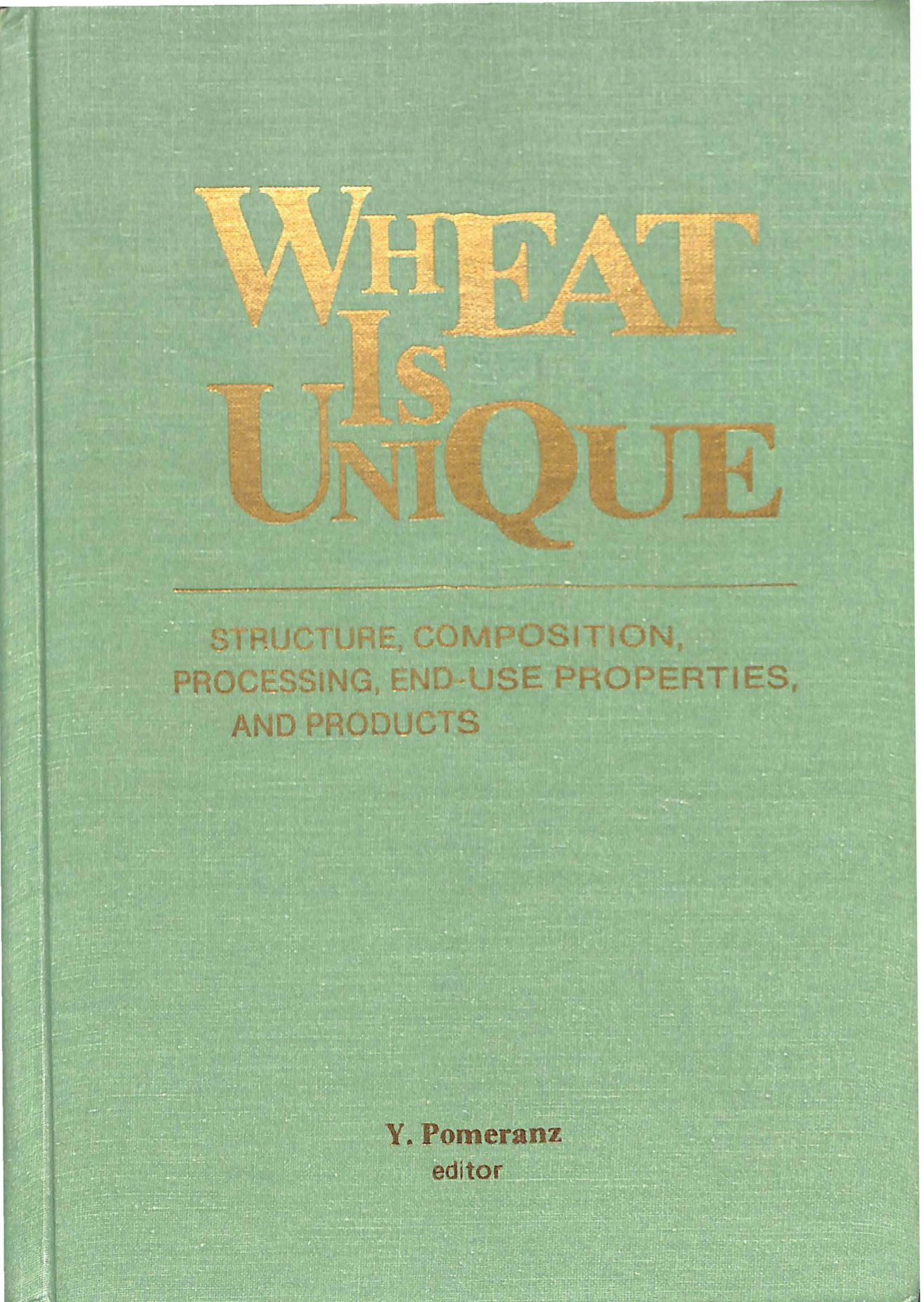


33

# THERMAL MODIFICATION OF GLUTEN AS RELATED TO END-USE PROPERTIES

J.C. Autran, O. Ait-Mouh and P. Feillet

Laboratoire de Technologie des Céréales
I.N.R.A., 2 place viala
34060 Montpellier Cedex, France

## INTRODUCTION

Most of cereal food processes are characterized by hydrothermic treatments under a range of temperature (55°C to 220°C) and humidity (35 to 70 %), depending on the type of product (bread, cookie, pasta, vital gluten). These treatments are important and critical steps in cereal technology and they strongly affect the quality characteristics of end-products.

On the other hand, gluten proteins play a predominant role in most of cereal foods, contributing to structures that are desirable to the consumer or the industry. Their multifunctional characteristics enable them to take part in several stages occuring between raw materials and products. They are strongly modified by heat treatments and one of the first consequences of the temperature raise is a physical change known as protein denaturation.

## THEORETICAL BASIS OF HEAT DENATURATION OF PROTEINS

As recently reviewed by Schofield and Booth (1983), Davies (1986) and Hoseney (1986), it is well known that exposing proteins to high temper-

atures for only short periods causes most of them to undergo conformational changes, of which the most visible effect is a decrease in solubility. Most globular proteins undergo denaturation when heated above 60 to 70°C, but considerable variations in the degree of change exist among proteins (Voutsinas et al. 1983). Formation of an insoluble white coagulum when egg white is boiled is a common example of protein denaturation but equally significant and profound is certainly the loss of biological activity of enzymes.

Since no covalent bonds of the polypeptide chain are broken during a relatively mild treatment, denaturation causes the native characteristic folded structure of the polypeptide chain to uncoil or unwind into a randomly looped chain. Then, thermal agitation contributes to new associations and sequentially alters polypeptides into aggregates and finally into insoluble components.

As unfolding occurs, functional groups previously associated within the molecule become available for external binding (increase of surface hydrophobicity), for interactions with other proteins or subunits, or other constituents (depending on heating temperature, heating time and humidity), giving rise to more highly aggregated complexes (Wall and Huebner 1981). If the denatured unfolded conformation has less free energy (i.e. is more stable) than the folded native conformation by only a very small margin, the change may be reversible. However, if the activation-energy barrier is high, the polypeptide may be irreversibly locked into the denatured (aggregated) conformation.

As the interactions of water molecules with ionic and polar groups strongly influence the folded conformation of proteins, the effect of heat on denaturation is markedly affected by the water content of protein : as the water content decreases, the amount of heat required to denature a protein significantly increases. Dry proteins are thus much more resistant to heat



denaturation than proteins in solution (Neucere and Cherry 1982).

Heat disrupts the non-covalent forces, particularly H bonds and electrostatic interactions. All chemical bonds are weakened as the temperature increases. However, hydrophobic bond formation is an endothermic process which may be favored by increasing temperature up to about 60°C.

In gluten proteins, where the structure is mainly stabilized through S-S bonds and hydrophobic interactions, two major phenomena have been described. First, heat-induced unfolding causes the apolar residues to move towards the outside, to make more difficult the contact of the molecule with water (the protein becomes insoluble in aqueous media) and contributes to aggregates strengthened through hydrophobic interactions. Simultaneously, an SH/S-S interchange can be facilitated between exposed S-S and SH groups in adjacent molecules (Schofield 1986), giving rise to a more highly aggregated structural state having less free energy and therefore more stable than the native state.

