Use of Spectroscopic and Fluorescence Techniques to Assess Heat-Induced Molecular Modifications of Gluten

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ABSTRACT

Fresh gluten isolated from soft wheat flour was heated at varying temperatures for different times: from 45°C for 1 hr, to 110°C for 18 hr; it was then freeze-dried. The solubility of the untreated and heated glutens in different solvents under nonreducing and reducing conditions was determined, and the extracts were analyzed for protein composition by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and acid PAGE. The intrinsic fluorescence, the fluorescence developed on titration with 8-aniline-1-naphthalene sulfonate (ANS), the UV spectra with second derivatives and solution stability were measured on acetic acid extracts of the glutens. Titration with ANS revealed sites with high and low probe affinity (high and low hydrophobicity). The results show: 1) a hydrophobic modification at 45°C, this in no way affected the electrophoretic patterns; 2) moderate changes at 65°C ascribable to conformational modifications; 3) heating at 90°C or above strongly affected protein solubility in acetic acid and produced disulfide supported aggregates. Conformational modifications were also evident. In these changes gliadins, except ω-gliadins, and low molecular weight albumins and globulins were the most involved. 4) Heating at 110°C for 18 hr produced insolubilization not reversed by dithiothreitol.

Thermal modifications of wet gluten are of special interest as they contribute to the stability of the protein film that develops during fermentation and retains CO_2 during baking. After gluten is separated from starch and other soluble components of flour, it can be affected by thermal denaturation during drying.

From the commercial point of view, gluten can be heated to 70°C without deleterious effect. However, any further increase in temperature may affect its functionality. Above 80°C there is even loaf volume depression when compared to unfortified base flour (Bent et al 1990). Denaturation has a detrimental effect on gluten vitality (McDermott 1985, Wadhawan and Bushuk 1989a). Flour vitality is significantly negatively correlated with the intrinsic fluorescence of the acetic acid soluble fraction of gluten and is positively correlated with the solubility of gluten proteins in acetic acid (Wadhawan and Bushuk 1989b). Furthermore, loss of vitality has been associated with a loss of gliadin solubility (Bent et al 1990).

Investigations have been made into the effect of heating glutens at different temperatures and it has been shown by size-exclusion high-performance liquid chromatography (SE-HPLC) fractionation of proteins extracted from heated gluten, that above 50°C, the glutenin fraction becomes insoluble in sodium dodecyl sulfate (SDS)-phosphate buffer, while the gliadin fraction remains unaltered (Schofield et al 1983, Bent et al 1990). Above 70°C, baking performance is lost and the gliadins begin to be affected, which is consistent with the changes observed in the viscosity modulus (G'') and viscosity data (Bent et al 1990). These changes in gluten have mainly been attributed to the formation of interpolypeptide disulfide bonds, that, at 50°C, begin in the glutenin fraction, and at 75°C also involve the gliadins. Thus, the proteins lose solubility (Schofield et al 1983).

At 60°C, gluten viscoelasticity is modified, and gluten solubility in 60% ethanol decreases (Hay and Every 1990, Jeanjean et al 1980). Higher temperatures increased these effects (Booth et al 1980).

Thermal damage, which depends on the moisture content of gluten, becomes evident above 20% (w/w). Glutenin is less extractable in SDS, and the number of free sulfhydryls in proteins with molecular masses ≥40 kDa decreases (Weegels et al 1994a,b). Thus, to understand and control the changes that develop during baking, all the above information must be taken into consideration.

The aim of the present work was to assess the changes in the structure and surface behavior of gluten protein during heating, starting from relatively low temperatures (45°C). Spectroscopic and fluorescence techniques were used, as well as differential solubility criteria in nonreducing and reducing media. The data were always correlated with the polypeptide composition of the fractions considered.

MATERIALS AND METHODS

Materials

All chemicals were of analytical grade. The commercial wheat flour (*Triticum aestivum*), supplied by Vigevano Milling Co., contained 14.1% water and 12.8% (dwb) protein. Gluten was prepared by the standard method of hand washing using 30 min resting time (AACC 1983). The acetic acid extracts of gluten for spectroscopic studies were used immediately after the extraction.

Heat Treatment of Gluten

Small samples of gluten (20 g, 60% moisture) were wrapped in aluminum foil, folded and sealed to prevent water loss during heating, and heated in an oven. After treatment, the samples were taken from the packets, frozen in liquid nitrogen, freeze-dried, ground, sieved (60 mesh), and stored at 4°C in vacuo.

Assay and Extraction of Gluten Proteins

Total protein in the gluten samples was determined in an automatic nitrogen analyzer (NA1500, Carlo Erba) with an atropine standard; the conversion factor was 5.7 (Tkachuk 1969).

Soluble proteins were quantified spectrophotometrically as described by Eynard et al (1994).

