Effect of High-Temperature Short-Time Treatment of Wheat Flour on Gluten Vitality and Structure

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ABSTRACT

The effect of high-temperature short-time (HTST) applications on gluten in its natural flour environment was investigated. Changes occurring in HTST-treated samples during storage and in samples subjected to severe thermal denaturation were studied. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) profiles of the gluten polypeptides, and the intrinsic fluorescence, were modified only in the heat-denatured preparation. On the other hand, after HTST treatment changes in the extrinsic fluorescence (8-aniline-1-naphthalene sulfonate [ANS] binding) revealed enhanced surface hydrophobicity, which however, regressed on storage. Consistent with these observations was the amount of the associated water. It appears that it is the gluten surface that is mainly affected by HTST treatment, and this could represent a very early stage of heat damage of gluten.

High-temperature short-time (HTST) treatments are a recent development in food technology. Material in finely particulate form is conveyed through a rotating heated cylinder by a turbulent stream of hot air. During the run, the particles undergo repeated rapid contact with the hot surface. Time of passage and temperature of the contact surface and conveying air are controlled. Due to the small size of flour particles, HTST treatments provide uniform thermal input. Heat transfer is minimized and water evaporation is facilitated.

HTST techniques applied to flour provide a useful model of heat transfer to gluten in conditions as close as possible to that of gluten's natural kernel surrounding. The effect of heat treatment on isolated gluten has been reported in the literature (Booth et al 1980, Weegels et al 1994b, Schofield et al 1983) and is currently under study in our laboratory (Cerletti et al 1994, Guerrieri et al 1996). In this article, we examine the behavior of gluten heated within flour, under conditions that are similar to those of the actual flour processing conditions. To remain as close as possible to the industrial situation, we preferred to apply the HTST treatment using industrial equipment under highly controlled conditions.

The most widespread test for vitality is to measure the change in loaf volume that results after the addition of gluten to flour, followed by baking. This is a cumbersome procedure for routine industrial use, and the gluten in the flour itself could possibly interfere. Other assays for vitality are water absorption by gluten and extensibility of the hydrated gluten, but neither of these methods has proven totally reliable (Wadhanwan and Bushuk 1989a).

Wadhanwan and Bushuk (1989a) studied gluten devitalization on 27 samples of commercial gluten and gluten isolated by hand washing in the laboratory. Dough development, dough stability, α-amylase activity, and other chemical parameters were studied, but no definite correlation was found between these results and loaf volume, except for sodium content, which could be affected by the industrial preparation of gluten.

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Positive correlations have been found between gluten vitality, gluten solubility in 0.05*N* acetic acid, and electrophoresis and fluorescence results (Wadhanwan and Bushuk 1989b). The turbidity of acid solutions of gluten has definitely been related to the thermal damage of gluten (Hay and Every 1990).

Czuchajowska and Pomeranz (1991) evaluated commercial vital gluten by near-infrared reflectance (NIR) spectroscopy and developed calibration equations to predict gluten functionality. Good correlation was found between NIR wavelengths and solubility in 3*M* urea and protein content, but further results are required to correlate composition and vitality.

In this study, properties related to vitality and protein structure were studied in gluten isolated from HTST-treated flours and, for reference purposes, in native or fully denatured gluten obtained from untreated flour. A comparison of the results can be used to assess whether the presence of starch and other flour components during HTST treatment influences the heat effects on gluten.

MATERIALS AND METHODS

Flour

Globofood S.A. of Agno, Switzerland, supplied the soft wheat flour (*Triticum aestivum*) and also performed the HTST treatment. The flour contained 13.8% water and 14.0% (dwb) protein. Flour 1 was untreated base flour. Flour 2 was heat-treated 10 min in a HTST line with contact surfaces at 90°C and flour at exit 80–90°C. Flour 2 was stored 15 days at 25°C before use. Flour 3 was the same as flour 2 but was stored globally three months. All samples were stored about one month at 4°C in vacuo before isolating gluten.

Gluten Preparation

Flour was hand washed according to the standard method (AACC 1983) with 30 min of resting time. Isolated gluten contained 80–85% protein. Samples were frozen in liquid nitrogen, lyophilized, ground, sieved (45 mesh), and stored in vacuo at 4°C.

Gluten was solubilized in 0.05*N* acetic acid (1:40, w/v). Instead of extracting twice (Wadhanwan and Bushuk 1989b), we collected the supernatant of only one extraction and centrifuged for 10 min at 15,000 × *g*.