## IMPORTANCE OF THERMAL DENATURATION IN CEREAL PRODUCTS

The phenomenon of thermal denaturation is of primary importance in relation to food products. It is extremely important and affects their preparation, processing, nutritional value, quality and safety. Depending upon the particular application, it may be desirable or undesirable.

For many uses, undenatured proteins have superior functional properties (such as solubility, emulsifying, foaming and thermosetting properties) compared to denatured proteins. Therefore protein denaturation is usually viewed in a negative sense by food-protein chemists, particularly in relation to preparation of functional ingredients.

However, denaturation may have positive effects and, in cereal foods, heat treatments are even necessary for starch digestibility, texturization and determination of the technological and organoleptic qualities of the final product. For instance, in bread-making, the thermal denaturation of the gluten film around the gas droplet is an essential step for the formation of the unique foam texture of bread. It also allows selective thermal inactivation of certain undesirable components (amylases, yeast enzymes, lipases, lipoxygenases, peroxidases).

In contrast to many food systems (egg white, soy and milk proteins), relatively few reports have concerned denaturation of wheat gluten proteins. Since the pioneering studies of Mecham and Olcott (1947) and Pence et al (1953), few, if any, in-depth analyses of the alterations produced in flour or semolina proteins as a consequence of heat treatments were reported in the literature.

The objectives of the present paper are to review the major effects of thermal processing on wheat proteins and to develop an understanding of the complex physico-chemical changes they undergo, based on the study of four different examples of heat treatments :

-Model systems : hand-washed gluten or purified gliadins or glutenins.
-Bread-baking and cookie-making.
-High-temperature pasta drying and pasta cooking.
-Industrial vital gluten production and drying.

In these studies, protein denaturation has been monitored by the following methods : solubility in various solvents, electrophoresis in polyacrylamide gel in acid buffer (A-PAGE) or in the presence of sodium dodecyl sulfate (SDS-PAGE), conventional size-exclusion chromatography, size-exclusion (SE-HPLC) or reversed-phase (RP-HPLC) high-pressure liquid chromatography, circular dichroism spectroscopy (CD), prediction of secondary structures, viscoelastic measurements and differential scanning calorimetry (DSC) experiments.



## MODEL SYSTEMS : HAND-WASHED GLUTEN OR PURIFIED GLIADINS OR GLUTENINS

Jeanjean et al (1980) extracted gluten from four common wheat and three durum wheat cultivars, cast it into a special cell of the Viscoelastograph and dipped it into boiling water for 0-7 min, after which viscoelasticity and protein solubility were examined. On heating, gluten compressibility decreased, gluten firmness and elasticity increased (Fig. 1); some proteins, soluble in 60 % ethanol were insolubilized. Results of SDS-PAGE showed that some subunits of salt-soluble and gliadin-like proteins participate in the formation of insoluble protein networks and oth-

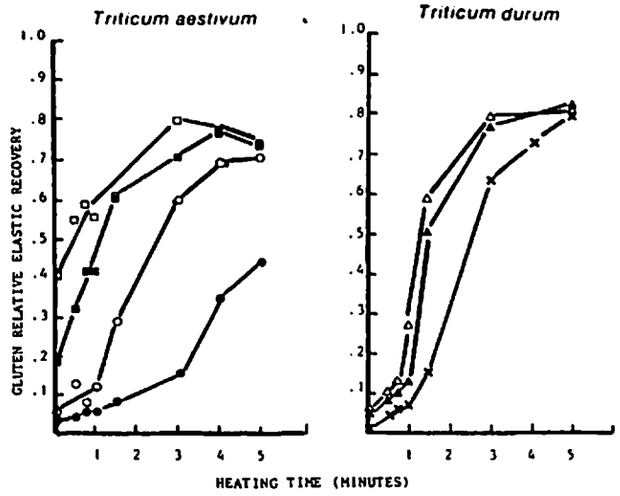


Fig. 1 Influence of heating time on gluten disk relative elastic recovery. Cultivars : ● = Clement, ○ = Maris Huntsman, ■ = Capitole, □ = Kolibri, × = V39, ▲ = Lakota, △ = Agathe (Reprinted, by permission, from Jeanjean et al 1980).

ers do not. Moreover, differences between cultivars were significant : in common wheats with better baking quality and in durum wheats with better pasta-cooking quality, the tendency of ethanol-soluble proteins to aggregate during heating was greater. Since the aggregates could be disrupted further only with mercaptoethanol, it was postulated that protein insolubilization occured through the formation of new bonds, possibly disulfide bonds.

Complementary observations were reported on bread wheat glutens heated 0-90 min in boiling water, specifically an insolubilization of proteins associated with a gradual disappearance of streaking material and of bands in A-PAGE patterns or unreduced SDS-PAGE patterns (Autran and al 1982). Similar results were obtained by Autran and Berrier (1984) from durum wheat glutens heated 0-30 min. Standard patterns were essentially restored upon treatment of gluten by a reducing agent before SDS-PAGE confirming that disulfide bonds could be involved in protein aggregation by heat. Some protein fractions were more susceptible to insolubilization than others. Streaks and slot material, that correspond to extractable LMW and HMW glutenin fractions, disappeared after less than 5 min of heat treatment, and patterns from glutens heated from 30 to as long as 90 min consisted essentially of an enhanced group of ω-gliadin bands (Fig. 2).

When comparing different glutens extracted from flours that varied in breadmaking potential, Sadouki and Autran (1987) observed different behaviours for the various HMW glutenin subunits. In general, those (viz. n° 1 or n° 5-10) whose presence is positively correlated with high baking quality exhibited a higher tendency to form insoluble complexes upon heat treatment than those (viz. n° 2-12) whose presence is negatively correlated with baking quality.

Recently, more accurate studies were carried out from purified gliadin fractions. Menkovska et al (1987) submitted gliadin fractions to heat



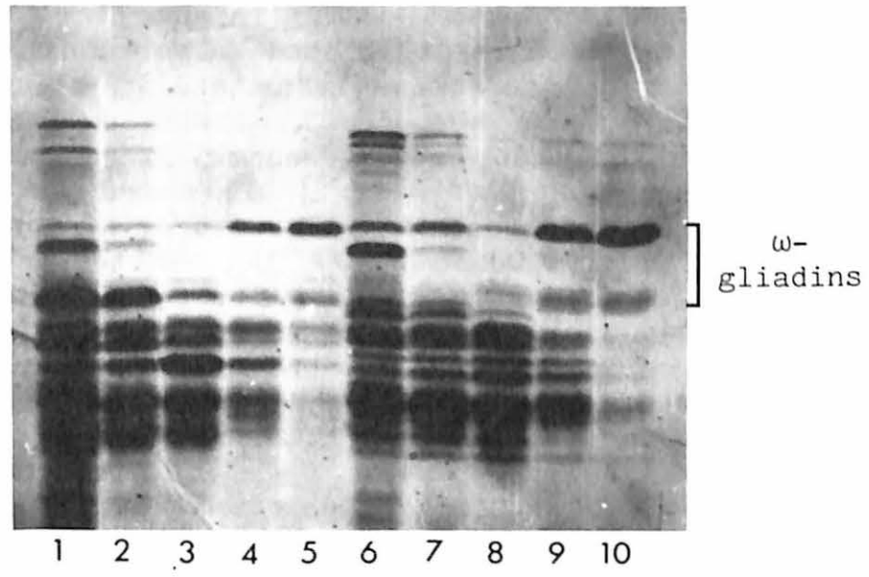


Fig. 2. SDS-PAGE patterns of ethanol-soluble fraction of gluten from two durum wheat cultivars: Montferrier (slots 1 to 5) and Calvinor (slots 6 to 10). Proteins were extracted from control semolina (1 and 6) and from gluten heated 0 min (2 and 7), 1.5 min (3 and 8), 10 min (4 and 9) and 30 min (5 and 10) (Reprinted, by permission, from Autran and Berrier 1984).