Gluten proteins were solubilized using four procedures. Procedure 1: sequential extraction at 60°C with 50% propanol followed by 50% propanol with 1% DTT added, both for 30 min, using the

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procedure of Singh et al (1991). Optimal extraction of gliadins was obtained with 0.8 ml propanol/milligram of gluten. This ratio differs from that used by Singh et al (1991) working on flour. Procedure 2: acid-soluble proteins were separated according to Wadhawan and Bushuk (1989a) with the following modification: 200 mg of gluten was extracted at room temperature with 5 ml of 50 m*M* acetic acid for 1 hr and centrifuged at 12,000 × *g* for 20 min. Procedure 3: gluten was extracted using SDS, 2%, in 50 m*M* tris buffer (pH 8.5) for 2 hr at room temperature. Procedure 4: gluten was extracted using SDS buffer as described in procedure 3, with 1% DTT added, for 2 hr at room temperature.

Spectral Studies

Fluorescence measurements. Fluorescence spectra of proteins solubilized in 50 m*M* acetic acid (0.25 mg/ml) were recorded and analyzed at room temperature with a luminescence spectrometer (LS50B, Perkin Elmer) using FLDM software (FL Data Manager, Perkin Elmer) with 290 nm excitation wavelength, 150 nm/min scan speed, and 2.5 nm slit width. Binding of the apolar ligand 8-aniline-1-naphthalene sulfonate (ANS) to the proteins was evaluated by titrating to saturation, with 1–10 μl of 1 m*M* or 10 m*M* ANS, the protein solution (0.25 mg/ml in acetic acid), and measuring the resulting fluorescence, λex 405 nm, λem 480 nm, slit width 2.5 nm. The titration curve was deconvoluted using Peakfit software (Jandel Scientific, Erkrath, Germany). The components were linearized by the Lineweaver Burk plot (low ANS concentration) and the Hill equation (high ANS concentration) (Cantor and Shimmel 1980). This allowed the determination of the ANS binding constants of both the low- and high-affinity sites in gluten.

UV Spectroscopy. Absorption between 240 and 350 nm of the acetic acid soluble gluten proteins (0.25 mg/ml) was recorded on a λ2 spectrophotometer (Perkin Elmer) and analyzed using PECSS software. Second-derivative analysis of the UV spectra was made according to Ragone et al (1984) by measuring the ratio *a/b*:

$$a/b = D_{287}–D_{283}/D_{295}–D_{290}$$

where D_x is the second derivative at the wavelength *x*.

Electrophoresis

SDS-PAGE was performed according to Laemmli (1970) on 12% polyacrylamide gels, in presence of 0.17*M* 2-mercaptoethanol when indicated. The apparent molecular mass of the separated polypeptides was determined by comparison with a standard mixture of proteins (myosin, β–galactosidase, phosphorylase b, BSA, ovo albumin, carbonic anhydrase, lysozyme) (Sigma).

Acid (A-)PAGE was performed according to Khan et al (1985) with the following modifications. Proteins were separated in 8% polyacrylamide gel (acrylamide to bis acrylamide in a 24:1 ratio). The acetic acid extracts were mixed in a 1:2 ratio with a solution containing 60% glycerol (w/v) and 0.05% pyronin G (w/v). Electrophoresis was run at 500 V at 10°C. The prerun was for 30 min; the separation took twice the run time of the tracking dye (pyronin G).

Proteins were stained with Coomassie brilliant blue R-250. SDS-PAGE and A-PAGE gels were scanned using a video-camera connected to a computer system, software Cream (Kem-en-Tech, Copenhagen, Denmark).

The illustrations of electrophoretic separations show only one tracing for those samples that produced identical patterns. The samples concerned are mentioned in the caption.

TABLE I
Solubility of Gluten Protein[a]

Sample	50 m*M* Acetic Acid	50 m*M* Tris/HCL + 2% SDS[b]	50% *n*-Propanol	50% *n*-Propanol +1% DTT
Native	80.0	76.0	25.0	22.0
45°C, 1 hr	67.0	74.0	31.0	18.0
65°C, 1 hr	59.0	65.0	34.0	21.0
90°C, 1 hr	2.4	10.0	1.4	21.0
110°C, 1 hr	1.6	6.0	0.5	10.0
110°C, 18 hr	0.5	nd[c]	0.3	11.0

[a] Percent of total. Relative standard deviation ≤5%.
[b] Sodium dodecyl sulfate.
[c] Not determined.

Solution Stability

The solution stability of acid soluble gluten proteins (1 ml, 0.25 mg/ml) was evaluated by measuring the turbidity at 350 nm during 100 sec after addition of 70 μl of 1*M* NaCl. Rate constants (*k*) of the process were calculated as:

$$kt = \ln (A_{max} - A_t/A_{max} - A_0)$$

where A_{max}, A_t, and A_0 are the maximum absorbance and absorbance at times *t* and 0 respectively.

RESULTS

All glutens had similar protein contents ranging in percent from 84.3–85.3 ± 1.0, except the sample treated at 110°C for 18 hr, which had 88.3% ± 0.7 ($n = 3$).

Solubility

As shown in Table I, various solvents extracted different amounts of proteins. On heating up to 65°C, the changes were moderate; the solubility increased in propanol, whereas it decreased in the polar solvents. After heating at 90°C, the amount of protein extracted in acetic acid and in propanol markedly diminished. When the extractant was the tris buffer containing SDS, the decrease was less sharp. Extraction in propanol + DTT partly diminished only on heating at 110°C. In all heated preparations, ≈94% of the total gluten dissolved in 2% SDS under reducing conditions (not shown).

