(SLIDE 1)

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

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(SLIDE 2) Most of cereal food processes are characterized by heat treatments under a range of temperature of 55°C to 200°C and final humidity of 5 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 quality characteristics of end-products.

On the other hand, gluten proteins play a predominant role in most of cereal foods, at several stages occuring between raw materials and end-product. But 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.

(SLIDE 3) THEORETICAL BASIS OF HEAT DENATURATION OF PROTEINS

It is well known that exposing proteins to high temperatures causes most of them to undergo conformational changes, whose the most visible effect is a decrease in solubility. Formation of an insoluble white coagulum when egg white is boiled is a common example of protein denaturation, but as much significant is certainly the loss of biological activity of enzymes.

Since no covalent bonds of the backbone of the polypeptide chain are broken during a 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 can permit new functional group associations and sequentially alters polypeptides to aggregates and finally to insoluble components.

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For instance, in gluten proteins (SLIDE 4), where the structure is mainly stabilized through S-S bonds and hydrophobic interactions, the apolar residues move towards the outside, making more difficult the contact of the molecule with water (the protein becomes insoluble in aqueous media), determining aggregates strengthened through hydrophobic interactions, and also facilitating SH/S-S interchanges and giving rise to new bonds and to a more highly aggregated structural state.

In some cases, the change may be reversible. However, if the unfolded conformation has less free energy (i.e. is more stable) and if activation-energy barrier is high (SLIDE 5), the polypeptide may be locked into the denaturated (aggregated) conformation. On the other hand, dry proteins are much more resistant to heat denaturation than proteins in solution.

(SLIDE 6) IMPORTANCE OF THERMAL DENATURATION IN CEREAL PRODUCTS

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

Protein denaturation is usually viewed in a negative sense by food-protein chemists because native proteins have often superior functional properties (such as solubility, emulsifying or foaming) compared to denaturated proteins. However, heat 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 texture of bread. It also allows selective thermal inactivation of certain undesirable components (amylases, yeast enzymes, lipases, lipoxygenases, peroxydases).

Let us turn now to a short review of the major effects of thermal processing on wheat proteins and to a discussion of the physico-chemical changes they undergo, with four different examples of heat treatments (SLIDE 7):

- Model systems: hand-washed gluten or purified gliadin fractions.

- Bread-baking and cookie-making.
- High-temperature pasta drying and pasta cooking.
- Industrial vital gluten production and drying.

I - MODEL SYSTEMS: HAND-WASHED GLUTEN OR PURIFIED GLIADIN FRACTIONS

At first, heat treatments have been applied to hand-washed gluten, or to purified gliadin fractions. For instance, (SLIDE 8) Jeanjean and coworkers, extracted gluten from several common wheat and durum wheat cultivars, and measured its viscoelasticity and protein solubility after gradual treatments of up to 7 min. in boiling water. On heating, gluten compressibility decreased, gluten firmness and elasticity increased (SLIDE 9), and some proteins, soluble in 60 % ethanol were insolubilized (SLIDE 10). Moreover, in wheats with better baking quality or better pasta-cooking quality, the tendency of ethanol-soluble proteins to aggregate during heating was greater. Since the aggregates could be further disrupted with mercaptoethanol, it was postulated that protein insolubilization occured through the formation of new bonds, possibly disulfide bonds.

This loss in solubility can be illustrated by SDS-PAGE electrophoresis: the patterns (SLIDE 11) show that heated products essentially consist of enhanced ω-gliadin fractions, while HMW- and LMW-glutenin subunits have rapidly disappeared.

Recently, Menkovska and coworkers and Meier and coworkers confirmed, by RP-HPLC and electrophoresis that different purified gliadin fractions did not have the same sensitivity to heat treatments: gliadins (especially the less hydrophobic fractions ω-), were much less affected than glutenins.

These different behaviours have been explained by Tatham and Shewry, using a physico-chemical approach based on circular dichroism (CD) measurements. For instance, increasing the temperature from 20° to 80° C resulted in a partial loss of the α-helical content (SLIDE 12), confirming earlier reports of Kasarda (1968) on A-gliadin. A different result, however, was obtained on ω-gliadins in which the conformational change consisted of an increase of β-turns. It was concluded that whereas the ω-gliadins are stabilized by strong hydrophobic interactions, the main stabilizing forces in the α-, β-, and γ-gliadins are covalent disulfide bonds and non-covalent hydrogen bonds.

II - BREAD-BAKING OR COOKIE-MAKING

In a regular bread-making process, the crust can reach a temperature of 200°C, while the crumb does not reach a temperature greater than 100°C. As soon as 1976, Wrigley and coworkers studied the effect of baking on heat denaturation by PAGE electrophoresis and were able to detect gluten in heated foods and baked goods through an identification of the extremely heat resistant ω-gliadins.

(SLIDE 13) In recent studies, attention has been focussed on solubility and conformational changes in gliadin fractions. For instance, Menkovska and coworkers (1987), using RP-HPLC, compared an extract of the flour to crumb and crust extracts. They showed that the intensity of all gliadin peaks in the crust was dramatically reduced, whereas in the crumb, the highly hydrophobic gliadins were more heat labile (and probably interacted more with other flour components) than the less hydrophobic ones.

(SLIDE 14) Menkovska and coworkers (1988) also demonstrated that the relative decrease from flour to crumb in gliadins was much greater in the good to intermediate breadmaking flours than in the poor-breadmaking flours and that this change concerned primarily gliadins of high mobility. It was postulated that α-, β-, and γ-gliadins, that are highly hydrophobic, are the more heat-labile and that the modification may be related, in part at least, to differences in breadmaking potential of flours. Similar results were obtained in 1987 by Pomeranz and coworkers who studied the changes in gliadin proteins resulting from cookie-making.

III - HIGH-TEMPERATURE PASTA DRYING AND PASTA COOKING

Pasta technology is another example, in which temperature has become an important parameter: first, because drying operations are more frequently performed above 70° or 90° C, and, second, because during cooking, pasta is left in boiling water for about 10 minutes.

High-temperature pasta drying

Recent pasta technologies using a high-temperature drying step have a strong effect on quality of cooked pasta, which consists of an improvement of both rheological characteristics and condition of surface of cooked pasta.

Dexter and coworkers and Feillet and coworkers showed that drying spaghetti above 70° C extensively denatures proteins, as shown by a steep decrease in solubility in acetic acid, in SDS or in soap (sodium myristate). (SLIDE 15) For instance, when pasta having 24 %, 18 % and 12 % moisture were left for 2 hours at 90° C, the stronger the hydrothermic treatment (i.e. the pasta humidity), the larger the loss of solubility in sodium myristate, which disrupts hydrophobic bonds. However, the initial solubility was restored by further extraction with mercaptoethanol, what could be explained again by formation of disulfide bonds between pasta proteins during heat treatment.