treatments (15 min at 100°C and 200°C) respectively, selected to represent the approximate temperatures that the crumb and crust approach during bread-baking. Subsequent analyses by A-PAGE and RP-HPLC showed that the highly hydrophobic fractions (which consist essentially of α-, β-, γ-gliadins, and possibly of extracted LMW-glutenin material) undergo major changes in the peak size of the RP-HPLC chromatograms and seem to be more heat labile than the less hydrophobic gliadins (which correspond to ω-gliadins). These results agree with SDS-PAGE data of Meier et al (1985) who

showed an increase in molecular weights of the gliadin proteins on heating and also confirm those obtained from crude gliadins and glutenins by Anno (1981).

Using a different physico-chemical approach based on circular dichroism (CD) measurements and prediction of secondary structure, Tatham and Shewry (1985) studied the effect of temperature on the conformation of native and cysteine-modified sulfur-rich $\alpha$ -, $\beta$ -, and $\gamma$ -gliadins and of sulfur-poor $\omega$ -gliadins in ethanolic solutions. Increasing the temperature from 20° to 80°C resulted in an increase in aperiodic structure, with partial loss of the $\alpha$ -helical content, confirming earlier reports of Kasarda et al (1968) on A-gliadin. A different result, however, was obtained on $\omega$ -gliadins in which the conformational change consisted of an increase of $\beta$-turns. It was concluded that whereas the $\omega$ -gliadins are stabilized by strong hydrophobic interactions, the main stabilizing forces in the $\alpha$ -, $\beta$ -, and $\gamma$ -gliadins are covalent disulfide bonds and non-covalent hydrogen bonds. Interestingly, the conformation of the modified gliadin was similar to that of the native protein, indicating that reduction and alkylation had little effect on the conformation. More recently, Tatham et al (1987) studied the effect of heating on the conformation of LMW subunits of glutenin (or aggregated gliadins) and found CD spectra more similar to those of $\alpha$ -, $\beta$ -, and $\gamma$ -gliadins than to those of $\omega$ -gliadins or HMW subunits of glutenins. No major differences in thermal stabilities of LMW fractions could be found to explain the differences in breadmaking quality between cultivars.

Therefore, irrespective of the experimental approach used for monitoring heat denaturation, all treatments clearly cause in gluten proteins a gradual loss in solubility associated with changes in functional properties (higher firmness and elastic recovery) that are likely to result from



structural modifications. The different fractions do not have the same sensitivity to heat treatments : gliadins (especially fractions $\omega$ ) are much less affected than glutenins whose thermal aggregation seems to involve the formation of new disulfide bonds.

## BREAD-MAKING OR COOKIE-MAKING

In a regular bread-making process, after mixing and fermentation steps, the dough is placed into an oven at about 220°C. A steep temperature gradient, 200°C $\longrightarrow$ 100°C, inward from the crust is established in the dough piece. Because bread is a moist product, its temperature cannot raise much above the boiling point of water unless it becomes completely dry. The only part of bread dried during baking is the crust, that can reach a temperature of 200°C, while the crumb does not reach a temperature greater than 100°C.

According to Hoseney (1986), the differences in characteristics and in size of the final baked product are determined by interactions that occur during heating. However, heat phenomena during the baking step are extremely complicated. The major change that takes place during the oven process is the redistribution of water from the gluten phase to the starch phase, thereby allowing the starch to undergo gelatinization. Gluten coagulation sets in at about 74°C and continues slowly until the end of baking. In the course of this process, the gluten matrix surrounding the individual gas cells is transformed into a semi-rigid film structure which becomes thinner as the gas cells expand and may rupture but not collapse.

In recent studies, attention has been focussed on solubility and conformational changes in both gliadin and glutenin proteins of bread. Meier et al (1985) and Menkovska et al (1987), using RP-HPLC and A-PAGE demonstrated that, in contrast to the apparent absence of modification of the gliadin fraction during mixing of fermenta-

tion step (as evidenced by absence of changes in electrophoretic patterns), interactions or degradations of gliadin proteins essentially occurred during baking. For instance, when comparing an extract of the crumb to a flour extract, the A-PAGE gliadin bands with low relative mobility (RM $<$ 40) were more intensely stained and those with RM $>$ 40 were less intensely stained, while the intensity of all gliadin bands in the crust was dramatically reduced. Menkovska et al (1987) also demonstrated that the highly hydrophobic gliadins (RP-HPLC elution times $>$ 23 min) extracted from crumb were more heat labile (and probably interacted more with other flour components) than the less hydrophobic gliadins (elution times $<$ 23 min).

When comparing flours that varied in breadmaking potential, Menkovska et al (1988) found that the change from flour to bread crumb was more pronounced in good breadmaking flours than in poor breadmaking flours (i.e. the relative decrease from flour to crumb in gliadins of high mobility was much greater in the good to intermediate breadmaking flours than in the poor-breadmaking flours). From RP-HPLC elution curves, the change in intensity of the peaks was not equal for various groups of gliadins and for various flours and the extent of reduction of peak intensity was also much higher in good- than in poor-breadmaking quality flours (Fig. 3), thus indicating differences in interactions or heat lability. Menkovska et al (1988) postulated that heat-labile (and highly hydrophobic) $\alpha$ -, $\beta$ -, and $\gamma$ -gliadins were modified during baking and that the modification may be related, in part at least, to differences in breadmaking potential of flours.

All these results agree with those of Wrigley et al (1980) who studied the effect of baking on heat denaturation by gradient-PAGE of gliadins and those of several authors who devised methods for detecting gluten proteins in heated foods



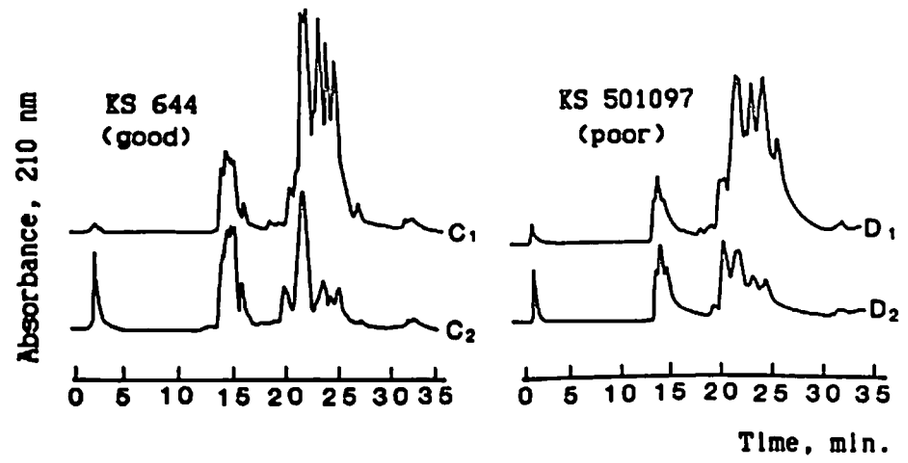


Fig. 3. Reversed-phase chromatography of gliadin proteins from flours (1) and corresponding bread crumbs (2) of lines KS 644 (C) and KS 501097 (D) (Reprinted, by permission, from Menkovska et al 1988).