Electrophoresis

Electrophoretic analysis of the extracts was performed under nonreducing and reducing conditions so as to identify the mono-

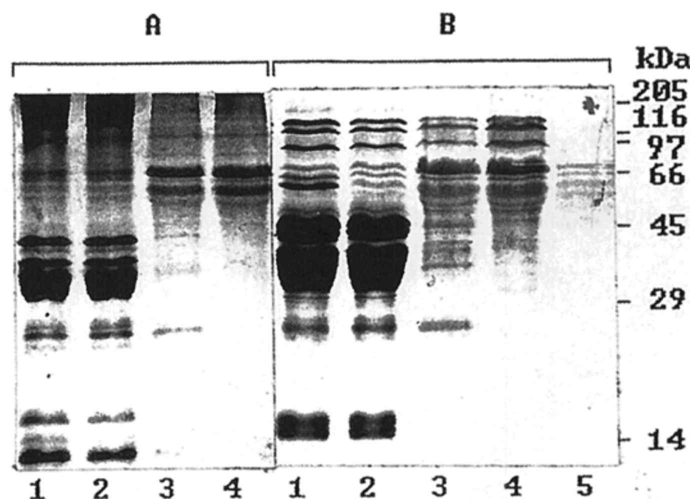

Fig. 1. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) patterns of unreduced **(A)** and reduced **(B)** acetic acid extracts of native (lane 1) and thermally treated glutens (lanes 2–5): 1 hr at 65°C; 1 hr at 90°C; 1 hr at 110°C; and 18 hr at 110°C, respectively. Sample heated at 45°C not shown as its patterns were identical to that of native gluten. Unreduced and reduced extracts from sample heated 18 hr at 110°C were identical.

meric or polymeric forms of the proteins; addition of DTT during extraction evidenced the role of disulfide bonds in the solubility behavior.

Native gluten and gluten heated at 45°C for 1 hr. The extracts from gluten heated at 45°C showed electrophoretic patterns identical to those of native gluten. Sample 1 in Figures 1–4 represents both these glutens. Acetic acid, SDS, and propanol extracts, run on SDS-PAGE under nonreducing conditions revealed gliadins ranging from 41 to 30 kDa (α-, β-, and γ-gliadins), 74–64 kDa (ω-gliadins), the low molecular weight (LMW) albumins and globulins (16–14 kDa), which are residues in hand-washed gluten (Pogna et al 1994), and some blurring at the origins of gels (Figs. 1–3, A, sample 1).

Blurring disappeared under reducing conditions, indicating that it derived from disulfide stabilized aggregates; high molecular weight (HMW) glutenin subunits (molecular masses ≥97 kDa) became clearly apparent, while the LMW glutenin subunits superimposed the gliadins with similar electrophoretic mobility (Pogna et al 1994). Components in the region of 60 kDa, possibly corresponding to HMW albumins (Gupta et al 1991) or D subunits of glutenin (Masci et al 1993), also appeared (Figs. 1–3, B, sample 1). The electrophoretic patterns of the reduced acetic acid, SDS, and propanol extracts were similar to that of the total protein extracted with SDS + DTT (Fig. 2, C, sample 1).

Extraction with propanol + DTT after propanol extraction allowed the separation of LMW and HMW glutenin subunits, together with the components in the region of 60 kDa, (Fig. 3, C, sample 1). The proteins that did not solubilize in propanol + DTT were dissolved by SDS + DTT (Fig. 3, D, sample 1).

The presence of gliadins in the acetic acid extracts was indicated by A-PAGE, which resolved α-, β-, γ-, and ω-gliadins (Fig. 4, sample 1).

Gluten heated at 65°C for 1 hr. The extracts from gluten heated at 65°C (Figs. 1–4, sample 2) had electrophoretic patterns that were generally similar to that of native gluten. However, the 60 kDa component was lacking in the reduced acetic acid and propanol extracts, while it was still present in the reduced SDS extract and in extracts obtained in the presence of DTT (Figs. 1–3, B, sample 2). Moreover, the extract in propanol + DTT (Fig. 3, C, sample 2) showed bands in the region of gliadins and LMW albumins and globulins, which were lacking in native gluten (Fig. 3, C, sample 1) and were quite abundant in gluten heated at 90°C (Fig. 3, C, sample 3). α-Gliadins were not resolved in A-PAGE (Fig. 4, sample 2).

