Effect of Heat Treatment on Protein Solubility and Viscoelastic Properites of Wheat Gluten

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ABSTRACT

Gluten was extracted from four common wheat and three durum wheat cultivars, cast into a special cell, and dipped into boiling water for 0–7 min, after which viscoelasticity and protein solubility were examined. On heating, gluten compressibility decreased, gluten firmness increased, and some proteins soluble in 60% ethanol were insolubilized through the formation of new bonds, possibly disulfide. Viscoelastic properties correlated highly with the content of proteins that were soluble in 60% ethanol or in 1% mercaptoethanol/0.025*M* borate buffer. Results of sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed that some subunits of salt-soluble and gliadinlike proteins participate in the formation of insoluble protein networks and others do not. Differences between cultivars were significant. In common wheats with better baking quality and durum wheats with better pasta-cooking quality, the tendency of the ethanol-soluble proteins to aggregate during heating was greater.

Relatively few studies have been designed to follow the denaturation of wet gluten by heat. The most exhaustive study, by Pence et al (1953), showed that gluten denaturation, measured either by protein solubility in dilute acetic acid or by baking performance, was essentially a first-order reaction; the relations among pH, temperature, and rate of denaturation were complex. More recently, Kent (1975) stated that heat treatment of wheat tends to make the gluten harsher and tougher. Moreover, little is known about the effect of drying conditions (freeze, drum, or spray drying) on the functional and biochemical properties of vital gluten. Doguchi and Hlynka (1967) showed that after dried glutens of hard red spring and durum wheats were heated at $\geq 70°C$ for 1 hr, the gluten ball had a definitively slower stretching rate.

Firmness and absolute recovery of durum wheat gluten balls were determined in our laboratory. Strong evidence was found that determination of the absolute recovery of a gluten ball heated 90 sec (98°C) could determine the intrinsic cooking value of durum wheat cultivars and thus be a promising breeding tool (Damidaux 1979, Damidaux and Feillet 1978). The present work was undertaken because more fundamental research on structural/functional relationships is needed to understand the biochemical basis of the denaturation of wheat gluten by heat.

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MATERIALS AND METHODS

Wheats and Flours

Four common wheat (*Triticum aestivum*) and three durum wheat (*T. durum*) cultivars, covering a wide range of intrinsic baking or pasta-making values, were used for this study. Quality data on these varieties are given in Table I.

Common wheats were milled into flour in an industrial mill

(Grands Moulins de Paris). Durum wheats were milled into semolina in a modified Brabender Junior mill in conjuction with a laboratory purifier (Alause 1971).

Isolation and Heating of Gluten

Gluten was extracted by the hand washing procedure described by Mauze et al (1972) except that deionized water instead of tap or salted water was used. Table II shows characteristics of unheated gluten.

Gluten (1 g) was cast into a special cell (Damidaux and Feillet 1978) and immersed in a boiling water bath. After heating, the cell was transferred to water at room temperature for 2 min. A disk of gluten was then removed from the cell for rheological and biochemical analysis. The diameter and the thickness of the gluten disk were 12 and 5.3 mm, respectively.

Determination of Viscoelastic Properties of Gluten

Viscoelastic properties of heated gluten disks were determined with a viscoelastograph, a new apparatus recently developed in our laboratory to evaluate the rheologic properties of viscoelastic products (Feillet el al 1977).

A gluten disk was put on the sample holder and compressed under a constant load (800 g for common wheat gluten, 500 g for durum wheat gluten) for 40 sec. Deformation (thickness) of the gluten disk was recorded during and after compression.

From the curve of strain vs time, the following were calculated (Fig. 1): Initial thickness (mm) of gluten disk (E); thickness (mm) of gluten disk 40 sec after load was put on (e_1); thickness (mm) of gluten disk 20 sec after load was removed (e_2); gluten compressibility (GC) = $(E - e_1)/E$; gluten relative elastic recovery (GRER) = $(e_2 - e_1)/(E - e_1)$; gluten absolute elastic recovery (GAER) = $(e_2 - e_1)$.

TABLE I
Protein Content and Intrinsic Baking or Pasta-Making Quality of Wheats

Wheat and Cultivar	Protein Content[a] (%, db)	Baking Quality[b]	Cooking Quality[c]
Triticum aestivum			
Clement	11.7	Very low[d]	···
Maris Huntsman	13.1	Very low[d]	···
Capitole	12.8	High	···
Kolibri	11.2	Superior	···
T. durum			
V 39	14.2	···	Low
Lakota	14.3	···	Medium
Agathe	15.9	···	High

[a] Flour or semolina protein content.
[b] From G.E.V.E.S. data (1977).
[c] From Abecassis and Alause (1977).
[d] Not intended for bread making.