Devitalized Gluten

Gluten separated from flour 1 was devitalized in an oven at 100°C for 20 hr according to the procedure of Wadhanwan and Bushuk (1989a).

Protein Assay

Protein content was determined in an automated nitrogen analyzer (NA1500, Carlo Erba). Samples were assayed in triplicate using atropine as the nitrogen standard. A conversion factor of 5.7 (Tkachuk 1969) was used to calculate total proteins.

Proteins in the acetic supernatant were determined spectrophotometrically using a modified Bradford procedure (Eynard et al 1994).

Electrophoresis

Total proteins were extracted from flour and gluten with sample buffer (31 m*M* tris HCl, pH 6.8, 66 m*M* sodium dodecyl sulfate [SDS], 0.17*M* 2-mercaptoethanol, and 2.9 m*M* glycerol) for 10 min at 100°C.

SDS-PAGE was performed according to Laemmli (1970) in 12% polyacrylamide gels in the presence of 0.17*M* 2-mercaptoethanol in the sample. Protein bands were stained with Coomassie brilliant blue R250. The gels were processed with a video-image computer system using Cream software (Kem-en-Tech). Protein markers were a standard mixture of proteins (myosin, β-galactosidase, phosphorylase b, BSA, egg albumin, carbonic anhydrase, trypsin inhibitor, lysozyme) (Sigma).

Fluorescence Measurements

Fluorescence spectra were determined on the acetic extracts of gluten proteins, (0.25 mg/ml) in a luminescence spectrometer (LS50B, Perkin Elmer) linked to a computer system using FLDM software (FL Data Manager, Perkin Elmer). Excitation wavelength was 290 nm, scan speed was 150 nm/min, emission and excitation slitwidth was 2.5 nm.

Binding of the hydrophobic ligand 8-aniline-1-naphthalene sulfonate (ANS) to gluten proteins dissolved in 0.05*N* acetic acid was evaluated by adding 1–10 µl 1 m*M* or 10 m*M* ANS to saturation and measuring the fluorescence (excitation 405 nm, emission wavelength 480 nm, slitwidth 2.5 nm).

The titration curves were deconvoluted into components with Peakfit software by Jandel. Components were linearized respectively by the Lineweaver Burk plot (high ANS concentration) and the Hill equation (low ANS) (Cantor and Schimmel 1980). This allowed the determination of dissociation constant (*K*d) of the ANS protein complex, i.e., the affinity for ANS of the sites in gluten (Cerletti et al 1994, Guerrieri et al 1996).

TABLE I
Composition (%) of Flour and Gluten

	Flour		Gluten	
Flour	**Water**	**Protein[a]**	**Water**	**Protein**
Untreated	13.8 ± 0.0	14.0 ± 0.0	16.8 ± 0.0	84.4 ± 0.7
HTST[b] treated	9.8 ± 0.2	12.5 ± 0.1	5.0 ± 0.0	79.1 ± 0.5
HTST treated, aged	10.6 ± 0.0	12.6 ± 0.1	1.6 ± 0.1	83.5 ± 0.5
Gluten from untreated flour, devitalized	...	...	nd[c]	85.9 ± 0.5

[a] N × 5.7 (dwb).
[b] High-temperature short-time.
[c] Not determined.

TABLE II
Solubility of Gluten Proteins

Gluten	Extracted Protein, %
Untreated	91.3 ± 3.2
HTST[a] treated	92.5 ± 5.2
HTST treated, aged	82.1 ± 4.1
Devitalized	1.1 ± 0.2

[a] High-temperature short-time.

Digestion of Flour and Starch with Amyloglucosidase

Purified starch (0.4%) and flours dissolved in water to reach 0.4% (w/v) starch were used either as such or gelatinized 1 hr at 100°C and then cooled at room temperature. Samples (1 ml) were heated at 60°C. Then 2 ml of 0.1*M* acetate buffer (pH 4.75) and 50 µl of amyloglucosidase, 1 mg/ml of 120 U/mg. After 10 min, the enzyme action was stopped by adding 0.5 ml of 2.5*M* NaOH and cooling. The glucose liberated was determined using a glucose oxidase/peroxydase kit from Sigma. The parallel blank run did not contain amyloglucosidase. Samples were assayed in triplicate.