Glutens heated at 90 and 110°C for 1 hr. The changes derived from heating at 90 or 110°C were conspicuous (Figs. 1–4, samples 3 and 4). They were more apparent under nonreducing conditions. In all extracts, the gliadins, except ω-gliadins, and LMW

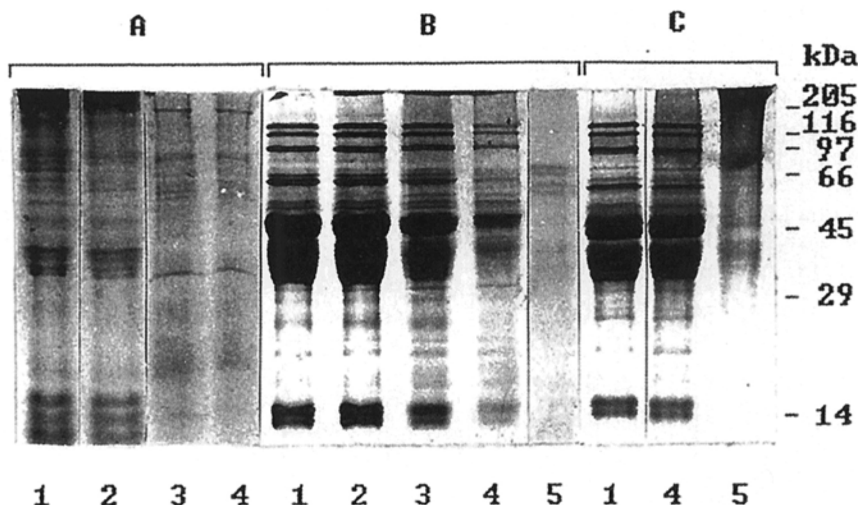

Fig. 2. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) patterns of unreduced **(A)** and reduced **(B)** SDS (2%) extracts of gluten and samples extracted in SDS buffer supplemented with 1% DTT **(C)**. Samples numbered as in Fig. 1.

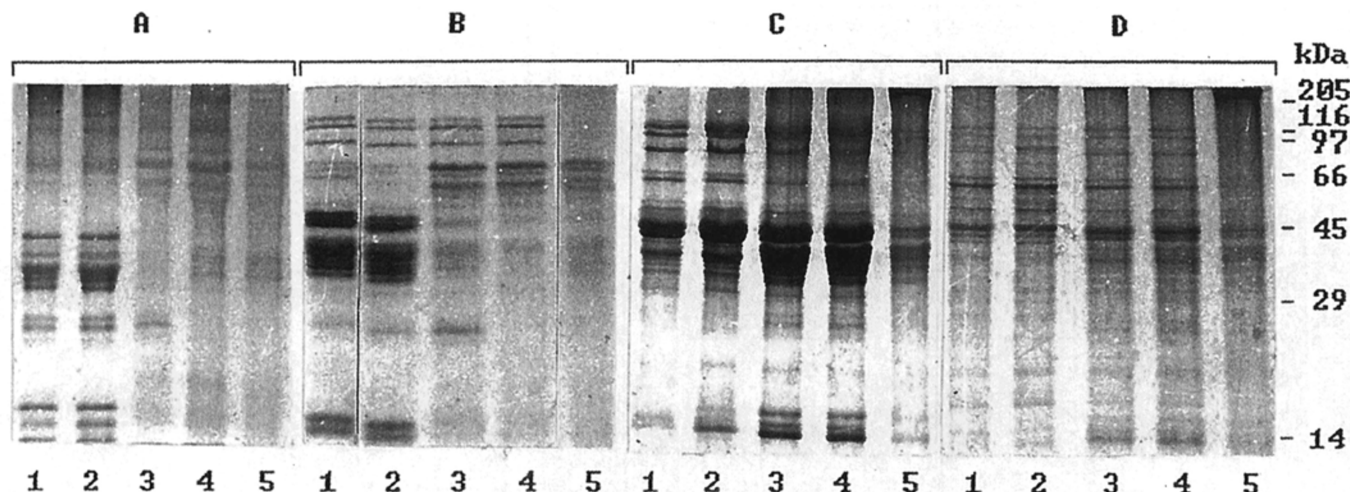

Fig. 3. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) patterns of gluten sequentially extracted in 50% *n*-propanol (unreduced [**A**], reduced [**B**]); 50% *n*-propanol containing 1% DTT (**C**); 2% SDS containing 1% DTT (**D**). Samples numbered as in Fig. 1.

albumins and globulins disappeared or diminished noticeably. Blurring was present at the top of lanes (Figs. 1–3, A, samples 3 and 4). Under reducing conditions, blurring disappeared and HMW glutenin subunits became apparent in all extracts, together with weak components in the region of α-, β-, and γ-gliadins and LMW glutenin subunits (Figs. 1–3, B, samples 3 and 4). These components were more evident in the reduced SDS extract, which also contained LMW albumins and globulins (Fig. 2, B, samples 3 and 4). Extracts prepared in the presence of SDS and DTT showed the same composition as native gluten (Fig. 2, C).

The proteins that were not extracted by propanol appeared in the subsequent extraction with propanol + DTT (Fig. 3, C, samples 3 and 4). The residues of the sequential propanol extractions differed from those in native gluten in having more components in the region of α-, β-, γ-gliadins and LMW glutenin subunits (Fig. 3, D, samples 3 and 4). These results indicate that on heating gluten at 90°C, part of the gliadins and LMW albumins and globulins required a reducing medium, or even the presence of SDS, to become soluble. In a solubility test, they are therefore accounted for as glutenins; separation of glutenins from other proteins according to Singh (Singh et al 1991) was no longer feasible. A-PAGE of the unreduced acetic extracts confirmed these results. α-, β-, and γ-gliadins were not resolved (Fig. 4, samples 3 and 4).