TABLE II
Moisture and Protein Content of Unheated Gluten Extracted from Wheats

Wheat and Cultivar	Moisture Content (% mb)	Protein Content (% db)
Triticum aestivum		
Clement	71.6	82.5
Maris Huntsman	71.4	82.8
Capitole	66.9	83.1
Kolibri	63.8	74.8
T. durum		
V 39	···	83.3
Lakota	65.7	74.2
Agathe	64.5	87.6

Extraction of Gluten Proteins

After heat treatments and viscoelasticity measurements, gluten disks were freeze-dried in a Virtis apparatus (freezing at $-55°C$) and ground in a Dangoumeau ball mill. Because of the experimental conditions, a temperature gradient developed in the gluten disk during heating, and consequently the dry gluten powder was slightly heterogeneous.

Commercial wheat starch (900 mg) was added to 300 mg of freeze-dried gluten to simplify the separation of insolubles during centrifugation. Gluten proteins were then extracted with 0.5*M* NaCl or sequentially with 60% ethanol and three 0.025*M* borate buffers: buffer alone (pH 10), buffer with 0.5% sodium dodecyl sulfate (SDS), and buffer with 1% mercaptoethanol. For each solvent, gluten was extracted with 25 ml of solvent once for 15 hr and once for 1 hr, with mild stirring, then mixed twice with 15 ml of solvent. After each extraction, the solutions were centrifuged at $38,000 \times g$ for 30 min.

Solubility of gluten in Na stearate was also examined by stirring 30 mg of gluten and 30 mg of Na stearate in 30 ml of water overnight at room temperature.

Analytical Procedure

Nitrogen content was determined by a semiautomatic Kjeldahl method described by Feillet (1976).

Polyacrylamide gel electrophoresis (PAGE) was conducted in 7% gels in aluminium lactate buffer, pH 3.2 (Kobrehel and Feillet 1971), or in 6.3% gels in aluminium lactate buffer, pH 3.1 (Bushuk and Zillman 1978). SDS-PAGE of reduced protein was performed in Tris borate buffer, pH 8.9, with 0.1% SDS (Bietz and Wall 1972).

Vertical SDS-PAGE of reduced proteins was performed as described by Payne and Corfield (1979) and slightly modified[1]. The polymerization was improved by using a higher catalyst content and pouring a thin layer of butanol onto the surface of the separation gel.

RESULTS AND DISCUSSION

Effects of Heating on Viscoelasticity of Gluten

GC, GRER, and GAER of heat-treated gluten disks were determined (Figs. 2–4).

[1] J. C. Autran, personal communication, 1979.

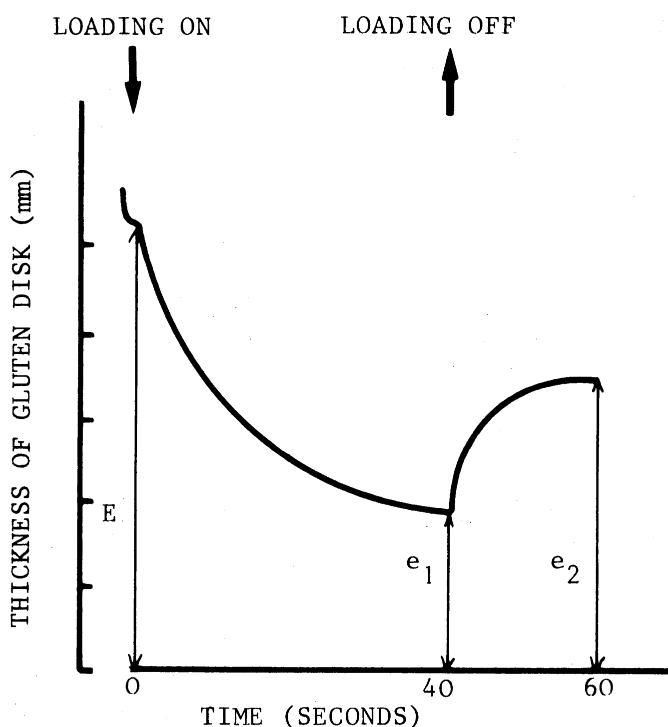

Fig. 1. Viscoelastogram of gluten disks. E = initial thickness of disk, e_1 = thickness of disk 40 sec after loading on, e_2 = thickness of disk 20 sec after loading off.