RESULTS AND DISCUSSION

The composition of the flours and freeze-dried glutens is given in Table I. The HTST flours and glutens showed significantly less water than did the untreated material. However, on storage this flour recovered part of the water but its isolated gluten was further dehydrated. All the treated and untreated samples contained <20% water, the level at which loss of vitality becomes manifest (Weegels et al 1994a,b). The permanent loss of total N observed in HTST-treated flour indicates loss, during HTST treatment, of (volatile) nitrogen components.

As shown in Table II. the solubility of gluten proteins in acetic acid was not greatly affected by HTST treatment, and only slightly affected by the subsequent storage, whereas in the denatured samples, the solubility was minimal.

Almost all the proteins (>95% of total) were solubilized in the presence of SDS and DTT and were analyzed by SDS-PAGE. The video image of the SDS-PAGE runs includes the unresolved aggregates that moved more slowly than the heaviest marker (M_r 205 kDa). Where the trace does not drop to zero, the peak areas considered in the calculations are identified by vertical lines (Fig. 1).

Table III shows the relative amounts of the various components in each run. In each case, the sum of the peaks adds up to 100. Components from 51 to 18 kDa, including α, β, and γ gliadins and low molecular weight (LMW) glutenins with some residual globulin, which may have contaminated gluten preparations after hand washing (Pogna et al 1994), diminished after HTST or

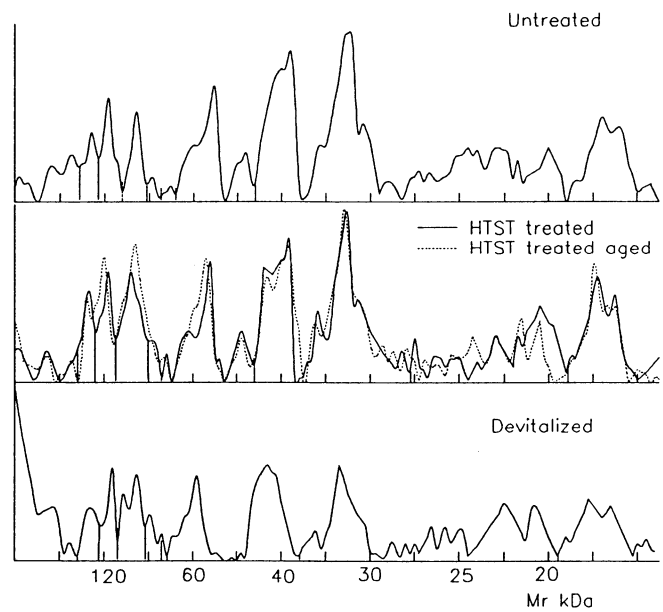

Fig. 1. Densitometric traces of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) separations of glutens from four samples: 1) untreated flour; 2) high-temperature short-time (HTST) treated flour; 3) HTST-treated flour stored three months at room temperature; 4) untreated flour heat-denatured 20 hr at 100°C.

denaturation. The components above M_r 60 kDa, an M_r range including predominantly ω-gliadins and high molecular weight (HMW) glutenins, and probably some oligomers, showed a relative increase. The new material may derive from dissolved aggregates or from newly aggregated low M_r monomers. Aggregates, considered as the areas with M_r > 139 kDa, diminished after HTST treatment, whereas they increased in the denatured samples. It seems possible that the bulky associations in the gluten block polymer structure are partially undone by HTST treatment, thus improving penetration into the gel.

The intrinsic fluorescence of acid-soluble gluten proteins of native and HTST-treated samples (maximum emission at 348 nm, excitation 290 nm) indicates a tryptophan chromophore (Fig. 2). Both extracts showed quite similar fluorescence intensity. The nearly linear increase in fluorescence with concentration showed that the chromophore did not significantly interact with other groups (Fig. 2). Proteins from heat-denatured gluten displayed twofold emission intensity, the maximum being displaced at 355 nm (Fig. 2). The concentration dependence of fluorescence was not linear (Fig. 2). Assays >0.4 mg/ml were not feasible because of limited protein solubility.