The only difference observed between glutens heated at 90 and 110°C for 1 hr was the polypeptide of 27 kDa, which was visible in the unreduced acetic and propanol extracts of gluten heated up to 90°C but disappeared from all extracts in samples heated at 110°C ,except where DTT was present (Fig. 2, C, samples 3 and 4).

Gluten heated at 110°C for 18 hr. When heating at 110°C was continued for 18 hr, only faint bands in the ω-gliadin region were visible on SDS-PAGE of acetic, SDS, and propanol extracts (Figs. 1–3, B, sample 5). The electrophoretic patterns did not change upon reduction. Extracts made with SDS + DTT contained proteins as aggregates, producing blurring at the origins (Fig. 2, C, sample 5).

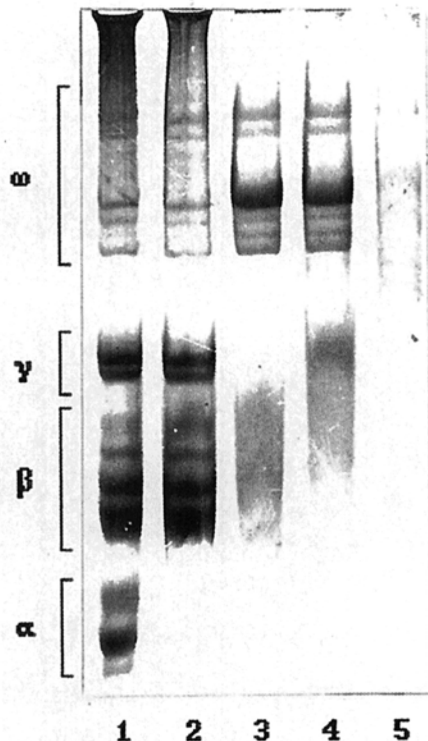

Fig. 4. Acid polyacrylamide gel electrophoresis patterns of acetic acid extracts. Samples numbered as in Fig. 1. Gliadins designated α, β, γ, and ω, according to Woychik et al (1961).

Spectral Studies

Intrinsic fluorescence. The intrinsic fluorescence (λex 290 nm) of the acetic acid soluble protein of native gluten had an emission maximum at 350 nm, suggesting a tryptophan chromophore. The sample treated at 90°C showed a modest increase in emission intensity and red shift of emission maximum (Fig. 5). The change became very evident on raising the temperature to 110°C for 1 hr, and the intensity more than doubled with 18 hr of heating at 110°C; the emission maximum was shifted to 355 nm.

Interaction with fluorescent probes. The fluorescence measured on titrating the protein extracts with ANS is shown in Fig. 6. Deconvolution of the titration curves disclosed a double exponential component in the early part of titration and a logistic one at higher ANS concentrations. Their sum represents the best fit for the experimental values. Their linearization occurred with very good correlation with r = 0.99878 for the double exponential component and r = 0.99984 for the logistic component, respectively. The maximal fluorescence developed by each component and the dissociation constant of the ANS protein complex, calcu-

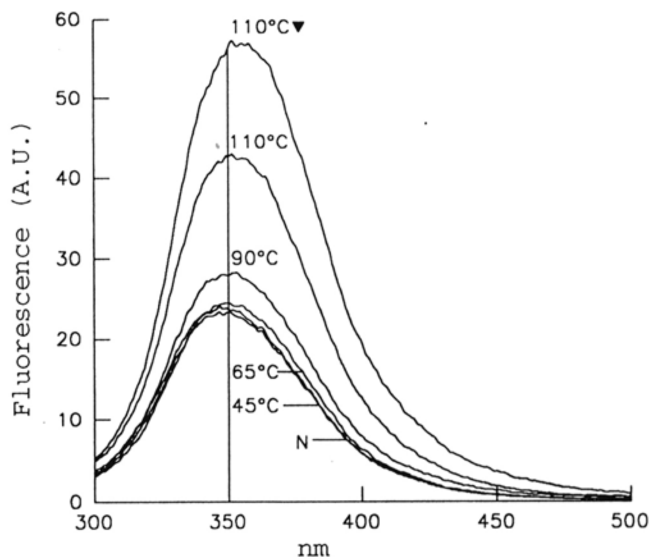

Fig. 5. Emission spectra (λex 290 nm)of native glutens (N) and glutens heated 1 hr and 18 hr (▼).