GC of unheated gluten disks, ranging from 0.76 (Clement, Kolibri, Agathe) to 0.82 (Maris Huntsman) were roughly identical for all wheat cultivars. GC was markedly affected by heating, especially after the 1-min treatment. Glutens isolated from cultivars with high baking quality (Kolibri) or high pasta-making quality (Agathe) were more rapidly and more highly modified than were glutens isolated from low quality cultivars (Clement, V 39); moreover, durum wheat gluten properties changed more rapidly than did those of common wheats. After 5 min, the disk of gluten isolated from Agathe (durum) became very firm and strong (ie, had low compressibility), whereas that of Clement (a nonbreadmaking common wheat) was still soft and weak.

The effect of heating time on GRER is shown in Fig. 3. For all samples, GRER increased with heating time, but differences between cultivars were large. After 3 min, GRER of Clement was still very low (0.15) but for Kolibri, Lakota, and Agathe it exceeded 0.75.

Large differences were also observed on unheated gluten. GRER values of wheat with high baking quality (Capitole and, particularly, Kolibri) were higher than those of nonbreadmaking and durum wheats.

With the exception of Clement and Kolibri, GAER values increased with heating time and then decreased. The maximum value was reached more rapidly with high quality wheats (both common and durum): in 2 min with Lakota and Agathe, 3 min with Capitole, 4 min with V 39 and Maris Huntsman. Possibly, GAER of Clement would decrease after heating more than 5 min. GAER of Kolibri stayed roughly constant but decreased very slightly with time.

The general effect of heating time on viscoelastic properties of gluten seems similar for all wheats, with the noteworthy exception that the times to maximum GAER value increased from the best to the poorest quality wheats.

TABLE III
Influence of Heating Time on Gluten Solubility[a] in NaCl[b]

Wheat and Cultivar	Heating Time, min			
	0	1.5	3	5
Triticum aestivum				
Clement	12.5	11.3	13.5	10.9
Maris Huntsman	7.7	6.7	7.2	5.4
Capitole	7.8	7.3	6.2	3.7
Kolibri	8.3	6.4	5.5	3.9
T. durum				
V 39	9.4	7.2	4.8	3.8
Lakota	10.7	6.2	4.7	4.3
Agathe	8.6	5.9	5.3	3.9

[a] Soluble protein, percent of total.
[b] 300 mg of gluten extracted in 25 ml of 0.5 *M* NaCl, once for 15 hr and twice for 15 min.

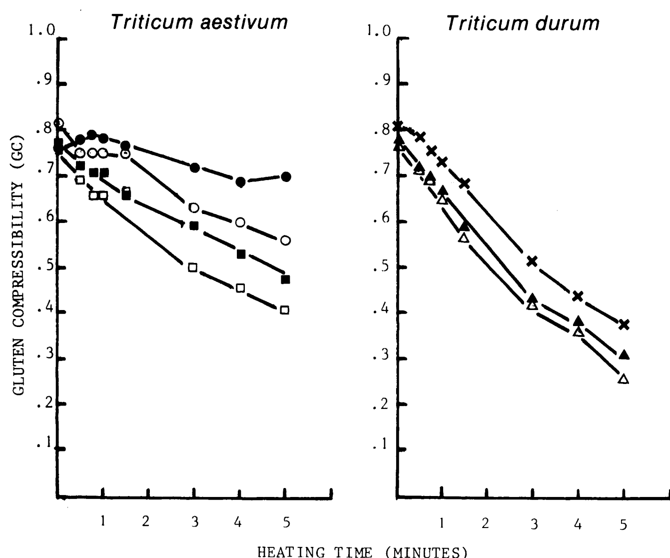

Fig. 2. Influence of heating time on gluten disk compressibility. Cultivars: ● = Clement, o = Maris Huntsman, ■ = Capitole, □ = Kolibri, x = V 39, ▲ = Lakota, Δ = Agathe.

Effects of Heating on Protein Solubility

The solubility of freeze-dried gluten in 0.5 *M* NaCl is presented in Table III. As expected, salt-soluble protein decreased as heating time increased. No major differences occurred among cultivars, except for Clement. The rye genes in this variety (I R substituted to 1 B) probably explain its high salt-soluble protein content.

Results of sequential extraction of gluten are shown in Fig. 5.