Fluorescence developed on titration with ANS was also examined. ANS interacts with the nonpolar regions on the surface of protein molecules (Arakawa et al 1991). The deconvoluted ANS titration curves revealed, in all samples, one component of logistic nature, predominating at high ANS concentration (Fig. 3B), and a double exponential component at low ANS concentrations (Fig. 3A). This behavior and the association constants of the ANS protein complex, calculated from the curves, indicated two types of binding site: low and high affinity (Table IV). Low-affinity sites produced more fluorescence, corresponding to hydrophobic areas spread on the surface of gluten molecules, whereas high-affinity sites correspond to regions with high specificity for ANS binding (Arakawa et al 1991). Heat treatments affected the sites differently. In the high-affinity sites, the fluorescence and the affinity (1/*Kd*) were moderately increased by HTST, but on aging, the fluorescence decreased and diminished greatly upon denaturation of gluten. In the low-affinity sites, the fluorescence increased after HTST treatment and was unaffected in denatured gluten, but the affinity for ANS increased considerably.

The effect of HTST treatment on starch was also assayed by determining starch availability to amyloglucosidase. Table V shows how, in ungelatinized flour, the HTST treatment slightly increased starch availability. Purified starch was digested less efficiently

TABLE III
Protomer Distribution in Flour from Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) Analysis

M_w (kDa)	Untreated Flour	HTST[a] Treated	HTST Treated, Aged	Gluten Devitalized
Aggregates	4.64	2.70	1.70	13.52
139	2.51	4.37	4.48	3.19
118	5.61	6.54	6.28	5.76
96	5.75	7.42	8.29	8.23
83	0.61	2.34	2.02	2.47
74	0.34	0.43	0.66	1.05
60	9.01	9.10	9.54	8.77
51	2.25	2.03	1.92	0.00
45	18.14	17.31	14.36	14.19
36	22.52	20.86	21.69	18.66
28–18	17.40	17.47	15.66	12.90
15	11.80	13.70	13.43	11.26

[a] High-temperature short-time.

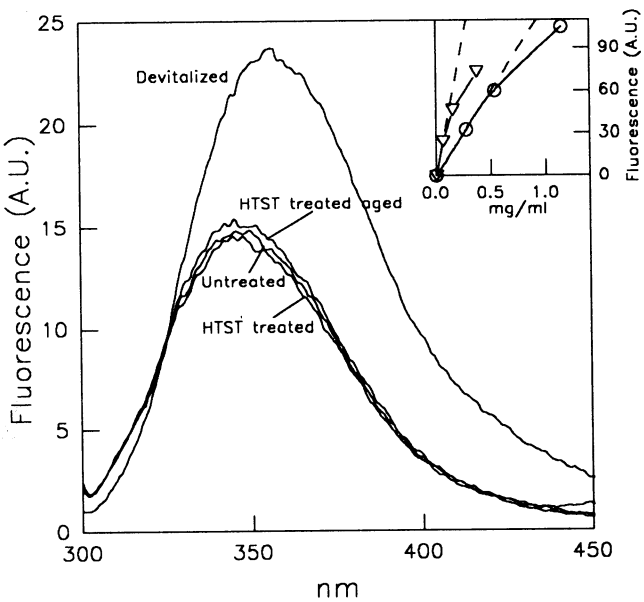

Fig. 2. Fluorescence emission of proteins extracted with 0.05*N* acetic acid from gluten of the indicated samples. Spectra were determined at 20°C using 0.25 mg of protein/ml. Excitation wavelength 290 nm. Inset: dependence of fluorescence on protein concentration for native and HTST-treated gluten (○),heat denatured (100°C) gluten (▽). Excitation and emission wavelength 290 and 350 nm.

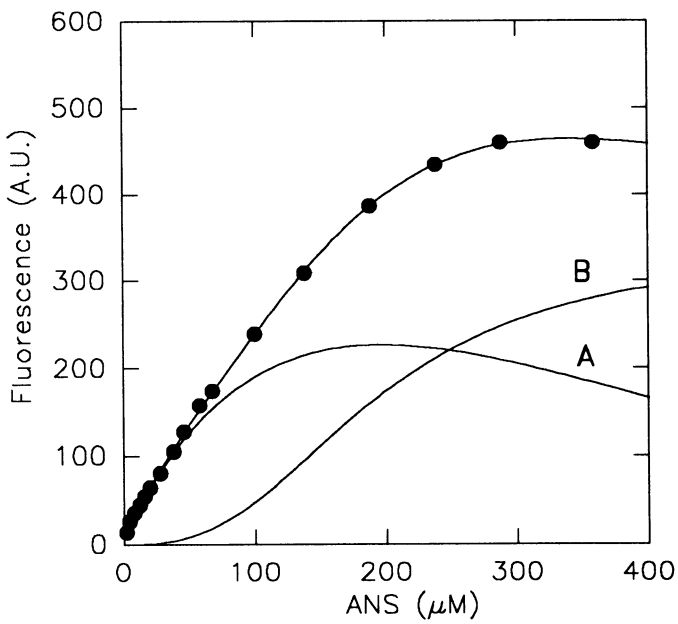

Fig. 3. Titration until saturation by 8-aniline-1-naphthalene sulfonate (ANS) of proteins solubilized from native gluten. Proteins, 0.25 mg/ml in 0.05*N* acetic acid. Double exponential component: high affinity for ANS (**A**). Logistic component: low affinity for ANS (**B**).