and baked goods (McCausland and Wrigley 1976; Wrigley 1977; Dysseler et al 1986). A complete gliadin pattern was obtained for the crumb of experimentally pan-baked bread, but the components of higher mobility did not stain as strongly as for flour. In contrast, only gliadins of low mobility were extracted with 2M urea from the crumb of hearth baked bread. On the other hand, less gliadin was extractable from bread crust, where protein denaturation due to heating was much greater than in the crumb. After extreme treatments only a few zones of very low mobility were noted. These probably represent polymerization products due to heating (McCausland and Wrigley 1976). The addition to 2M urea or of a 0.2 % reducing agent, $\beta$-mercaptoethanol, greatly increa-

sed the intensity of crumb protein staining in the gliadin mobility range. It was concluded that the reducing agent probably exerts this effect both by aiding the extraction of the gliadin proteins themselves and by reducing the S-S crosslinked glutenin polypeptides, thereby making them extractable also.

A comparison between the changes in gliadin proteins resulting from bread-making and cookie-making has been also carried out by Pomeranz et al (1987) using A-PAGE and RP-HPLC fractionations. It was observed that both in bread and in cookies, there was a reduction (from flour to baked product) in the intensity and resolution of highly hydrophobic (long elution time) HPLC peaks and that the decrease in resolution seemed to be more pronounced in good than in poor cookie-making flours. A possible involvement (probably through interaction with proteins or with other components) of gliadins in production of satisfactory products was proposed. A reduction in intensity and resolution of HPLC short elution time peaks (from flour to baked products) in cookies was also noted. These changes seemed to be primarily governed by the effect of high temperatures exerted during the baking of cookies and might be also related to functional properties of the flours.

## HIGH-TEMPERATURE PASTA DRYING AND PASTA COOKING

Temperature is an important parameter in pasta technology. Firstly, because drying operations are more frequently performed above 70° or 90°C and, secondly, because during cooking, pasta is left in boiling water for about 10 minutes (depending on shape). Pasta making, however, differs from bread-making : if dough development is defined as formation of a continuous network of protein sheets and fibrils, then at the dough-water content of pasta goods, no full gluten development takes place (Dexter and Matsuo 1979).



Such an absence of full gluten development would explain why differences in cooking quality between samples of semolina may be essentially accounted for by the manner in which the proteins are modified during the drying or the cooking step.

### High-temperature pasta drying

Recent pasta technologies using a drying step at 70° or 90°C may have a strong effect on quality of cooked pasta, which involves improvement of rheological characteristics and surface conditions (Dexter et al 1981; Feillet 1987).

While most of the information concerns the influence of temperature on lysine availability of pasta products (Cubadda et al 1968; Dexter et al 1984), only few reports relate to modifications of pasta proteins.

Drying spaghetti at 80°C extensively denaturates proteins, as demonstrated by solubility in dilute acetic acid (Dexter et al 1981; Ibrahim and McDonald 1981) and changes in gel filtration elution profiles. Increasing duration (from 1 to 9 hr) and temperature (from 70° to 90°C) of drying decreases proteins solubility in $MgSO_4$ and 0.1 % acetic acid (Resmini et al 1976). Additional information gained from microscopy studies showed that high temperatures contribute to the formation of a protein network and result in better pasta performance during cooking (Resmini et al 1976).

In other studies of heat treatment on pasta processed from both type $\gamma$-gliadin 45 and type $\gamma$-gliadin 42 durum varieties, Autran and Berrier (1984) confirmed the losses in ethanol or acetic acid solubility but also reported a weakening of gliadin bands in A-PAGE patterns compared to pasta dried at lower temperatures. However, this phenomenon essentially concerned the fast-moving components, while the slow-moving $\omega$-gliadin bands remained present, what allowed to propose a method for detecting bread wheat flour in heat-treated pasta products based on quantitation

of ω -gliadin group from A-PAGE electrophoresis (Kobrehel et al 1985).

-In a recent study, Feillet (1987) and Feillet et al (1987) investigated the effects of moisture content, and duration and temperature of pasta drying. For instance, submitting pasta (30 % moisture content) to heat treatment for 120 minutes led to a steep decrease of solubility in SDS, the initial solubility being restored only by further extraction with mercaptoethanol (Fig. 4); the changes could be explained by formation of disulfide bonds between pasta proteins during heat treatment. In another experiment, pasta samples with 24 %, 18 % and 12 % moisture contents were left for 2 hours at 90°C. The stronger the hydrothermic treatment (i.e. the pasta humidity), the larger was the loss of solubility

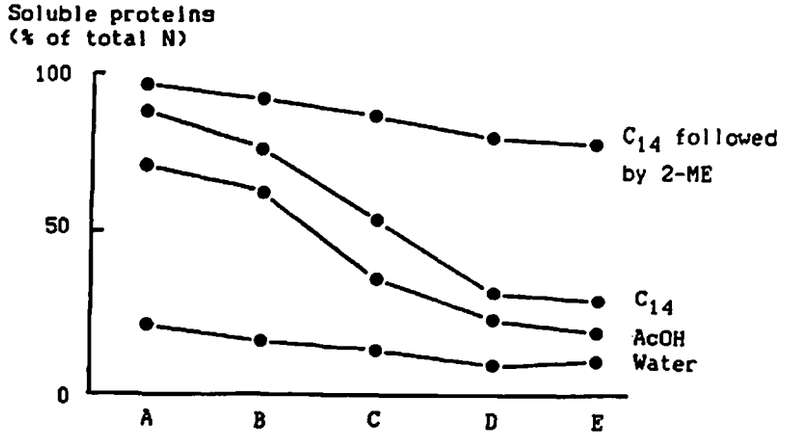


Fig. 4. Effect of heat treatment (2 hrs at 90°C) on pasta protein solubility. A-Semolina; B-Pasta dried at 55°C; Pasta left for 2 hrs at 90°C at 13 % (C), 18 % (D), and 24 % (E) moisture content. AcOH : Acetic acid; C14 : Sodium myristate; ME : 2-mercaptoethanol (Reprinted, by permission, from Feillet 1987).



in sodium myristate, which disrupts hydrophobic bonds. In addition, most residual proteins were soluble in β -mercaptoethanol.

A-PAGE fractionations of unreduced protein extracts revealed which proteins aggregate during heat treatment. ω -Gliadins, which have a very low sulfur content, are highly heat-resistant. Streaks and slot-proteins (which essentially consist of proteins with molecular weights from 35,000 to 50,000 daltons, i.e. in the range of major LMW subunits of glutenin) rapidly disappear from electrophoretic patterns upon gradually increased heat treatments (Feillet et al 1987).

The heat sensitivity of LMW glutenins was confirmed by SE-HPLC of SDS-phosphate (unreduced) extracts. All protein peaks decreased when the intensity of heat treatment was increased, but the phenomenon especially affected peaks 1 and 2 (which essentially consist of LMW glutenin aggregates), which rapidly disappeared from the elution curves (Fig. 5).