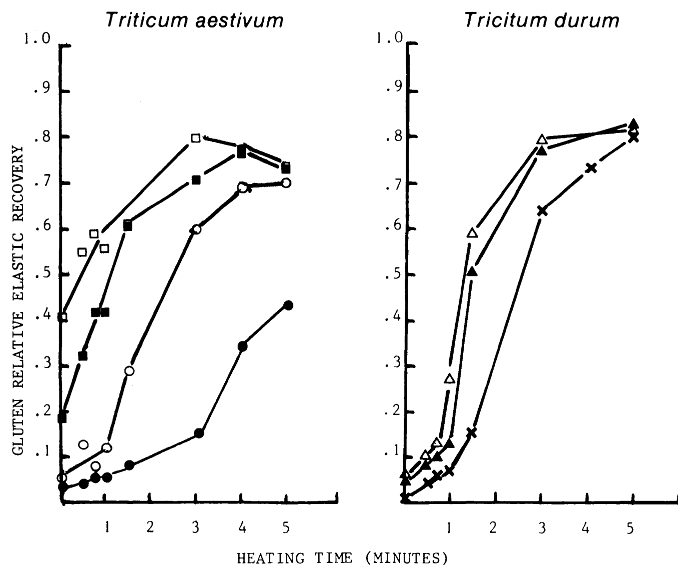

Fig. 3. Influence of heating time on gluten disk relative elastic recovery. Cultivars: ● = Clement, o = Maris Huntsman, ■ = Capitole, □ = Kolibri, x = V 39, ▲ = Lakota, Δ = Agathe.

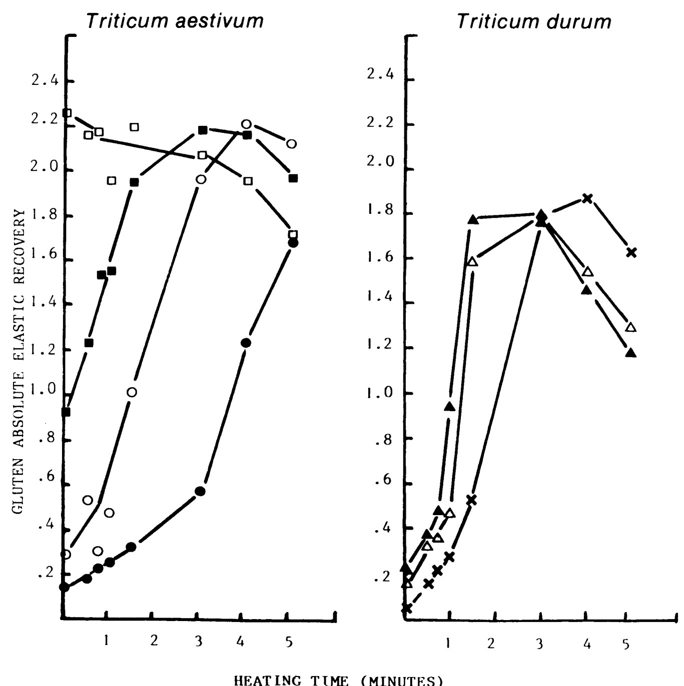

Fig. 4. Influence of heating time on gluten disk absolute elastic recovery. Cultivars: ● = Clement, o = Maris Huntsman, ■ = Capitole, □ = Kolibri, x = V 39, ▲ = Lakota, Δ = Agathe.

Triticum aestivum

CLEMENT

MARIS HUNTSMAN

CAPITOLE

KOLIBRI

Triticum durum

V 39

LAKOTA

AGATHE

PERCENTAGE OF TOTAL PROTEINS

HEATING TIME (MINUTES)

Fig. 5. Influence of heating on gluten solubility in sequential extraction with 60% ethanol (•), 0.025 *M* borate buffer (o), 0.5% sodium dodecyl sulfate/0.025 *M* borate buffer (*), and 1% mercaptoethanol/0.025 *M* borate buffer (**x**).