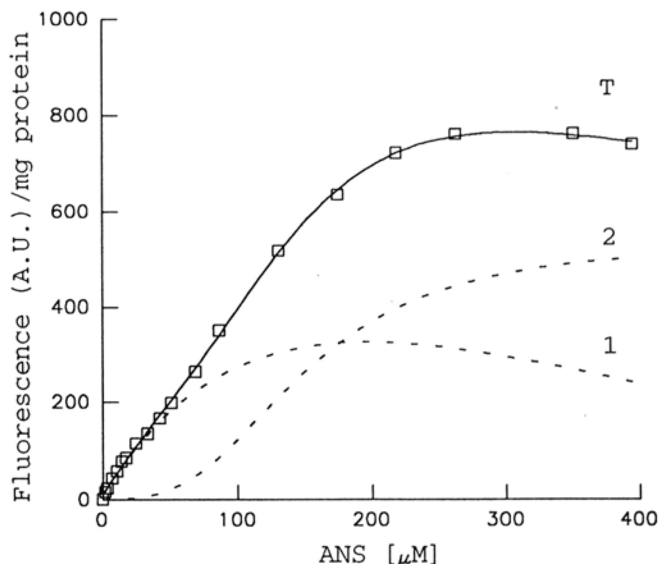

Fig. 6. Titration with 8-aniline-1-naphthalene sulfonate (ANS) of native gluten (□) extracted in 50 mM acetic acid. 1 = double exponential component (high affinity); 2 = logistic component (low affinity); T = sum of 1 and 2.

lated from the linearized curves, are given in Table II. Such data give evidence for sites with high- or low-probe affinity, measured by the reciprocal of the dissociation constant.

A peculiar behavior of gluten heated at 45°C is suggested by the changes in fluorescence. As shown in Table II, the maximal fluorescence increased in the sample heated at 45°C, whereas it decreased after heating at 65°C; this depended on the behavior of low-affinity sites. The dissociation constant of ANS from the high-affinity sites, i.e., the reciprocal affinity, decreased at 45°C but was unchanged at 65°C. In low-affinity sites, it had a larger increase at 45°C than at 65°C. At temperatures above 65°C, there was a general decrease in extrinsic fluorescence. The dissociation constant of ANS from low-affinity sites varied irregularly.

UV spectra. The relative spectral contribution of tyrosine and tryptophan chromophores was estimated according to Ragone et al (1984). Figure 7 gives the UV spectrum of native gluten and the second derivative spectrum, with indicated *a* and *b*. The *a/b* ratio depends on the exposure of tyrosine to the solvent. Values of *a/b* in the various samples are reported in Table III. A specific change occurred in the tyrosine microenvironment at 45°C, indicating decreased exposure of the tyrosine chromophore, whereas in the proteins remaining soluble after 1 hr of heating at 110°C, the exposure of tyrosines significantly increased. Continued heating at 110°C did not change the value.

TABLE II
Fluorimetric Parameters of ANS[a] Bound to Gluten

	Maximal Fluorescence[b]			Dissociation Constant	
Sample	Total[c]	1[d]	2[e]	*kd*[d], μM	*kd2*[e], M
Native	760	330	471	94	2.6
45°C, 1 hr	800	240	620	77	44.0
65°C, 1 hr	650	190	484	90	22.0
90°C, 1 hr	360	160	204	29	350.0
110°C, 1 hr	300	91	263	15	0.012
110°C, 18 hr	180	77	112	19	0.078

[a] 8-aniline-1-naphthalene sulfonate (ANS).
[b] Arbitrary units.
[c] From the titration curve of Fig. 6.
[d] Double exponential component.
[e] Logistic component. Values of fluorescence in the presence of 400 μM ANS.

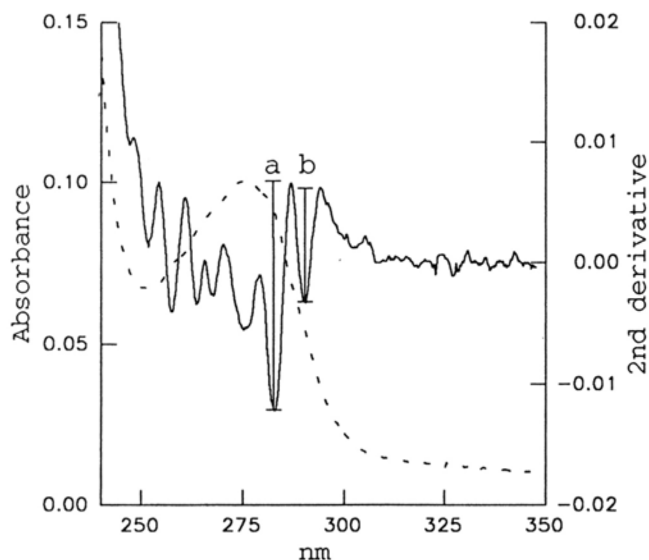

Fig. 7. UV absorbance and second derivative of UV absorbance of native gluten. Thermally treated glutens gave similar tracings, except for the value of *a/b*.

Solution Stability

An optimal rate of turbidity increase was produced by adding 70 μl of 1*M* NaCl. As shown in Fig. 8, a decrease in stability was observed in gluten treated at 90°C, first announced by faint modifications at 65°C. The change became consistent on heating at 110°C. The inset of Figure 8 indicates the increase of *k*.

Macroscopic Aspect

The macroscopic aspects of heat-treated glutens are shown in Fig. 9. Pictures were taken immediately after heating. The most evident changes were established between 65 and 90°C. Diffuse browning occurred when heating was continued for 18 hr.