TABLE IV
Maximal Fluorescence ANS[a]-Protein Bound

Gluten	High Affinity[b]	*Kd* (μM)[c]	Low Affinity[b]	*Kd* (M)[c]
Untreated	226.6	80.5	308.1	1.6
HTST[d] treated	249.2	59.3	387.7	4.4
HTST treated, aged	220.0	75.0	381.9	1.5
Devitalized	112.1	78.3	298.0	0.004

[a] 8-aniline-1-naphthalene sulfonate.
[b] Maximal fluorescence, arbitrary units.
[c] Dissociation constant.
[d] High-temperature short-time.

TABLE V
Starch Digestion (%) by Amyloglucosidase

Samples	Without Gelatinization	After Gelatinization
Purified starch	3.8 ± 0.1	55.8 ± 1.2
Untreated flour	5.3 ± 0.2	47.6 ± 1.7
HTST[a] treated	5.9 ± 0.2	45.3 ± 1.4
HTST treated, aged	6.1 ± 0.1	45.5 ± 2.1

[a] High-temperature short-time.

than the starch in flour. However after gelatinization, the situation was reversed and no differences were apparent between HTST-treated and untreated flours.

CONCLUSIONS

The changes in water distribution induced by HTST treatment denote a more labile association of water to gluten than to other flour components, and the dehydration of gluten is irreversible. The water recovered by the HTST-treated flour during storage is presumably bound to starch. One possibility is that in HTST-treated flour the starch-gluten interaction is changed due to surface modifications in the gluten.

The intrinsic fluorescence indicates that the HTST treatment did not induce relevant conformational changes in the proteins, whereas heat denaturation did. Modifications in reactivity to ANS indicate effects on the nonpolar regions at the gluten surface. Sites of high and low hydrophobicity were exposed. Liberation of trapped water, reflected in the decreased water content of treated gluten, is a plausible consequence of the surface rearrangements. Thus, gluten becomes unstable. Enhanced surface hydrophobicity favors aggregation of molecules in a water medium, leading to the observed decrease in solubility and extractability, a slow process as was evident in aged preparations. The decreased availability to ANS of sites of high hydrophobicity during aging probably depends on slow conformation or aggregation changes.

The overall parameters that denote loss of vitality (solubility in acetic acid and intrinsic fluorescence) (Wadhanwan and Bushuk 1989b) are scarcely affected during the HTST treatment, even at 90°C. This is because the initial water content is below the threshold for loss of vitality (Weegels et al 1994a). Moreover, the small dimensions of the flour particles and the instant contact with the heat source facilitate water evaporation. The modifications evidenced by ANS on the surface of the HTST-treated gluten complex indicate that vitality does not necessarily coincide with the native state of the complex. Evidently, the induced modifications did not interfere with the viscoelastic behavior peculiar to native gluten. On the other hand, Guerrieri et al (1996) have shown that the characteristics related to vitality were severely modified when isolated gluten was heated at 90°C. Thus it appears that, within its natural environment in flour, gluten is more heat resistant.

Thermal input at denaturation is far more drastic and induces permanent modifications in conformation and association, affecting the protein structure and producing interchain disulfide binding with important negative effects on functional properties (Schofield et al 1983).

Only a few of the parameters that changed in denatured gluten appear affected by HTST treatment. Thus, it can be excluded that the moderate effects of HTST depend on a full denaturation of a limited number of gluten units.

Availability to amyloglucosidase is an indicator of the state of starch molecules. Untreated purified starch has poorer digestion, compared to flour starch, which is ascribable to a partial crystallization of the pure starch. In flour gels, the nonstarchy components and the starch itself probably interact, causing weaker hydrolysis than in gels of purified starch. Increased digestion in HTST-treated flour can be attributed to the destructuring of the starch granule: indeed no difference appeared in the gelatinized samples.

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