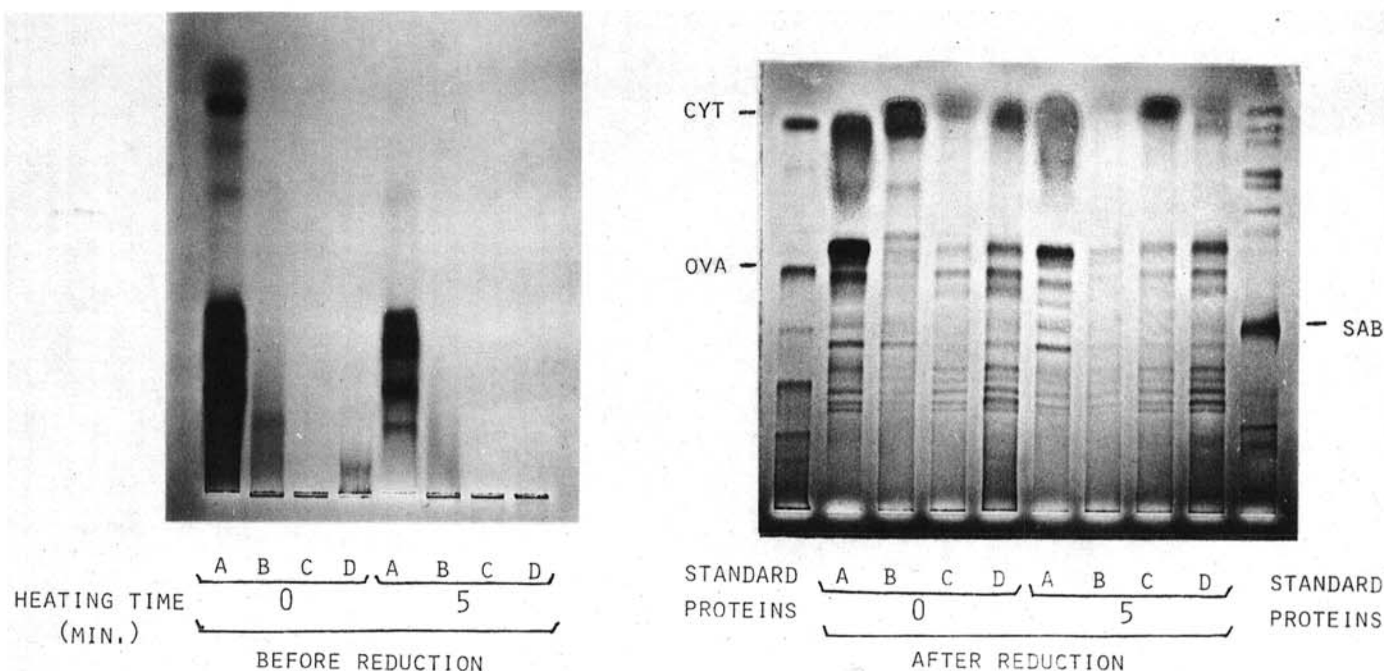

Fig. 6. Polyacrylamide gel electrophoresis of gluten proteins (**left**) before (aluminium lactate buffer, pH 3.2) or (**right**) after (Tris/sodium dodecyl sulfate buffer) reduction by mercaptoethanol. Proteins were sequentially extracted from unheated and heated (5 min) gluten isolated from Kolibri (*T. aestivum*). **A,** 60% ethanol solubles; **B,** 0.025 *M* borate buffer solubles; **C,** 0.5% sodium dodecyl sulfate/0.025 *M* borate buffer solubles; **D,** 1% mercaptoethanol/0.025 *M* borate buffer solubles.

For all samples, the most striking variations were in the ethanol-soluble (ES) and mercaptoethanol-soluble (MES) fractions. With increased heating time, the amount of ES decreased markedly and the amount of MES increased. The changes in the two other fractions (borate buffer solubles and SDS solubles) were less important.

In *T. aestivum* cultivars, the amount of MES in Capitole and Kolibri untreated gluten was twice the amount in Maris Huntsman gluten. No such difference was observed among durum wheat cultivars. Mercaptoethanol solubility of gluten reached a maximum value in the heat treatment 300–420 sec range with durum wheat and Kolibri. A longer heating time would be necessary to observe a maximum value of mercaptoethanol solubility with the other cultivars.

Moreover, the point at which gluten solubility in mercaptoethanol became higher than gluten solubility in ethanol was reached sooner with high quality than with low quality wheats.