PAGE fractionations of unreduced pasta protein extracts revealed (SLIDE 16) which proteins aggregate during heat treatment. ω-gliadins, which have a very low sulfur content, are very heat-resistant. Interestingly, streaks and slot-proteins (which essentially consist of LMW subunits of glutenin) rapidly disappear from electrophoretic patterns upon gradually increased heat treatments.

SLIDE 17 gives an illustration of the particular heat sensitivity of LMW glutenins through SE-HPLC curves 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). These peaks rapidly disappeared from the elution curves. LMW-glutenins could be directly involved in varietal differences in gluten viscoelasticity since cultivars belonging to type γ-gliadin 45 have twice as much LMW-glutenins than those with type γ-gliadin 42. However, the proteins having a high tendency to coagulate upon heat treatment and to prevent stickiness during pasta cooking, may not be necessarily those that contribute to the formation of a viscoelastic network during mixing and extrusion steps. So, a new hypothesis (SLIDE 18) has been proposed by Feillet and coworkers, based on a new protein fraction called DSG (durum wheat sulfur-rich glutenin) by Kobrehel and Alary. These proteins may prevent pasta stickiness in contributing to aggregation of LMW glutenins (and possibly of HMW glutenins) through both hydrophobic and disulfide bonds.

Pasta cooking

Similar but more intense denaturating effects were observed upon pasta cooking. The most striking results are the very rapid disappearance of

streaks from A-PAGE patterns and the remarkable heat resistance of ω-gliadin fractions (SLIDE 19). Even after 10 min of cooking, the ω-gliadin bands remain obviously visible (and are enhanced due to a possible extra uncoiling). This is a basis of present investigations intended to accurately determine the amount of bread wheat flour in high-temperature dried (or cooked) pasta.

IV - INDUSTRIAL VITAL GLUTEN PRODUCTION AND DRYING

The use of commercial gluten by the Western European milling industry to replace strong imported wheats in the production of flours for conventional breadmaking and more especially for other speciality breads has become an increasingly important commodity in the EEC and especially in France and in the UK. It is essential that the gluten be vital, that it retains the desirable viscoelastic properties required for gas retention. However, gluten is obtained through wet processes and then dried in driers operating at elevated (50-75° C) temperatures. (Drying is an extremely critical stage in the processing of wheat gluten since, as to be expected for a protein, too high a temperature can be extremely damaging for its functional properties). The commercial quality of these glutens is extremely variable and, according to Schofield, excessively high drying temperatures are a major factor in the reduced and variable baking performances of commercial glutens. Excessive heat produces a very tough gluten that is slow to hydrate, and in the extreme, the gluten loses its cohesive properties completely.

Schofield, Booth and coworkers showed, for instance, that extractability in SDS buffer diminished and that glutenin of highest molecular weight (in SE-chromatography) were affected predominantly. Gliadins were also affected but at higher temperatures: α-, β-, and γ-gliadin fractions were made progressively unextractable, unlike ω- that were essentially unaffected.

(SLIDE 20) As far as functional properties are concerned, baking performance, measured from reconstituted flours, declined progressively between 50 and 70°C (with a large increase in mixing time and a reduction in loaf volume) and most of the functionality was lost by 75°C.

Again, the protein solubility and the functionality of heat-denaturated gluten can be restored, at least partially, by dough

mixing in the presence of reducing agent, which tend to support the involvement of reactions involving S-S bonds in the denaturation process. However, (SLIDE 21) Schofield demonstrated that the level of total SH groups remained constant up to 100°C and that there was only a transfer of SH groups from the soluble to the insoluble form. Accordingly, gluten drying is causing the polymer system to become effectively more cross-linked through SH/S-S interchange between exposed S-S and SH groups in adjacent molecules, but without additional S-S and 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 stable. This results in a tougher, harsher, more elastic gluten, whose baking performance is decreased.

CONCLUDING REMARKS

Although a number of models have been proposed to account for elasticity, viscosity and extensibility of gluten proteins, their molecular basis is not completely understood. Obviously, the balance between fractions that respectively aggregate by hydrogen bonding, hydrophobic interactions, or by formation of new disulfide bonds is very critical in determining satisfactory dough strength and loaf volume and this balance is dramatically changed upon heat treatment. A possible question, therefore, is why the higher levels of unextractable protein found in heat treated gluten do not confer better quality on the samples since a higher content in insoluble or residual protein is generally associated to a higher baking quality (Orth and Bushuk). The obvious answer could be that the physico-chemical basis of protein insolubility are different in both cases. But it is still necessary to elucidate which bonds are involved in these differences.

Regardless of the type of heat treatment and of the methods used for monitoring denaturation, several general conclusions can be drawn (SLIDE 22+23).

1 - Heat denaturation of gluten : causes conformational changes in gluten proteins: decrease in solubility; changes in HPLC or electrophoretic patterns; changes in functional properties.

2 - The different gluten fractions do not have the same tendency to interact or to cross-link through thermal denaturation: glutenins are extremely sensitive and aggregate much more rapidly than gliadins. Heat causes changes especially in glutenins, that allow new, but not necessarily additional, intermolecular disulfide bonds to be formed. They give increased molecular size to the gluten aggregate, decreased extractability, and inferior baking performance because of the increased toughness.

3 - Sulfur free (and less hydrophobic) ω-gliadins are extremely heat-resistant

4 - The tendency of proteins to aggregate is greater in good than in poor-breadmaking flours. So, the susceptibility of proteins to heat modification may explain some differences in quality potential of wheat varieties.

5 - The study of changes that result from heat treatments is a dynamical and powerful way for investigating physico-chemical basis of technological quality and functional properties of proteins.

6 - Many aspects concerning interactions are not clearly understood and more sophisticated physico-chemical and molecular methods are now required, including the determination of complete sequences of HMW- and LMW-glutenin subunits through DNA sequencing.

