in 0.1 M tris-HCl buffer pH 7.5.

Institut National de la Recherche Agronomique, Unité de Biochimie et Technologie des Protéines, and *Unité de Physico-Chimie des Macromolécules, Nantes, France.

Correspondence to:

Dr. Colette Larré, Institut National de la Recherche Agronomique, Unité de Biochimie et Technologie des Protéines, B. P. 71627, F-44316 Nantes Cedex 03, France.

2.2 Selective gluten solubilization

Freeze-dried native and modified glutes were dispersed in 0.1 M acetic acid at a concentration of 1 mg/ml. The suspension was centrifuged at 16 000 g for 20 min. The supernatant was freeze-dried. The fractions were subjected to SDS-PAGE [2] and their protein content determined by Kjeldahl procedure [2].

2.3 Rheological measurements

The rheological properties of the reaction mixtures were studied by dynamic measurements in shear with a Carrimed SL 100 rheometer [2]. A sample of 300 mg hydrated gluten was deposited on the rheometer plane previously thermostated at 40 °C. The transglutaminase reaction was followed by measuring the storage (G') and loss (G'') moduli. The frequency dependence of G' and G'' was measured on gluten samples after 18 hours incubation in the range of 0.001 to 10 Hz at 20 °C.

3 Results and discussion

3.1 Biochemical characterisation of the reaction products

The three glutes were freeze-dried and subjected to electrophoresis. Fig. 1 shows reduced gluten proteins fractionated in

Figure 1. SDS-PAGE of native gluten (NG) and their acidic extract (NGA), treated gluten (EG) and their acidic extract (EGA) for three near-isogenic lines.

SDS polyacrylamide gel. The lines n° 3, 7, 11 corresponding to the treated Tgase glutes clearly show the presence of very large molecules unable to penetrate the stacking or the separation gels and the complete disappearance of the bands corresponding to HMW-GS. Some low molecular weight glutenin subunits (LMW-GS) and gliadins still remained but in much lower proportions than when compared to the electrophoregram of native glutes (lines n° 1, 5, 9). As around one third of amino acid residues in gluten are glutamine, we can assume that the limiting substrate in the transglutaminase reaction is the number of available lysines. The lysine content in gliadins and in LMW-GS varies from 0 to 3 residues per mole whereas that of HMW-GS can reach 6 to 8 per mole. This difference can explain the complete disappearance of HMW subunits from SDS-PAGE after the reaction.

3.2 Protein extractability of transglutaminase treated gluten

In order to characterise the reaction products, acetic acid-soluble gluten proteins were extracted, quantified (Table 1) and analysed by SDS PAGE (Fig. 1). The double null line which has a reduced content in HMW-GS gave the highest extraction yield. The 5 + 10 line which has the highest level of polymer content exhibited the lowest extractability. In the case of 2 + 12 and 5 + 10 lines the electrophoretic analysis of the acetic soluble fractions (line 8, 12) clearly showed the unextractability of the highest Mr glutenin subunits. The extractability of acetic acid-soluble proteins decreased substantially after transglutaminase treatment, only one half to one third of the total protein remained extractable indicating that most of the polymers formed become insoluble. The SDS-PAGE patterns of the acetic acid-extracts (lines 4, 8, 12) showed only some gliadin components and small amphiphilic proteins. Staining on the sample wells and blurred migration lines indicated that some polymers were also extracted. These analyses provide further evidence that high Mr glutenin subunits are better substrates for the Tgase reaction and indicate that some of the polymers are soluble.

Table 1. Percentage of gluten protein solubilized in 0.1 M CH₃COOH, results calculated on the basis of Nx5.7.

Line	Native	Tgase treated
Double null	90.8	45.6
2 + 12	89.4	32.7
5 + 10	70.9	29.6

3.3 Effect of Tgase on the viscoelastic properties of gluten

As shown in Fig. 2, G' increases markedly with incubation time, as does G'' (not shown) but in a lower extent. The curves obtained with the three near isogenic lines clearly showed the development of a rheologically significant structure induced by Tgase reaction. The early development of G' was fitted to first order reaction kinetics in order to allow description with few numerical parameters. This analysis was previously performed for protein gelation kinetics induced by Tgase [6]. An empirical formula $G' = G'_{\infty} (1 - e^{-kt}) + G'_{ini}$ was a suitable choice of fit for the data where G' is the storage modulus at time t , G'_{∞} is a constant reflecting the increase in G' at infinite t , k is a constant rate of network development, G'_{ini} is the sum of the initial G' of native hydrated gluten and the increase of G' during the mixing