Since these phenomena occur in a similar way in both types of wheats, another question was why poor varieties can be improved through such treatments, especially as far as surface condition (absence of stickiness and surface deterioration) is concerned. It is now accepted (Resmini and Pagani 1983; Feillet 1984) that during cooking in boiling water there is a competition between (1) protein coagulation into a continuous network and (2) starch swelling. If (1) prevails, starch particles are trapped in a continuous network, promoting high firmness and little stickiness of cooked pasta, while, if (2) prevails, proteins coagulate in discrete regions lacking a continuous network, giving soft and sticky pasta. High temperature pasta drying might partially overcome this competition by producing a coagulated protein network in dry pasta without starch swelling (Feillet 1984).

It is generally believed that gluten is not fully developed when pasta are prepared from doughs containing 25-35 % water. Still, in poten-

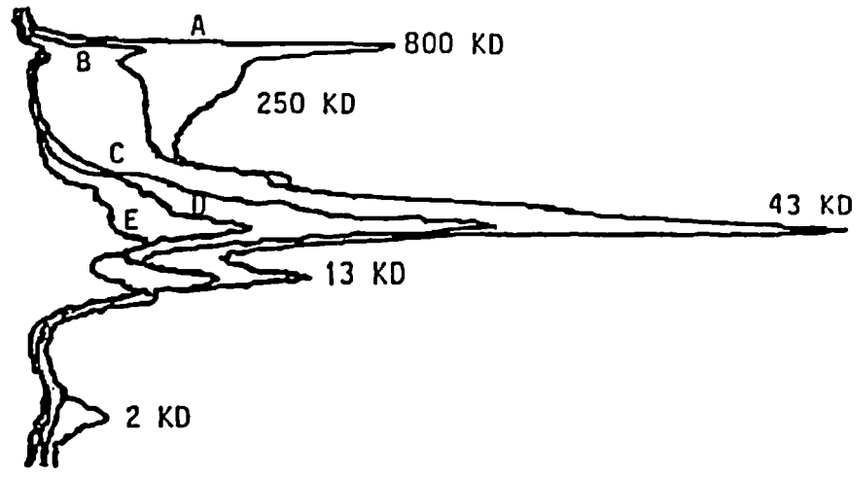


Fig. 5 Size exclusion HPLC chromatography of SDS-phosphate (unreduced extracts). A - Semolina; B - Pasta dried at 55°C; Pasta left for 2 hrs at 90°C at 13 % (C), 18 % (D), and 24 % (E) moisture content, respectively (Reprinted, by permission, from Feillet 1987).

tially strong varieties (type $\gamma$ -45), a viscoelastic network might be already formed at the mixing or extrusion stage (before any heat treatment) due to pronounced functional properties (or more probably due to the very high proportion of LMW subunits of glutenin in the varieties belonging to type $\gamma$ -45 (Autran et al 1987)). In contrast, poor varieties (type $\gamma$ -42) have a lower quality potential and lack a viscoelastic network, because of the reduced tendency to aggregate (or the small amount) of their LMW proteins. To affect an improved firm structure, these varieties require a heat treatment, which may cause a rapid aggregation of their LMW (and HMW) glutenin subunits.

However, the proteins having a high tendency to coagulate upon heat treatment and to promote the retention of a good surface condition of



pasta during cooking, may not be necessarily those that contribute to the formation of a viscoelastic network during mixing and extrusion. A new hypothesis has been proposed by Feillet et al (1988), following the discovery by Kobrehel and Alary (1988) of a new protein fraction called DSG (durum wheat sulfur-rich glutenin). SH + S-S content of DSG proteins and surface condition of cooked pasta were significantly correlated and DSG proteins were not extractable by sodium myristate from heat-treated pasta, but were extractable by mercaptoethanol. Consequently, Feillet et al (1988) proposed a functional role of DSG in preventing disaggregation of the surface of cooked pasta. They postulated that DSG proteins contribute to aggregation of LMW glutenins (and possibly of HMW glutenins) through hydrophobic and disulfide bonds and that these bonds are sufficiently strong to prevent starch leaching during pasta cooking and to maintain a satisfactory surface condition of cooked pasta.

## Pasta cooking

The effect of cooking on protein solubility of pasta was thoroughly examined by Wasik (1978) who found that cooking decreased the amount of soluble proteins recovered by the Osborne procedure ; 10-15 % of the total recovered protein was soluble in water, saline, alcohol and acid solvents, in contrast to about 65-75 % from the uncooked product. Cooking reduced the solubility in AUC solvent from 95 % to 50 % and radically modified Sephadex G200 gel filtration profiles. Nevertheless, no change was observed in the SDS-PAGE patterns of proteins after reduction by mercaptoethanol.

According to Dexter and Matsuo (1979), protein extractability in dilute acetic acid rapidly decreased during spaghetti cooking up to about 12 min. At each cooking time examined, the poorer quality wheats exhibited significantly greater

protein extractability than the better ones. This was explained by the greater proportion of extractable gluten protein in the poor-quality wheats. However, gel filtration elution profiles of acetic acid extracts at each cooking time revealed no significant quantitative differences in the pattern of protein denaturation.

In another study, spaghetti processed from several durum wheat varieties of a wide range of cooking quality were cooked in boiling water, frozen and freeze-dried, and examined for protein solubility and electrophoretic patterns by Autran and Berrier (1984). Upon cooking, a gradual loss of solubility in ethanol of several gliadin bands was observed. They included firstly some γ-gliadins (A-PAGE mobilities 40-51), then some β- and α-gliadins, whilst the whole slow moving group (ω-gliadins 20-23-26 and 33-35- 38) remained obviously visible ; they dominate the patterns (and are even enhanced) after 10 min of pasta cooking. Also, streaks in the gliadin patterns disappear after only 1-2 min of cooking (Fig. 6).

A similar behaviour was observed for both good (Bidi 17) and poor (Tomclair) cooking quality cultivars. In particular, γ-gliadin 45 seemed to be insolubilized according to the same kinetics than γ-gliadin 42. It was especially interesting, however, to note that the partition between components that are resistant or susceptible to heat denaturation occurs within a genetic linkage group (where recombinations are usually not observed). The bands 40-42, or 45 are typical γ-gliadins and aggregate upon heat treatment; on the other hand the bands 33-35-38, or 35, are typical ω-gliadins and behave as highly resistant proteins. It is not clear how components coded by a cluster of closely linked genes, that presumably derive from an ancestor gene through point mutations, can differ so widely in functional properties and behaviour upon heat treatments.