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(1)

EXAMPLES OF HEAT TREATMENTS IN CEREAL FOOD PROCESSES

(2)

	MAXIMUM TEMPERATURE (°C)	FINAL HUMIDITY (%)
BREAD-BAKING	98 (CRUMB)	34
COOKIE-MAKING	140 (CRUST)	5
PASTA DRYING	55-90	12
PASTA COOKING	100	70
GLUTEN DRYING	55-70	9

THEORETICAL BASIS OF HEAT DENATURATION OF PROTEINS

(3)

Most visible effects :

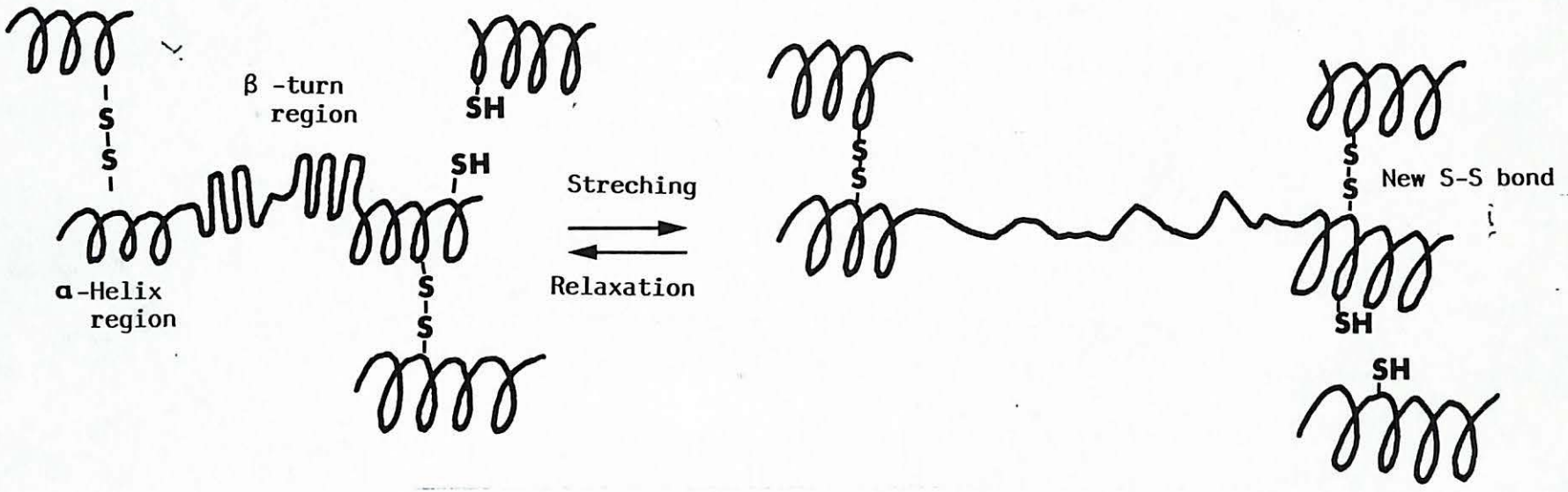
- Decrease in solubility (example : formation of an insoluble coagulum when egg white is boiled).
- Loss of activity of enzymes

Denaturation :

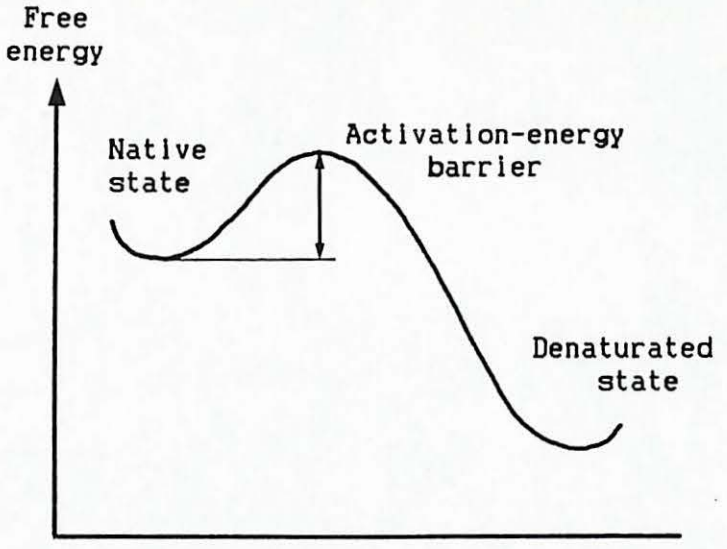
- causes the native characteristic folded structure of the polypeptide chain to uncoil or unwind into a randomly looped chain.

Thermal agitation :

- can permit new functional group associations (SH/S-S)interchange) and sequentially alters polypeptides to aggregates and finally to insoluble components



HEAT-INDUCED UNFOLDING CAN FACILITATE SH/S-S INTERCHANGE



(5)

IMPORTANCE OF THERMAL DENATURATION IN CEREAL PRODUCTS

(6)

Thermal denaturation is of primary importance in relation to food proteins.

It affects their :

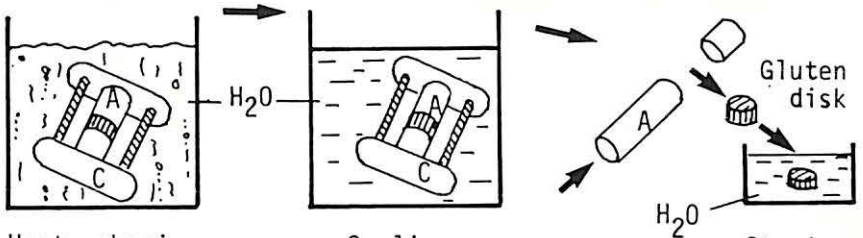
- preparation
- processing
- nutritional value
- quality
- safety

Depending upon the particular application, it may be desirable or undesirable.

MAJOR EFFECTS OF THERMAL PROCESSING ON WHEAT PROTEINS

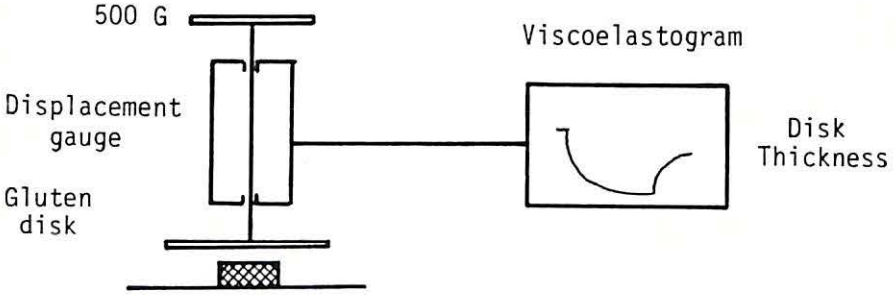
(7)

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



Heat shaping	Cooling	Storing
T° 100°C	T° 20°C	T° 20°C
90 sec	120 sec	60 sec

1a. Disk gluten shaping by heat treatment.
A = Piston, C = Clamp.



1b. Evaluation of gluten viscoelasticity.