Figure 2. Changes in storage modulus of the glutes of near-isogenic Siccó lines during 4 h transglutaminase reaction at 40 °C. Deformation: 3%; frequency: 1 Hz. O: 5 + 10 line; □: 2 + 12 line; Δ: double null line; — calculated storage modulus by curve fitting.

time prior the first G' measurement. This equation was fitted to data by a non-linear regression procedure. The unknown constant was estimated and the adjusted correlation coefficient calculated. Final values of the storage modulus (G'_F) of the reaction products were estimated by $G'_F = G'_{\infty} + G'_{ini}$ (Table 2). In all cases high correlation coefficients were obtained, indicating that the fitted model describes the data well. The slowest rate constant as well as the lowest final storage modulus value were obtained for line double null suggesting that the loss of subunits 1, 5 and 10 significantly decreased the susceptibility of gluten to Tgase induced polymerisation. In the case of line 2 + 12, the reaction progressed more rapidly than that of line 5 + 10 but its final estimated G'_F value was lower than that of line 5 + 10. The G'_F value may be closely related to the maximum number of covalent linkages that could be enzymatically added to the gluten. This suggests that subunits 5 and 10 are better substrate for Tgase than subunits 2 and 12. The lower initial rate obtained for the 5 + 10 line is certainly more related to the fact that this gluten presents a network with a higher initial viscoelasticity than line 2 + 12 which could slow down the enzyme diffusion and action in the first stage of the reaction. The values of $G'_{F exp}$ at 1 Hz, obtained for each near isogenic line after 18 h incubation (Table 2) are in agreement with the G'_F values previously estimated.

Table 2. Results obtained after fitting the first four hours rheological data to the equation $G' = G'_{\infty} (1 - e^{-kt}) + G'_{ini}$, experimental G' values (G'_{exp}) obtained after 18 h incubation.

	$k[h^{-1}]$	$G'_F = G'_{\infty} + G'_{ini}$	R^2	$G'_{F exp}$
5 + 10	0.66	21869	0.995	25420
2 + 12	1.40	14624	0.997	12410
Double null	0.39	10943	0.999	10270

3.4 Frequency dependence of reaction products

The enzyme treatment resulted in a substantial increase in storage modulus values which appeared to be less frequency dependent than the G' values of the corresponding untreated gluten. The effect was more pronounced with the double-null line, then on the 2 + 12 line and to a lower extent on the 5 + 10 line. After treatment the G' vs frequency curves of the three glutes became very close each other. As shown in Fig. 3, $\tan \delta = G''/G'$ displayed the same large decrease for the three isogenic lines over the whole frequency range, indicating that

Figure 3. Variation with frequency of the tangent of loss angle measured at 20 °C, 3% deformation. Untreated gluten (filled symbols) and enzymatically treated (open symbols) gluten of three near-isogenic lines. O: 5 + 10 line; □: 2 + 12 line; Δ: double null line.

the system tends to a more solid like state. The order of the untreated samples, from the double-null line to 5 + 10 line was hold but the curves became closer and their shape very similar after the enzymatic treatment. The $\tan \delta$ curves of enzymatic treated gluten presented a minimum around 0.01 Hz indicating a displacement of the elastic plateau towards higher frequencies.

4 Conclusion

The lysyl residues of gluten formed Glu-Lys bonds which participate to the connectivity of the gluten network. The formation of permanent connections reinforced the network structure and modified the viscoelasticity properties of the gluten.

References

- [1] Payne, P. Y., L. M. Holt, K. Harinder, D. P. MacCartney and G. J. Lawrence, Proceedings of the 3rd international Workshop on Gluten proteins, Ed R. Lasztity and F. Bekes, 216–226 (1987).
- [2] Popineau, Y., M. Cornec, J. Lefebvre and B. Marchylo, *Journal of Cereal Science* 19 (1994) 231–241.
- [3] Sakamoto, H., Y. Kumazawa and M. J. Motoki, *Food Sci.* 59 (1994) 866–871.
- [4] Larré, C., M. Chiarello, Y. Blanloeil, M. Chenu and J. Guéguen, *J. Food Biochem.*, 17 (1994) 267–282.
- [5] Cornec, M., Y. Popineau and J. Lefebvre, *Journal of Cereal Science* 19 (1994) 131–139.
- [6] Chanyongvorakul, Y., Y. Matsumura, H. Sakamoto, M. Motoki K. Ikura and T. Mori, *Biosci. Biotech., Biochem.* 58, 5 (1994) 864–869.

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