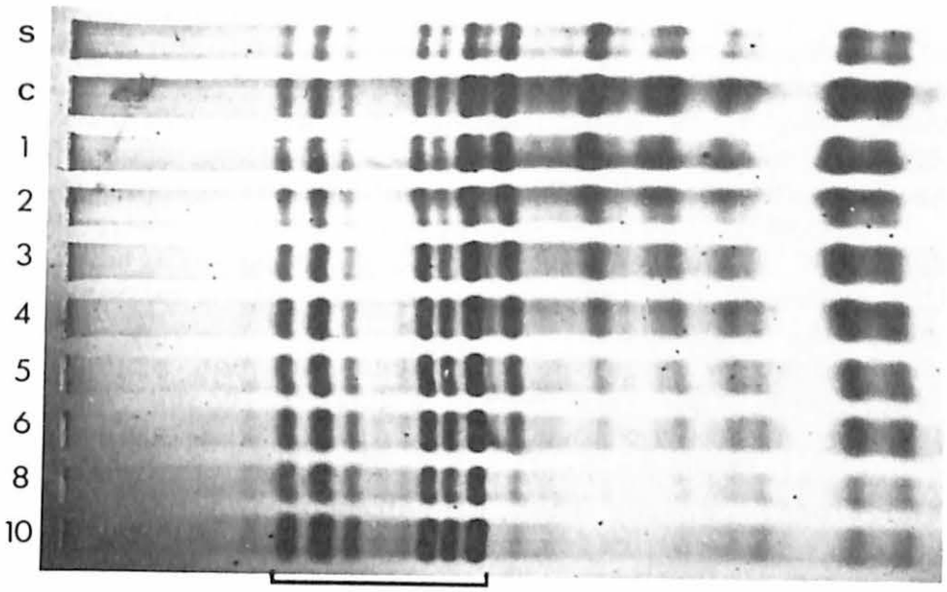


Fig. 6. A-PAGE of gliadins extracted from (S) semolina, (C) control uncooked spaghetti and spaghetti cooked 1, 2, 3, 4, 5, 6, 8 and 10 min, respectively (from Autran, unpublished results).

## INDUSTRIAL VITAL GLUTEN PRODUCTION AND DRYING

The use of gluten by the Western European milling industry to replace strong imported wheats in the production of flours for conventional breadmaking and especially for other specialty breads has increased rapidly in recent years. It is essential that the gluten be vital, e.g., that it retains the desirable viscoelastic properties required for gas retention (Stenvert et al 1981; McDermott 1985). However, gluten is obtained through wet processes and then dried in driers operating at elevated (50-75°C) temperatures. The commercial quality of these glutens is extremely variable. According to Wu and Inglett

(1974), Schofield et al (1983) and Schofield (1986), the degree of heat denaturation during drying is generally the main source of variation. Although little is known about the exact effect of drying conditions (freeze, drum, or spray drying) on the functional and biochemical properties of vital gluten, excessively high drying temperatures are thought to be a major factor in the reduced and variable baking performances of commercial glutens.

As laboratory heating of gluten may not exactly represent the commercial process, Booth et al (1980), Schofield and Booth (1983) and Schofield et al (1983) have studied the chemical and functional changes that gluten proteins undergo as a result of different conditions employed during industrial drying. They showed that extractibility in SDS buffer decreased and that glutenins of highest molecular weight (in SE-chromatography) were affected predominantly. Gliadins were also affected but at higher temperatures : $\alpha$ -, $\beta$ -, and $\gamma$ -gliadin fractions were made progressively inextractable, unlike $\omega$ -gliadins that were essentially unaffected.

Baking performance, assessed in reconstituted flours, declined progressively between 50 and 70°C and most of the functionality was lost by 75°C (Schofield et al 1983). When a gluten dough was heated to 80°C, a large increase in mixing time and a reduction in loaf volume resulted. Also, after steaming, the gluten was both more difficult to deform and relatively more elastic (Dreese et al 1988).

The fact that the functionality of heat-denaturated gluten can be restored, at least partially, by dough mixing in the presence of a reducing agent (Schofield et al 1984) tends to support the involvement of S-S bonds in the denaturation process. However, Schofield et al (1983) concluded that the level of total SH groups remained constant up to 100°C and that there was a transfer of SH groups from an SDS-extractable to an SDS-inextractable form (Fig. 7). Accordingly, gluten drying is causing the polymer system to become effectively more cross- linked, but without any decrease of the total SH groups. Thermal agitation, which allows to explore all possible conformations, may therefore promote the formation of new bonds through SH/S-S interchange and to form a more highly polymerized state with less free energy and more stability.

Similar conclusions were drawn by Hansen et al (1975) and, recently by Davies et al (1987). They suggested that heat setting of proteins results from an increase in branching from chain interactions through difulfide bond formation; they explained the increase in breaking stress by postulating the formation of new protein cross-links between cystein residues exposed by temper-

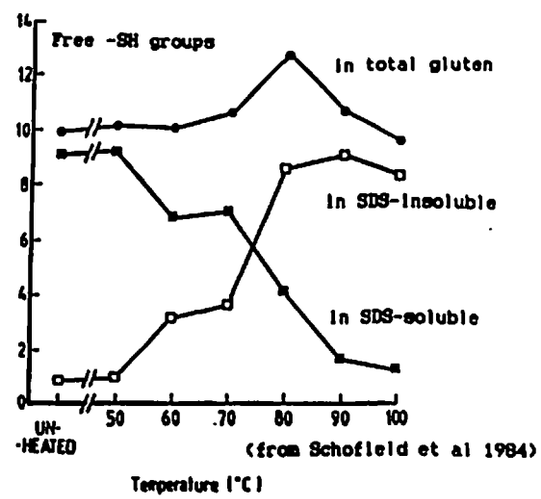


Fig. 7. Effect of heat on free sulfhydryl groups in SDS extractable ( ■——■ ) and SDS-inextractable ( □—□) fractions of gluten and in the total gluten protein ( ●——● ) (Reprinted, by permission, from Schofield et al 1984).

ature-induced unfolding of the proteins. The authors proposed, however that differences in tensile properties among varieties are determined primarily by the number and reactivity (accessibility) of cystine residues.

Additional, alternative, mechanisms have been reported in an attempt to describe the phenomena involved in heat modification of functional properties. For instance, Dreese et al (1988) compared rheological characteristics of laboratory hand-washed and lyophilized gluten and commercial gluten and concluded that the washing effect was more important than the drying effect. Also, Eliasson and Hegg (1980), studying heated gluten by differential scanning calorimetry (DSC), recorded a major thermal transition in the starch component, rather than in the protein fraction, probably because the method was not adapted to follow protein modifications. Further experiments of Schofield et al (1984), who demonstrated that inclusion of fungal $\alpha$ -amylase did not protect the gluten against loss of baking performance on heating up to 70°C, suggested that the effect on gluten functionality in this temperature range was due to changes in the protein component and not in the starch component.

In summary, as succinctly reviewed by Schofield (1986), all the available data suggest that the loss of functionality in heated gluten is likely to occur through heat-induced unfolding of glutenin polypeptides that facilitates SH/S-S interchange between exposed S-S and SH groups in adjacent molecules. This results in a tougher, harsher, more elastic gluten, whose baking performance is decreased. It was demonstrated that the glutenin fraction is more susceptible to heat denaturation than the gliadin fraction.

## CONCLUDING REMARKS

The functional properties (elasticity, extensibility, viscosity) of most of the wheat products



are largely determined by the gluten protein viscoelastic behaviour ; gliadins contribute to viscosity and extensibility of gluten, while glutenins impart elasticity. Although a number of models have been proposed to account for those properties, their precise molecular basis is still not known. Nevertheless, it is well established that, among gluten proteins, gliadins are monomers that aggregate by hydrogen bonding and hydrophobic interactions, while glutenins are part of very high molecular size complexes that are additionally stabilized by interpolypeptide disulfide bonds.