TABLE IV
Influence of Heating[a] on Gluten Solubility[b] in Na Stearate[c] and on Total Gluten Solubility from Sequential Extraction

Wheat and Cultivar	Heating Time (min) Before Extraction: In Stearate 0	In Stearate 5	Sequentially in Ethanol, Borate Buffer, and SDS/Borate Buffer 0	Sequentially in Ethanol, Borate Buffer, and SDS/Borate Buffer 5
Triticum aestivum				
Clement	89.5	44.4	75.3	49.2
Maris Huntsman	93.7	31.8	81.6	37.9
Capitole	88.9	28.3	65.8	32.8
Kolibri	89.1	23.1	64.8	32.8
T. durum				
V 39	92.2	28.7	80.3	36.2
Lakota	93.9	25.5	80.5	28.8
Agathe	92.0	20.8	75.7	26.4

[a]5 min in boiling water.
[b]Soluble protein, percent of total.
[c]30 mg of gluten extracted by 30 mg of Na stearate in 30 ml of water.

According to the electrophoretic pattern (Fig. 6), the ES proteins are gliadins contaminated by fast moving components (water-soluble or salt-soluble proteins). The streak in the pattern seems to indicate high molecular weight proteins in the ethanol solution. This was confirmed by SDS-electrophoresis of protein subunits. The proteins solubilized by mercaptoethanol did not enter the gel and were consequently high molecular weight proteins. High molecular subunits were also present in borate and SDS-borate extracts.

The fast moving components become almost entirely insoluble in 60% ethanol after heating (Fig. 6). Furthermore, by Bushuk and Zillman's procedure (1978), the pattern of the gliadins isolated from glutens heated for various times did not change significantly.

We postulate that, by heating, the ES fraction is converted into insoluble proteins through the formation of new bonds. Because these are further disrupted with mercaptoethanol, they could be disulfide bonds. The gluten protein solubility of untreated gluten suggests that such associations may have occurred before heating.

Another hypothesis cannot be ruled out. According to Kasarda et al (1976), the insoluble wheat protein would aggregate through hydrogen, hydrophobic, and ionic bondings. Reducing agents, such as mercaptoethanol, that disrupt disulfide bonds would

TABLE V
Correlation Coefficients[a] Between Protein Fractions of Gluten and Gluten Compressibility (GC), Gluten Relative Elastic Recovery (GRER), and Gluten Absolute Elastic Recovery (GAER)

	Protein Fractions: From Sequential Extraction of Gluten by 60% Ethanol	0.025M Borate Buffer: Alone	With SDS[b]	With ME[c]	Soluble in 0.5M NaCl
GC	0.91	0.37	0.62	−0.93	0.76
GRER	−0.91	−0.26	−0.54	0.90	−0.76
GAER	−0.62	−0.06	−0.29	0.59	−0.51

[a]$n = 48$; r at 1% significance level = 0.45.
[b]Sodium dodecyl sulfate, 0.5%.
[c]Mercaptoethanol, 1%.