DISCUSSION AND CONCLUSIONS

Hydrophobicity and solubility are not limited to the reactivity of specific groups. They derive from cooperative effects and, in complexes like gluten, are influenced by molecular interactions. ANS titrations of the gluten complex evidenced sites with high or low hydrophobicity, i.e., a high- or low-affinity for the probe. The independent behavior of these sites to temperature stress indicates that separate regions of the complex are involved.

The hydrophobic probe ANS made it possible to detect modifications in gluten proteins heated at temperatures as low as 45°C. Supporting evidence for this transition at ≤45°C was also observed in gluten solubility in propanol, and in the exposure of tyrosine to solvent. These results indicate an increased surface hydrophobicity in samples heated at 45°C. This could be due to conformational changes in gluten that exposed previously unavailable

TABLE III
Tyrosine Exposure

Sample	*a/b*[a]
Native	2.35
45°C, 1 hr	2.22
65°C, 1 hr	2.37
90°C, 1 hr	2.53
110°C, 1 hr	3.93
110°C, 18 hr	3.88

[a] See Fig. 7.

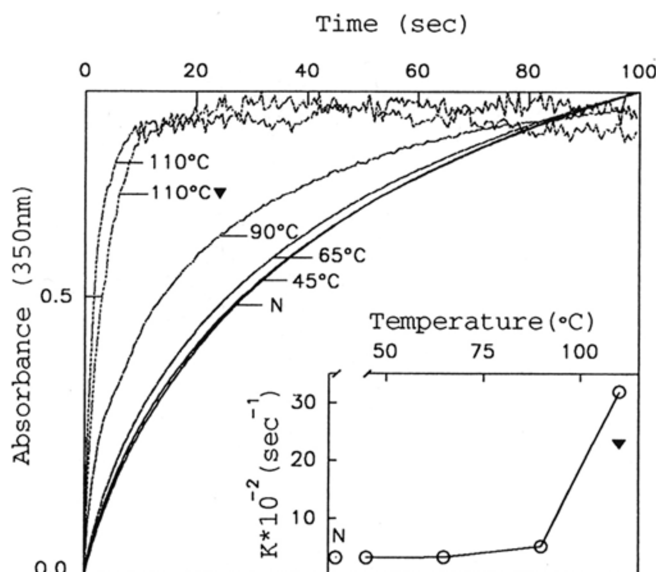

Fig. 8. Turbidity kinetics at 20°C of native gluten (N) and glutens heated at various temperatures for 1 hr and 18 hr (▼), after the addition of 70 μl of 1*M* NaCl to acetic acid extracts (1 ml, 0.25 mg/ml of protein). Inset: rate constants calculated as described in text.

areas. It is quite possible that the strong interactions between gliadins and glutenins, present in native gluten, is decreased. Further indication that hydrophobicity is a highly sensitive property of the gluten complex comes from the work of Weegels et al (1994b). These authors found that for gluten heated at 80°C, the hydrophobicity was affected at a lower moisture content than are other properties like conformation, extractability in SDS, or the ratio of free sulfhydryl groups to total half cysteine.

The increased protein extraction with propanol from mildly heated glutens probably derives from a modification in gliadin solubility. At 65°C, early changes appeared in the electrophoretic behavior of gliadins, particularly the α-gliadins. These data indicate that gliadins are modified by heating at 65°C and probably even at 45°C, a lower temperature range than that reported by Schofield at al (1983). According to the model of Huebner (1977), as put forward by Eckert et al (1993), gliadins are located at the surface of the gluten strands. Therefore, they are liable to be the site for early modifications. Moreover, according to Tatham et al (1985), α-gliadins have a less regular structure than do β- and γ-gliadins. This may be the reason for the lower thermal stability.

The changes that occur in the 55–75°C temperature range appear to be responsible for the parallel changes in loaf volume (Schofield et al 1983), rheological behavior (Dreese et al 1988), surface properties (Eliasson and Silverio 1991), and functionality (Booth et al 1980, Hay and Every 1990). Relevant information on the molecular events that occur at this temperature can be obtained by considering the solubility of polypeptides as indicated by SDS-PAGE, together with gluten spectral and stability properties. In gluten heated at 65°C, solubility modifications were minor, as indicated by the loss of the 60 kDa protein. The modifications depended mainly on conformational changes because they were less evident in the presence of SDS. Modifications such as increased polarity of the tyrosine microenvironment, decreased stability, and decreased fluorescence of bound ANS were minimal, but such modifications did forecast the changes that became evident at higher temperatures. They developed in a way that is typical of protein denaturation.

It was observed by Wadhawan and Bushuk (1989b) that the intrinsic fluorescence of both dry gluten and gluten acetic acid extracts behaved similarly (the fluorescence increased) upon heating at 110°C. Our data indicate that the earliest changes are apparent at 90°C, but the beginning of the process can be recognized even at 65°C.

The trend towards lower hydrophobicity apparent from the ANS titration and tyrosine microenvironment of proteins still soluble in acetic acid after heating at 90°C or above indicates coalescence of nonpolar regions. On titrating with ANS, a similar decrease in hydrophobicity was observed for the proteins unextracted in acetic acid buffer from gluten heated at 80°C (Weegels et al 1994b). This result was attributed to aggregate formation. The increased affinity for ANS in sites that remained exposed at high temperatures indicates that the conformationally rigid regions are nonpolar.