(9)

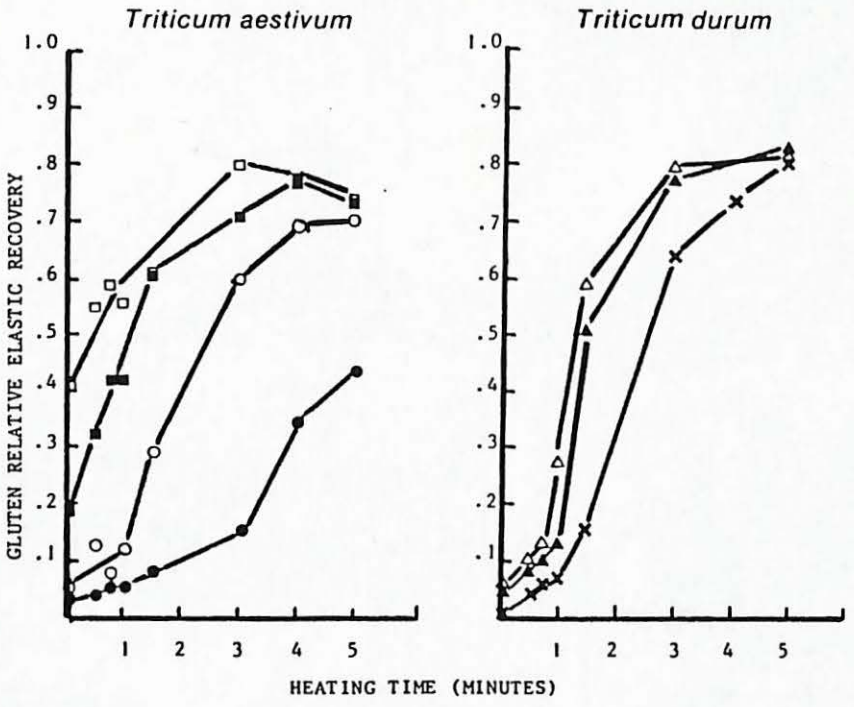
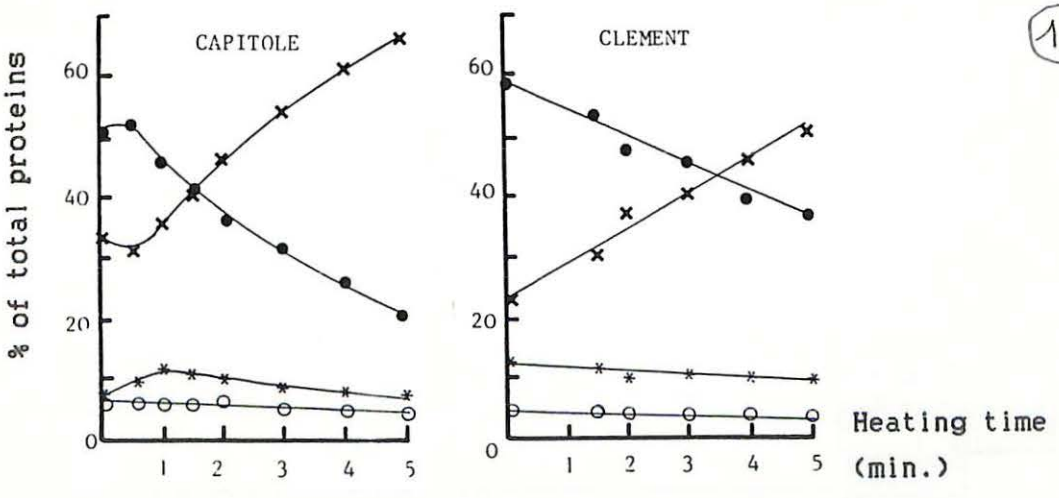


Fig. 3. Influence of heating time on gluten disk relative elastic recovery. Cultivars: ● = Clement, o = Maris Huntsman, ■ = Capitole, □ = Kolibri, x = V 39, ▲ = Lakota, Δ = Agathe. (from Jeanjean et al 1980)

(10)

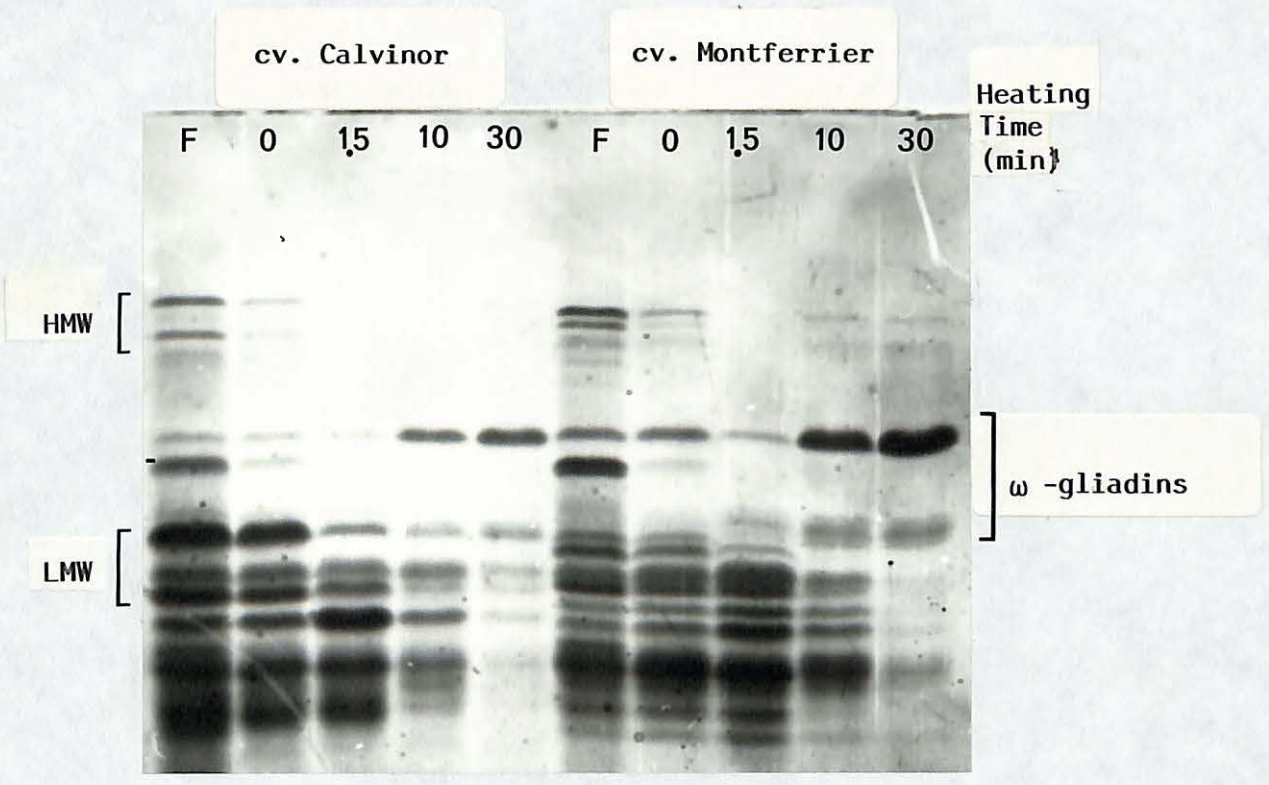


Influence of heating on gluten solubility in sequential extraction with 60% ethanol (●), borate buffer (o), SDS-borate buffer (*), ME-borate buffer (×).