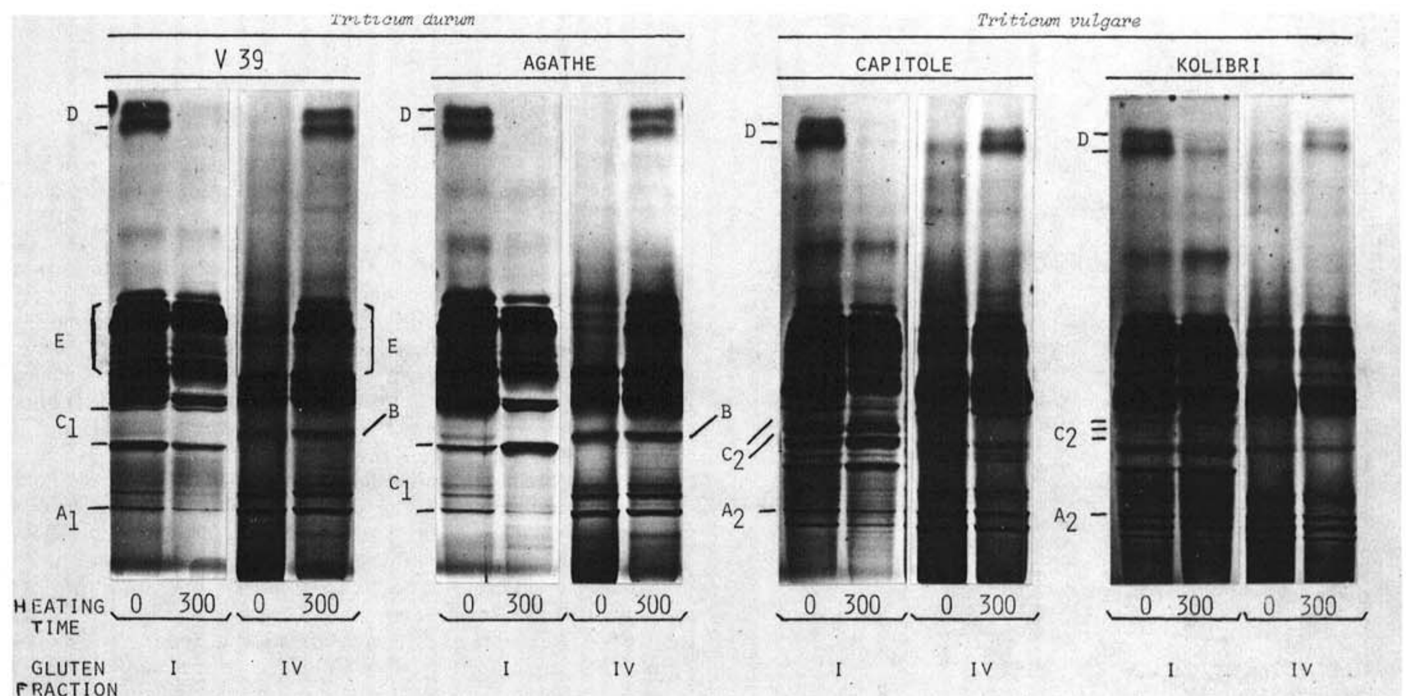

Fig. 7. Vertical sodium dodecyl sulfate-polyacrylamide gel electrophoresis patterns, showing influence of heating time (0 and 300 sec) on subunit composition of 60% ethanol-soluble (I) and 1% mercaptoethanol-soluble (IV) gluten proteins isolated from wheats. **A_1** (durum) and **A_2** (common), subunits of fractions I and IV before and after heating; **B,** subunit of durum fraction IV only; **C_1** (durum) and **C_2** (common), subunits of fraction I only; **D** and **E,** subunits of fraction I before heating and fraction IV after heating.

change the protein's three-dimensional structure and destroy the secondary bonding forces. Moreover, heating is known to reinforce the strength of hydrophobic bonds. Consequently, gliadinlike components and possibly salt-soluble proteins may interact together or with glutenin fractions through hydrophobic bonding and form new insoluble high molecular weight protein complexes. To support this hypothesis, we tried to dissolve the gluten proteins with Na stearate (Kobrehel and Bushuk 1977). Data are listed in Table IV.

Approximately 90% of the total protein was solubilized by extracting unheated gluten once with Na stearate; differences among cultivars and between species were not significant. After 300-sec heating, the solubility of gluten proteins in Na stearate was sharply reduced and was slightly lower than the sum of the proteins soluble in 60% ethanol, borate buffer, and 0.5% SDS-borate buffer. Consequently, we postulate that bonds other than hydrophobic ones were responsible for the insolubilization of gluten proteins during heating.

Furthermore, whatever the reasons are for the insolubilization of normally ES fractions, the results (Fig. 5, Table IV) indicate that the ability of gluten proteins to aggregate during heating differs markedly according to the wheats. The data suggest that the capacity for proteins to associate easily and quickly into insoluble complexes and possibly to form an insoluble network is characteristic of high quality baking wheat (ie, Kolibri) and high quality pasta-making wheat (ie, Agathe).

This finding is consistent with the finding that the cooking quality of a pasta product is related to the capacity of its proteins to aggregate and form a network that prevents loss of gelatinized starch during cooking (Feillet 1977, Feillet and Abecassis 1976). A higher percentage of MES fraction or a lower percentage of sodium stearate-soluble fraction would indicate a higher quality of durum or common wheat.

Recent advances in the separation of wheat protein subunits (or polypeptide chains) by vertical SDS-PAGE (Autran[1], Payne and Corfield, 1979) allowed us a closer look at the subunit composition of ES and MES fractions isolated from gluten before and after 300-sec heating. Fractions isolated from two durum wheats (V 39, Agathe) and two common wheats (Capitole, Kolibri) were examined (Fig. 7).

Subunits can be classified into four groups: 1) subunits, such as A_1 (durum wheats) and A_2 (common wheats), that contribute to the composition of ES and MES fractions both before and after heating; 2) specific subunits of the MES fraction, such as B (durum wheats only), that are absent from ES patterns; 3) specific subunits of the ES fraction, such as C_1 (durum wheats) and C_2 (common wheats), that are absent from MES patterns even after gluten is heated; and 4) specific subunits of the ES fraction before heating, such as D and E, that are present in the MES fraction after heating.