Obviously, the balance between fractions that aggregate by hydrogen bonding, hydrophobic interactions, or by formation of new disulfide bonds is critical in determining satisfactory dough strength and loaf volume and this balance is dramatically changed upon heat treatment. The changes that result from heat treatment may be much higher than the regular differences among varieties. A possible question, therefore, is why the higher levels of inextractable protein in heat treated gluten do not confer better quality on the samples since a higher content in insoluble or residual protein is generally associated with a higher baking quality. The obvious answer is that the physico-chemical basis of protein insolubility is different in both cases. Still, it is necessary to elucidate which bonds are involved in those differences. According to Booth et al (1980), either inextractable proteins differ in character from residue protein found naturally in wheat flours - a strong positive correlation between level of residue protein and baking quality has been reported by Orth and Bushuk (1972) - or that they are similar in character but present in excess of the level required for baking performance thus resulting in a tough gluten.

Regardless of the type of heat treatment and of the methods used for monitoring denaturation,

several general conclusions can be drawn.

1 - Thermal agitation causes a native folded structure to uncoil or unwind into a randomly looped chain causing different chemical groups to react or to interact (sulfhydryl into disulfide bonds, disulfide interchange reactions, non polar groups brought together giving rise to hydrophobic interactions) and to form aggregate or network structures that can be at least partly disrupted by reducing agents or detergents. The most visible effect of heat denaturation of gluten is therefore a decrease in protein solubility and a weakening or a disappearance of certain components from unreduced electrophoretic patterns, associated with changes in functional properties (higher firmness and elastic recovery) that are likely to result from structural modifications.

2 - The different gluten fractions or subunits do not have the same tendency to interact or to cross-link through thermal denaturation. Gluteninsare extremely sensitive and strongly aggregate upon even mild heat treatments, while the most hydrophobic $\alpha$ -, $\beta$ -, and $\gamma$ -gliadins also become insoluble but in a more gradual way. The solubility of most fractions can be generally restored upon the use of reducing agents, indicating the occurence of new S-S bonds (possibly through SH/S-S interchange) in heat-treated pasta products.

3 - Sulfur-free (and less hydrophobic) $\omega$ -gliadins, the structure of which is more a random coil type, have a very low chemical reativity and therefore a very high resistance to heat denaturation. Extra uncoiling is however a possible reason why they can bind more dye and give more intense bands.

4 - Varietal differences have been observed in the overall decrease in solubility. Among bread wheats or durum wheats, the tendency of proteins to aggregate is greater in good- than in poor-breadmaking flours or -pastamaking semolinas. This indicates that the susceptibility of proteins



to heat modification may explain some differences in quality potential of flours. Among durum wheats, LMW glutenins (whose relative amount makes a major difference between the two quality classes of durum wheats) could be more directly involved in varietal differences in cooking quality than $\gamma$ -gliadin markers 42 and 45, or HMW glutenins.

5 - Wheat proteins make an extremely complex model since proteins are synthesized in a wet environment that becomes drier upon grain development; they are hydrated again upon mixing and then modified upon drying, baking or cooking. The manner in which the structure can change during various heat denaturation treatments (flexibility, loosening of the overall conformation) could provide a powerful dynamic approach for investigating the physico-chemical basis of technological quality and for understanding the basis of functional properties in food processing.

6 - Many aspects are still elusive and more sophisticated physico-chemical methods are now required. Since most of the biochemical work in model systems has focussed on gliadin proteins, future investigations should concern glutenins which are the ones primarily involved in heat aggregation phenomena.

## LITERATURE CITED

ANNO, T. 1981. Studies on heat-induced aggregation of wheat gluten. J. Jpn Soc. Food Nutr. 34: 127-132.

AUTRAN J.C., and BERRIER, R. 1984. Durum wheat functional subunits revealed through heat treatments. Biochemical and Genetic implications. In : Gluten Proteins : Proc. 2nd Int. Symp. Workshop on Gluten Proteins, Wageningen The Netherlands, A. Graveland A. and J.H.E. Moonen, eds. : 175-183.

AUTRAN, J.C. HOULIAROPOULOS, E., and LAIGNELET, B., 1982. Viscoelastic properties of bread wheat gluten : measurements, use in breeding and biochemical basis. Cereal Foods World 27 :

469.
AUTRAN, J.C., LAIGNELET, B., and MOREL, M.H. 1987. Characterization and quantification of low-molecular-weight glutenins in durum wheats. Biochimie 69 : 699-711.
BOOTH, M.R., BOTTOMLEY, R.C., ELLIS, J.R.S., MALLOCH, G., SCHOFIELD, J.D., and TIMMS, M.F. 1980. The effect of heat on gluten physico-chemical properties and baking quality. Ann. Technol. Agric. 29 : 399-408.
CUBADDA, R., FABRIANI, G., and RESMINI, P. 1968. Variazioni della lisina utilizzabile nelle paste alimentari indotte dai processi technologici d'essicamento. Quad. Nutr. 28 : 199-208.
DAVIES, A.P. 1986. Protein functionality in bakery products. In : Chemistry and Physics of Baking, Special Publication No. 56, The Royal Society of Chemistry, Burlington House, London; J.M.V. Blanshard, P.J. Frazier and T. Galliard, eds.: 89-104.
DAVIES, A.P., PATIENT, D.W., INGMAN, S.J., ABLETT, S., DRAGE, M., ASQUITH, M., and BARNES, D.J. 1987. Wheat protein properties and puff pastry structure. In : Proc. 3rd International Workshop on Gluten Proteins, Budapest, Hungary, R. Lasztity and F. Bekes, eds. World Scientific Singapore : 466-477.
DEXTER, J.E., and MATSUO, R.R. 1979. Changes in spaghetti protein solubility during cooking. Cereal Chem. 56 : 394-398.
DEXTER, J.E., MATSUO, R.R., and MORGAN, B.C. 1981. High temperature drying : effect on spaghetti properties. J. Food Sci. 46 : 1741-1756.
DEXTER, J.E., TKACHUK, R., and MATSUO, R.R. 1984. Amino acid composition of spaghetti : effect of drying conditions on total and available lysine. J. Food Sci. 49 : 225-228.
DREESE, P.C., FAUBION, J.M., and HOSENEY, R.C. 1988. The effect of different heating and washing procedures on the dynamic rheological properties of wheat gluten. Cereal Foods World, 33 : 225-228.



DYSSELER, P., JACQUAIN, D., and KARYDAS, J. 1986. Détermination électrophorétique des gliadines dans les produits alimentaires cuits. Belgian J. Food Chem. Biotechnol., 41 : 143-149.
ELIASSON, A.C., and HEGG, P. O. 1980. Thermal stability of wheat gluten. Cereal Chem. 57: 436-437.
FEILLET, P. 1984. The biochemical basis of pasta cooking quality. Its consequences for durum wheat breeders. Sciences des Aliments 4 : 551-566.
FEILLET, P. 1987. Séchage à haute et très haute température des pâtes alimentaires: rapport de synthèse. Institut National de la recherche Agronomique, Ministère de l'Agriculture, 269 p.
FEILLET, P., AUTRAN, J.C., and AIT MOUH, O. 1987. Role of low molecular weight glutenin in determining cooking quality of pasta products. Cereal Foods World 32 : 670.
FEILLET, P., AIT MOUH, O., KOBREHEL, K., and AUTRAN, J.C. 1988. Role of low molecular weight glutenins in determining cooking quality of pasta products : an overview. Cereal Chem. (in press).
HANSEN, L.P., JOHNSTON, P.H., and FERREL, R.E. 1975. Heat-moisture effect on wheat flour. I. Physical-chemical changes of flour proteins resulting from thermal processing. Cereal Chem. 52 : 459-472.
HOSENEY, R.C. 1986. Component interaction during heating and storage of baked products. In: Chemistry and Physics of Baking, Special Publication No. 56, The Royal Society of Chemistry, Burlington House, London. J.M.V. Blanshard, P.J. Frazier and T. Galliard, eds.) : 216-226.
IBRAHIM, R.H., and McDONALD, C.E. 1981. Experimental high-temperature drying of spaghetti Cereal Foods World 26 : 507.
JEANJEAN, M.F., DAMIDAUX, R., and FEILLET, P. 1980. Effect of heat treatment on protein