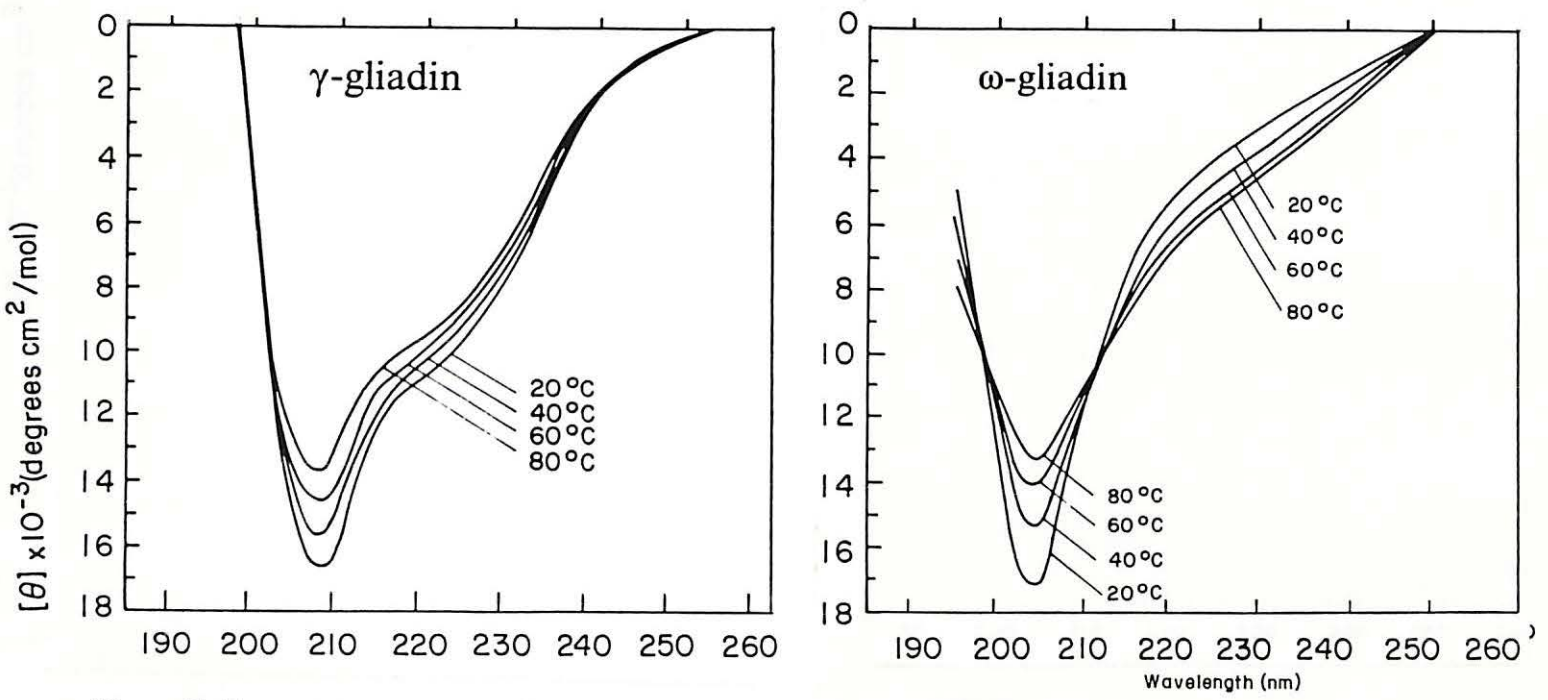
(from Jeanjean et al 1980)

SDS-PAGE PATTERNS OF ETHANOL-SOLUBLE
FRACTION EXTRACTED FROM
HEAT-MODIFIED GLUTENS

(11)



EFFECT OF TEMPERATURE ON THE CD SPECTRA OF
γ-GLIADIN AND ω-GLIADIN IN 70 % ETHANOL



(from Tatham and Shewry 1985)