The differences in the abilities of ES fraction subunits to aggregate with heating and to form insoluble materials through new bonds must be emphasized. In V 39 gluten, for instance, the high content of subunit C_1—and possibly of other similar polypeptides—together with the low content of subunits D and E might restrain the association of gliadin components in the MES fractions (Fig. 6). Subunits similar to D and E would, however, contribute strongly to the formation of the MES fraction by heating.

Moreover, low molecular weight subunits, such as D, which probably originate from the salt-soluble proteins, clearly do participate in the formation of new aggregates.

Correlations

Simple correlation coefficients (Table V) were calculated between protein fraction contents and viscoelasticity values within all gluten samples (n = 48).

Several correlation coefficients were highly significant. The r values were highest for GC and GRER in the ES and MES fractions (Table V). GC was the highest and GRER the lowest when the content in the ES fraction was the highest or that in the MES fraction was the lowest, but the relationships were not linear (Fig. 8).

Two factors can exert an influence on these values: heat treatment (time) and wheat samples (cultivar, species). Analysis of variance indicates that both factors were highly significant. Interestingly, the relationships demonstrated by comparing all data also occurred within each cultivar.

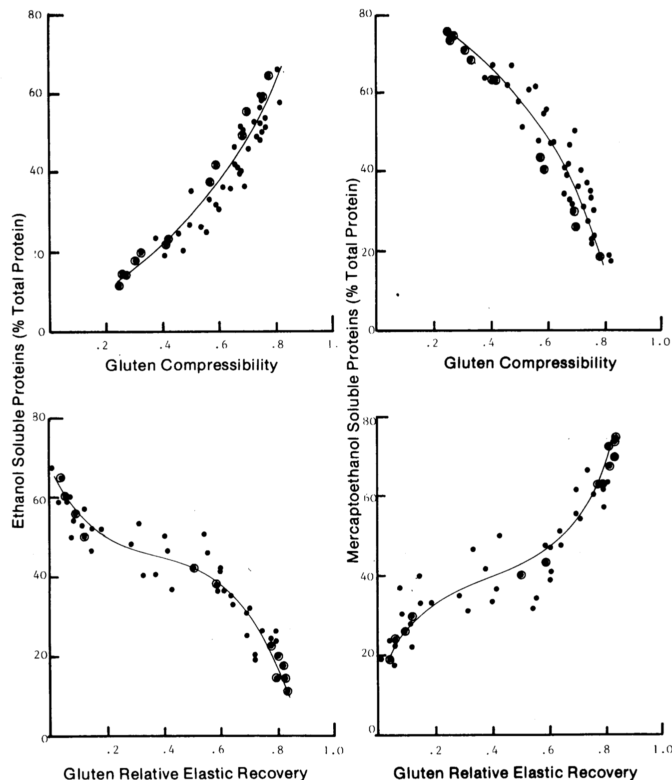

Fig. 8. Content of 60% ethanol-soluble and 1% mercaptoethanol-soluble gluten proteins vs gluten compressibility and gluten relative elastic recovery (n = 48) in seven wheat cultivars. o = durum wheat, • = common wheat.

CONCLUSION

The protein solubility and the viscoelastic properties of gluten are markedly modified by heating in boiling water. Gluten compressibility decreases and gluten relative elastic recovery increases. Changes in viscoelastic properties of gluten are highly related to changes in protein solubility.

The ES proteins are insolubilized through the formation of new bonds between polypeptide chains. New disulfide bonds are quite probably involved in this phenomena.

A few polypeptide chains, or subunits, specific for the ES proteins do not interact during heating and therefore do not participate in the formation of the new insoluble protein complex.

Wheat cultivars have different behaviors. The better the baking quality of common wheat and the pasta-cooking quality of durum wheat, the higher is the ability of ES proteins to aggregate during heating. The ability of proteins to form an insoluble network during cooking prevents the release of gelatinized starch and is responsible for a high pasta-cooking quality.

Because gluten was almost entirely dissolved in Na stearate, our results confirm the role of hydrophobic—and possibly hydrogen—bonds in the structure of native gluten, as outlined by others (Kasarda et al 1976, Kobrehel and Bushuk 1977). Moreover, they suggest that disulfide bonds are markedly involved in the final gluten structure after heating.

We need further work to understand how the tendancy of gluten proteins to aggregate by heating differs from one variety to another and to examine the hypothesis that varietal differences in the content and properties of subunits that do participate in the formation of the insoluble protein network are responsible for these differences between varieties. The physicochemical characteristics of these subunits must also be determined.

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