The electrophoretic behavior of the heated glutens and the effect of DTT addition indicate that disulfide stabilized aggregates were formed at 90 and 110°C. According to Schofield et al (1983), disulfide and sulfhydryl interchange reactions are responsible for the heat-induced polymerization of glutenins and, at higher temperatures, of gliadins. Jeanjean et al (1980) attributed the decrease in extractability of gliadins and cysteine rich albumins in heated gluten to disulfide bond formation.

In gluten heated at 90°C or above, monomeric proteins, with the exception of ω-gliadins, were not extracted by acetic acid. However, in the SDS buffer extracts, they became evident after addition of DTT. This indicates the importance of conformational changes especially in the aggregation of gliadins and albumins and globulins. As suggested by Schofield et al (1983), the high temperature solubility of ω-gliadins, which lack cysteine, is a further indication that the sulfhydryl and disulfide system is involved in causing the insolubilizing aggregations. If heating at 110°C was continued for 18 hr, the interactions changed and the aggregates could not be solubilized by DTT and SDS. The gluten complex underwent further modifications as indicated by the spectral behavior.

Glutenins are naturally polymerized by a disulfide network in native gluten. Accordingly, SDS PAGE ± DTT did not show any heat-induced, disulfide-stabilized aggregation. Information on the state of the glutenins is available only when the solubilities of the compared samples differ. This is probably why we observed initial changes in the glutenins at a higher temperature than that observed by Schofield et al (1983) using gel filtration. Given the changes in solubility of gliadins heated above 65°C, and the aggregate formation leading to increased molecular mass, the

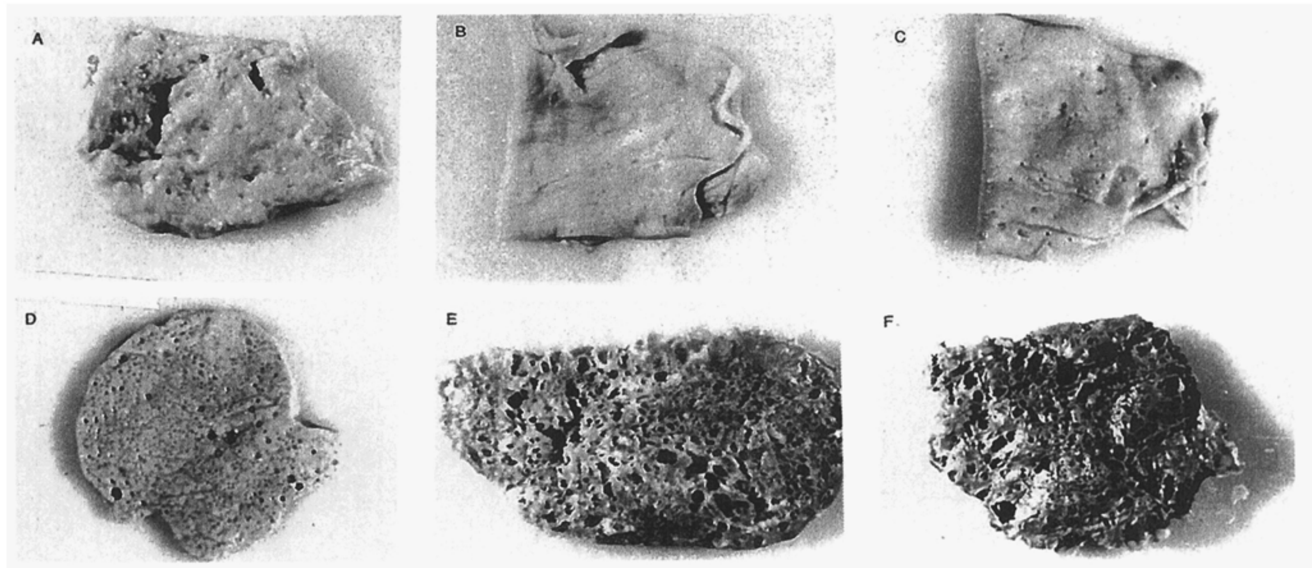

Fig. 9. Macroscopic aspect of native and heat-treated glutens before freeze-drying. **A,** native gluten; **B,** 1 hr heating at 45°C; **C,** 1 hr heating at 65°C; **D,** 1 hr heating at 90°C; **E,** 1 hr heating at 110°C; **F,** 18 hr heating at 110°C.

PAGE cannot be over emphasized.

A comparison of the composition of acetic acid extracts of glutens heated at high temperatures revealed that the predominance of ω-gliadins is the probable cause of the changes in spectral properties in the glutens heated at 110°C for 18 hr. Glutens heated at 90 and 110°C for 1 hr showed a less modified composition. The different behavior is attributed to differences in conformation and aggregation.

In the gluten samples heated at 90 and 110°C, cells and alveola formed are due to water evaporation and gas retained during the kneading of the gluten. The modifications of surface hydrophobicity and structure very likely affected the structured water and the molecule aggregation and thereby contribute to cell formation.

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