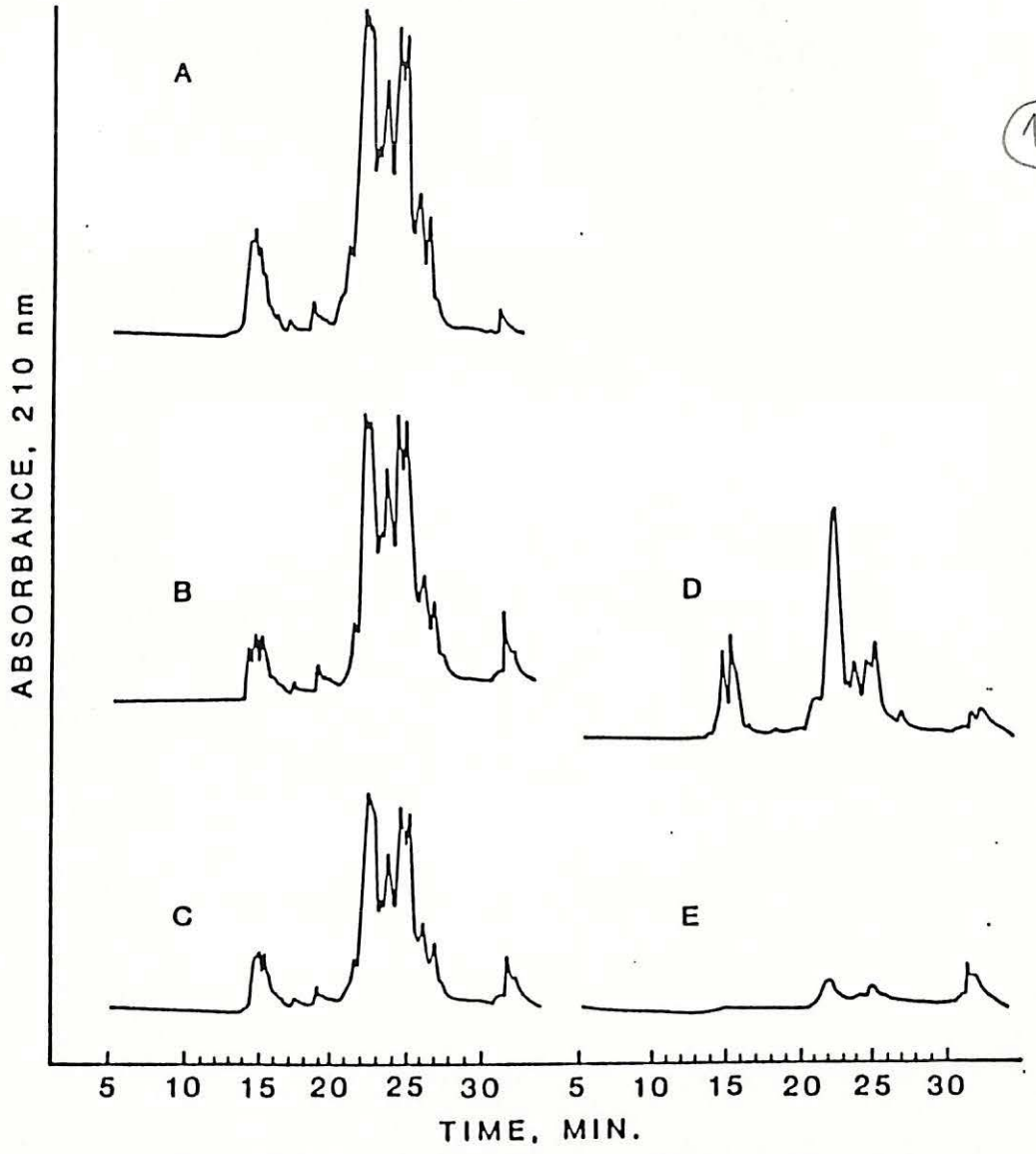
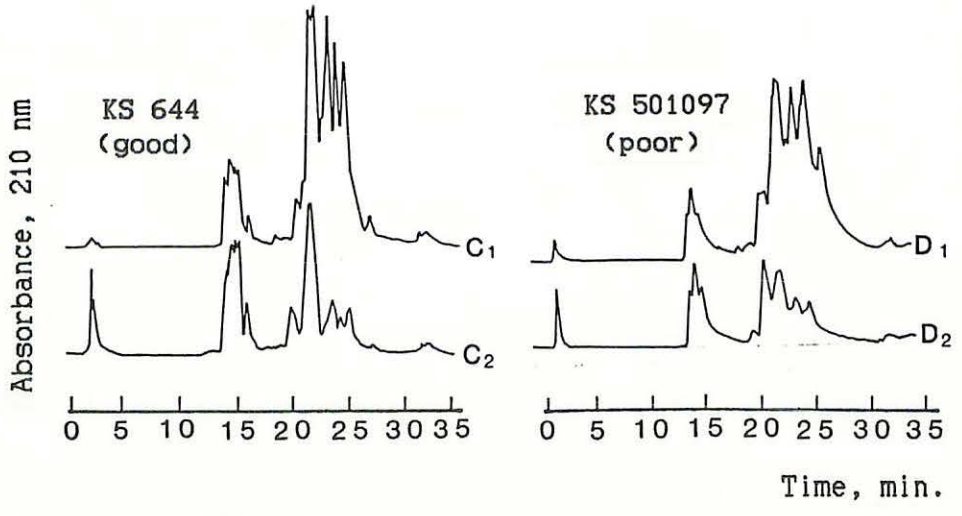
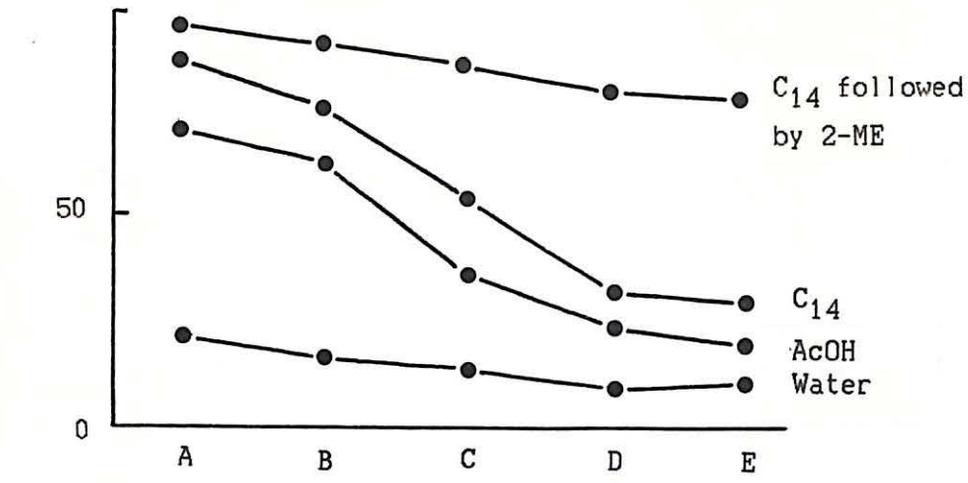


Fig. 2. High-performance liquid chromatography patterns of gliadins extracted from RBS-78 flour (A), nonfermented dough (B), fermented dough (C), bread crumb (D), and bread crust (E). The absorbance scale was the same for all samples. from Menkovska et al (1987)



RP-HPLC elution patterns of gliadin proteins from flours (1) and corresponding bread crumbs (2) of wheat lines KS 644 (good) and KS 501097 (poor). From Menkovska et al (1988)

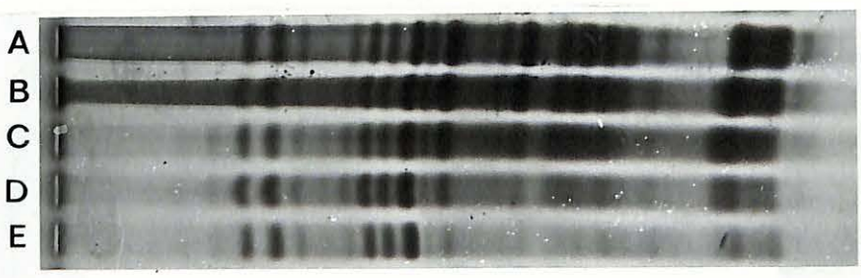
(15)



Effect of heat treatment (2 hrs at 90° C) on pasta protein solubility. A - Semolina; B - Pasta dried at 55°C; Pasta left for 2hrs at 90°C at 13 % (C), 18 % (D), and 24 % (E) moisture content respectively.