solubility and viscoelastic properties of wheat gluten. Cereal Chem. 57 : 325-331.
KASARDA, D.D., BERNARDIN, J.E., and GAFFIELD, W. 1968. Circular dichroism and optical rotatory dispersion of α -gliadin. Biochemistry 7 : 3950-3957.
KOBREHEL, K., AGAGA, D., and AUTRAN, J.C. 1985. Possibilité de détection de la présence de blé tendre dans les pâtes alimentaires ayant subi des traitements thermiques à haute température. An. Fals. Exp. Chim. 78 : 109-117.
KOBREHEL, K., and ALARY, R. 1988. The role of a low molecular weight glutenin fraction in the cooking quality of durum wheat pasta. J. Sci. Food Agric. (in press).
McCAUSLAND, J. and WRIGLEY, C.W. 1976. Electrophoretic analysis of wheat and rye mixtures in meal, flour and baked goods. J. Sci. Food Agric. 27 : 1197-1202.
McDERMOTT, E.E. 1985. The properties of commercial glutens. Cereal Foods World 30 : 169-171.
MECHAM, D.K., and OLCOTT, H.S. 1947. Effect of dry heat on proteins. Ind. Eng. Chem. 39: 1023-1027.
MEIER, P., WINDEMANN, H., and BAUMGARTNER, E. 1985. Auftrennung von Gesamtgliadin aus unterschiedlich hitzebelasteten Weizenmehlen mittels Phasenumkehr-Hochdruckflüssig-Chromatographie. Z. Lebensm. Unters. Forsch. 180 : 467-473.
MENKOVSKA, M., LOOKHART, G.L., and POMERANZ, Y. 1987. Changes in the gliadin fraction(s) during breadmaking : isolation and characterization by high-performance liquid chromatography and polyacrylamide gel electrophoresis. Cereal Chem. 64 : 311-314.
MENKOVSKA, M., POMERANZ, Y., LOOCKHART, G.L., and SHOGREN M.D. 1988. Gliadin in crumb of bread from high-protein wheat flours of varied breadmaking potential. Cereal Chem. 65 : 198-201.
NEUCERE, J.N., and CHERRY, J.P. 1982. Structural changes and metabolism of proteins following



heat denaturation. In : Food Protein Deterioration, ACS Symp. Series No. 206, J.P. Cherry ed. American Chemical Society, Washington, 135-162.
ORTH, R.A., and BUSHUK, W. 1972. A comparative study of the proteins of wheats of diverse baking qualities. Cereal Chem. 49 : 268-275.
PENCE, J.W., MOHAMMAD, A., and MECHAM, D.K. 1953. Heat denaturation of gluten. Cereal Chem. 30 : 115-126.
POMERANZ, Y., LOOKHART, G.L., RUBENTHALER, G.L., and ALBERTS, L.A. 1987. Changes in gliadin proteins during cookie making. Cereal Foods World, 32 : 670.
RESMINI, P. and PAGANI, M.A. 1983. Ultrastructure of pasta. Food Microstructure, 2 : 1-12.
RESMINI, P., De BERNARDI, G., and MAZZOLINI, C. 1976. Influenza delle condizioni di essicazione su alcune caratteristiche della pasta alimentare. Bull. Lab. Chim. Prov. 27 : 283-293.
SADOUKI, H., and AUTRAN, J.C. 1987. Mise en évidence du rôle de certaines gluténines de haut poids moléculaire dans la qualité boulangère des blés tendres en Algérie. Lebensm.-Wiss. u.-Technol., 20 : 180-190.
SCHOFIELD, J.D. 1986. Flour proteins : structure and functionality in baked products. In : Chemistry and Physics of Baking, Special Publication No. 56, The Royal Society of Chemistry, Burlington House, London, J.M.V. Blanshard, P.J. Frazier and T. Galliard, eds.) 14-29.
SCHOFIELD, J.D., and BOOTH, M.R. 1983. Wheat proteins and their technological significance In : Developments in Food Proteins, Vol. 2, Applied Science Publishers, London, B.J.F. Hudson, ed. : 1-65.
SCHOFIELD, J.D., BOTTOMLEY, R.C., TIMMS, M.F., and BOOTH, M.R. 1983. The effect of heat on wheat gluten and the involvement of sulphydryl-disulfide interchange reactions. J. Cereal Sci. 1 : 241-253.
SCHOFIELD, J.D., BOTTOMLEY, R.C., LEGRYS, G.A. TIMMS, M.F., and BOOTH, M.R. 1984. Effect

of heat on wheat gluten. In : Gluten Proteins, Proc. 2nd Int. Workshop on Gluten Proteins, Wageningen, The Netherlands. A. Graveland and J.H.E. Moonen, eds. TNO, The Netherlands: 81-90.

STENVERT, N.L., MOSS, R., and MURRAY, L. 1981. The role of dry vital gluten in breadmaking. Bakers Digest 55 (2) : 6-12.

TATHAM, A.S., and SHEWRY, P.R. 1985. The conformations of wheat gluten proteins. I. The secondary structures and thermal stabilities of α-, β -, γ - and ω-gliadins. J. Cereal Sci. 3 : 103-113.

TATHAM, A.S., FIELD, J.M., SMITH, S.J., and SHEWRY P.R. 1987. The conformations of wheat gluten proteins. II. Aggregated gliadins and low molecular weight subunits of glutenin. J. Cereal Sci. 5 : 203-214.

VOUTSINAS, L.P., NAKAI, S., and HARWALKAR, V.R. 1983. Relationships between protein hydrophobicity and thermal functional properties of food proteins. Can. Inst. Food Sci. Technol. J. 16 : 185-190.

WALL, J.S., and HUEBNER, F.R. 1981. Adhesion and cohesion. In : Protein Functionality in Foods, ACS Symp. Series No. 147, American Chemical Society, Washington. J.P. Cherry, ed. : 111-129.

WASIK, R.J. 1978. Relationship of protein composition of durum wheat with pasta quality and the effects of processing and cooking on these proteins. Can. Inst. Food Sci. Technol. J. 11 : 129-133.

WRIGLEY, C.W. 1977. Characterisation and analysis of cereal products in foods by protein electrophoresis. Food Technology in Australia 29: 17-20.

WRIGLEY, C.W., Du CROS, D.L., ARCHER, M.J., DOWNIE, P.G., and ROXBURGH, C.M. 1980. The sulfur content of wheat endosperm proteins and its relevance to grain quality. Aust. J. Plant Physiol. 7 : 755-766.



WU, Y.V., and INGLETT, G.E. 1974. Denaturation of plant proteins related to functionality and food applications. A review. J. Food Sci. 39 : 218-225.