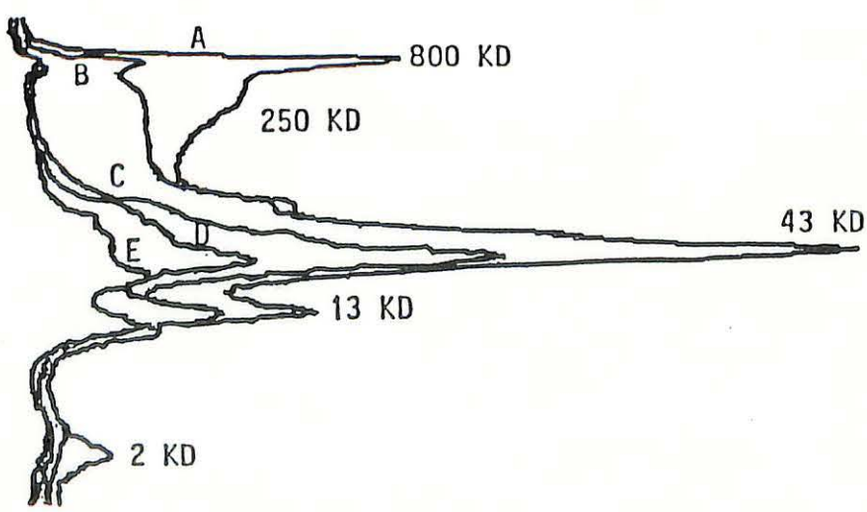
(16)

EFFECT OF PASTA DRYING ON PROTEIN AGGREGATION
A-PAGE OF ETHANOL-SOLUBLE FRACTIONS



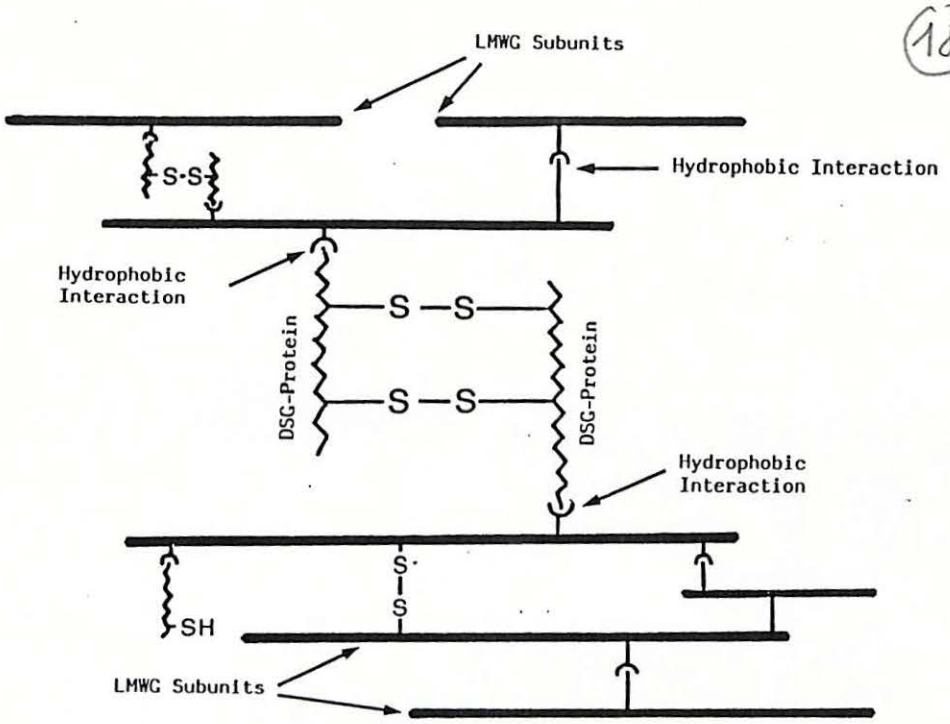
A - Semolina
B - Pasta : 55°C
C - Pasta : 90°C 13 % moisture
D - Pasta : 90°C 18 % moisture
E - Pasta : 90°C 24 % moisture

(17)



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

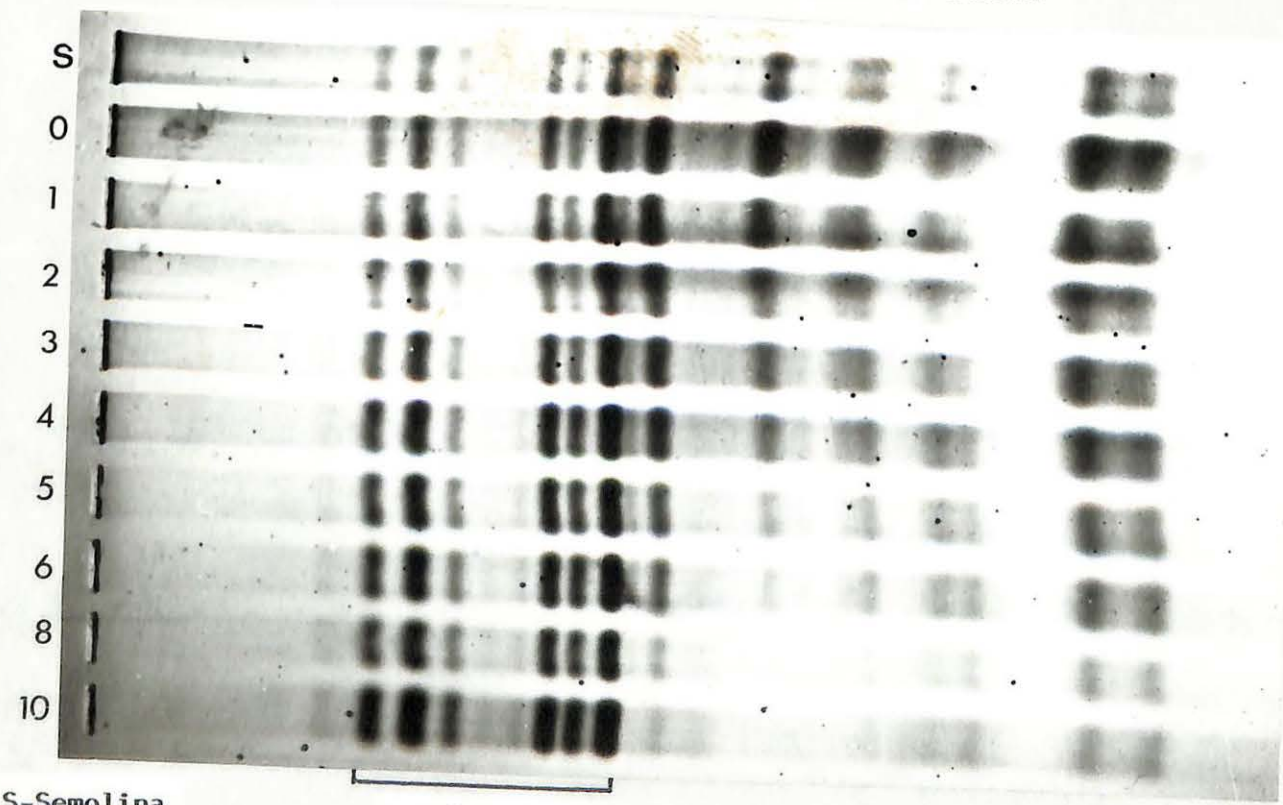
(18)



Proposed role of DSG proteins in aggregation of LMWG (after Alary, 1988).

(19)

A-PAGE OF GLIADINS EXTRACTED FROM COOKED SPAGHETTI

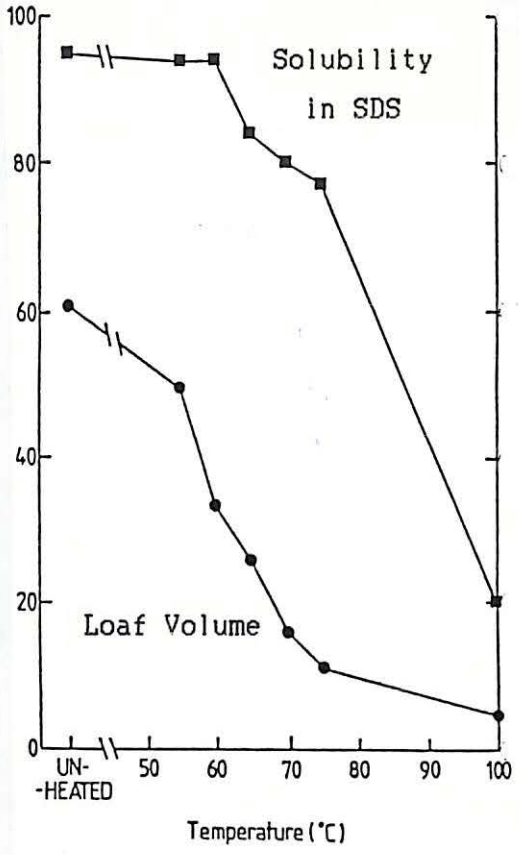


S-Semolina

0-10 : Cooking Time

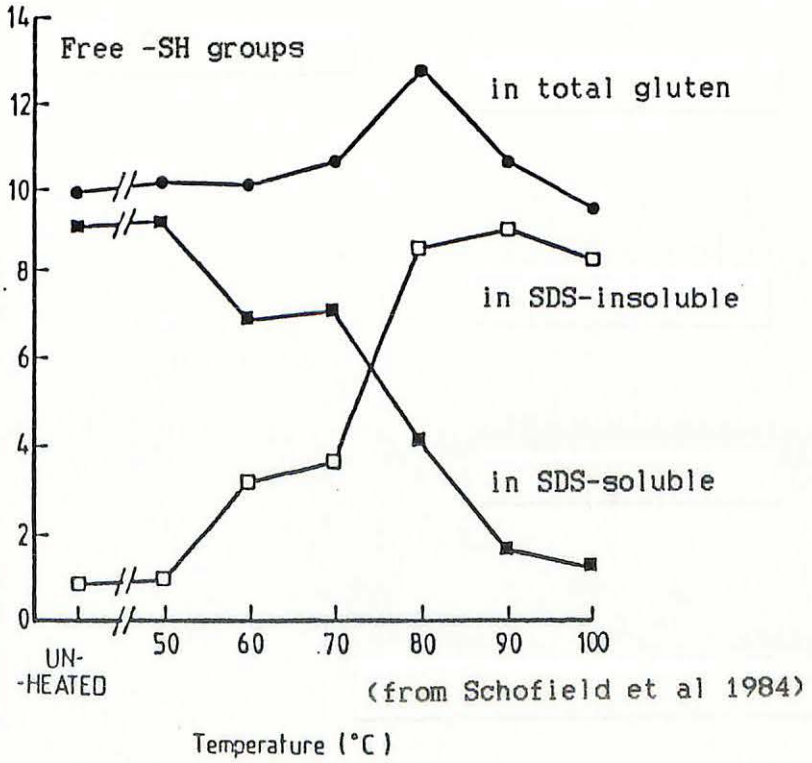
ω -gliadins

(20)



(from Schofield et al 1984)

(21)



(from Schofield et al 1984)

CONCLUSIONS

(22)

1 - Thermal agitation causes a native folded structure to uncoil into a randomly looped chain, causing disulfide interchange reactions and forming insoluble aggregates involving hydrophobic interactions.

Visible effects : decrease in solubility, change in electrophoretic patterns and in functional properties.

2 - Glutenins are extremely sensitive to heat treatments and aggregate more strongly than gliadins.

3 --ω -gliadins (random coil structure) have a higher resistance to heat denaturation than other groups of gliadins.

(23)

4 - Varietal differences : the tendency of proteins to aggregate is greater in good - than in poor-breakmaking flours or - pastamaking semolinas. Protein fractions that impart good quality are generally more susceptible to heat denaturation.

5 - The study of structural changes during heat treatments makes a dynamical way for investigating physico-chemical basis of technological quality and understanding functional properties in food processing.

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Heat treatments under a range of 55°C to 220°C and 35 to 70 % humidity according to the type of product (bread, cookie, pasta, vital gluten) are important and critical steps in cereal technology and they strongly affect gluten proteins. After reviewing some theoretical aspects of heat denaturation of proteins and presenting the methodology (proteins solubility, electrophoresis, HPLC), three different examples are developed. 1) In bread-making, attention is focussed on conformational changes in glutenins indicating that new intermolecular bonds facilitated by SH/SS interchanges were formed which led to increased molecular size of the aggregates and to a locked protein structure in the denaturated state. 2) Submitting durum wheat pasta to a high temperature drying also caused a more or less steep decrease of solubility in SDS or acetic acid depending on initial moisture content, but which could be restored by reducing solvents, indicating a formation of new disulfide bonds between proteins during heat treatments. 3) In industrial gluten production, excessively high drying temperatures resulting in tough samples are thought to be a major source of loss of functionality and commercial quality. Consequences on wheat quality requirements and miscellaneous aspects such as other uses of gluten or enzyme inactivation through heat treatments are also discussed.

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