GLUTEN PROTEINS

Proceedings of the 2nd International Workshop on Gluten Proteins

Wageningen
the Netherlands
1 - 3 May 1984

Editors: A. Graveland
J.H.E. Moonen

DURUM WHEAT FUNCTIONAL PROTEIN SUBUNITS REVEALED THROUGH HEAT TREATMENTS. BIOCHEMICAL AND GENETICAL IMPLICATIONS.

Jean-Claude Autran
Renée Berrier

Laboratoire de Technologie des Céréales, INRA
Montpellier, France

I - INTRODUCTION

Durum wheat is the raw material of choice for the manufacture of pasta products because of the ideally suited color and cooking quality of durum wheat pasta. Unlike common wheat, an important part of which can be used for animal feed, the sole use of durum wheat is for human food and, with the exception of few minor goods such as couscous, its only opening is pasta. Since pasta, at least in countries such as France and Italy, must be manufactured from pure durum wheat semolina, it is essential that durum wheat quality, and more especially pasta cooking quality, meets requirements of pasta making industries and consumers.

It is largely accepted that pasta cooking quality includes the following parameters :

- swelling
- cooking losses
- rheological properties : firmness, resilience (Damidaux and Feillet, 1978).
- state of surface : stickiness, surface desintegration (Kobrehel et al, 1982)

and is associated with quantity and quality of semolina proteins. However, the biochemical basis of cooking quality and more especially of varietal differences in cooking quality are still not entirely understood. Although we do not rule out that some carbohydrates or lipid fractions might contribute to the expression of cooking quality, our present paper is dealing with a classical view of the involvement of proteins only in cooking quality.

There are two reasons for improving our knowledge on biochemical basis of quality :

1) it will help to control and improve technological processes of pasta making,
2) it will help in taking into acount quality in breeding programs through efficient screening tests based on specific biochemical components.

Two ways can be imagined in order to discover proteic fractions or components involved in intrinsic quality of wheats.

First, to estimate relationships or correlations between the presence or the ratio of proteic fractions, proteic components or proteic "blocks" and quality data of genotypes.

Second, to demonstrate a functional property (i.e. a direct causal effect) of some proteic components in investigating the modifications they undergo upon technological processes or dynamical studies simulating the different technological steps.

II - RELATIONSHIPS OR CORRELATIONS BETWEEN THE PRESENCE OR THE RATIO OF PROTEIC FRACTIONS OR PROTEIC COMPONENTS AND QUALITY DATA OF GENOTYPES.

This approach, which has been reported many times (Bietz and Wall, 1972; Sozinov et al, 1974; Wasik and Bushuk, 1975; Damidaux et al, 1978; Rousset et Branlard, 1980), does not allow necessarily to find out biochemical basis of quality since correlations do not mean explanations. It can be used however in a practical point of view, for developing breeding tests for quality.

One of the most typical results of this approach is the excellent agreement between gliadin electrophoregrams (presence of gliadin n° 45 or n° 42 in Bushuk and Zillman's PAGE) and intrinsic cooking quality (measured through gluten viscoelasticity (Damidaux et al, 1978). The determination of gliadin pattern has become this five last years in France and other countries, an efficient breeding test allowing a screening for a high potential of cooking quality in early generations (Feillet, 1979; Damidaux et al, 1980; Feillet, 1980; Kosmolak et al, 1980; Du Cros et al, 1982).

In addition to 45 and 42 gliadin, other proteic components were investigated either in order to bring a modulation in the unbroken relationship between gamma gliadins 45 or 42 and gluten viscoelasticity (since some breeders found this relationship too much clear-cut) or in order to evidence specific components associated to other parameters of cooking quality (for example stickiness or surface desintegration of cooked data) which are not directly linked to gluten rheological properties. Until now however this latter attempt has failed but several other components were discovered which were also linked to gluten viscoelasticity.

Total proteins were reduced and fractionated by SDS-PAGE according to Payne et al (1979) and the three regions HMW, MMW, and gliadin were investigated (Figure 1).

In HMW, in contrast to bread wheats, no relationship was found. For example Bidi 17 and Tomclair or Agathe and Lakota showed the same HMW pattern (Autran et al, 1982).

In MMW, two subunits (MW 68000 and 70000 were found associated to gliadin 45 and 42 respectively. Actually these subunits turned out to agree respectively with omega gliadins 33-35-38-40 and 35 (Autran, 1981).

In the slow moving gliadin region an especially strong subunit (MW estimated to 50000), present in type 45 varieties only, drew our attention, but could not be identified to band 45 itself since Cottenet et al (1983) isolated pure gliadin 45 and demonstrated that it had lower MW (44000) and was almost undistinguishable from gliadin 42 in SDS-PAGE patterns.

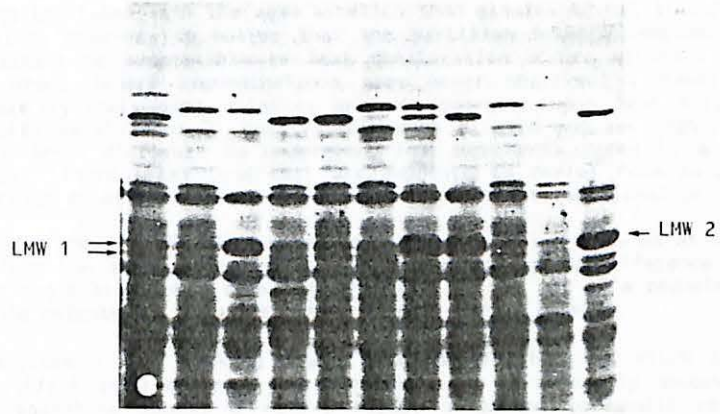

Figure 1 : S.D.S.-P.A.G.E. patterns of S.D.S.+M.E. extracted proteins from 11 french durum wheats : 1-Durtal; 2-Poinville; 3-Agathé; 4-Tomclair; 5-Wells; 6-Lakota; 7-Valdur; 8-Blondur; 9-Kidur; 10-Clandur; 11-Bidi.

After chromatography of whole durum wheat gliadin on CMC (umpublished results) the only bands that migrate at the same location in SDS-PAGE were two omega gliadins (PAGE mobilities : 20 and 23) but they could not account for the strong 50000 subunit since they are faint bands common to most of the 45 and 42 type durum wheat patterns.

Finally, it appeared (Payne et al, 1984a) that this strong subunit was not detectable in unreduced gliadin and that it was an alcohol-soluble material that gave rise to streaks in aluminium lactate PAGE and to a band upon reduction only. It can be considered as an aggregated gliadin or a LMW glutenin. This subunit is likely to correspond to LMW 2 glutenin identified by Payne et al (1984a) on two-dimensional maps of durum wheat gliadins. Varieties with gliadin 42 allele seem to contain similar subunits (LMW 1) but fainter and having higher mobility (MW estimated to 47000).

To summarize this first part it can be said that in durum wheats having strong gluten, gamma gliadin 45 is allways present associated to a MMW subunit which is the omega 35 (MW 68000) and to a very strong LMW glutenin subunit (MW 50000). The other varieties with gliadin 42 present, possess another MMW (MW 70000) which comprises the four omega 33-35-38-40 and a faint LMW glutenin (MW 47000). It is likely that the chromosome 1B$_s$ linkage group consists of these gamma, omega and LMW-glutenins (Payne et al, 1984b) and that the Sozinov's concept of block of closely linked gliadin genes must be extended to some LMW-glutenin subunits.

Either of these gamma, omega or LMW component can be used as a genetic marker, but according to us, the easiest screening from SDS-PAGE patterns could be obtained using LMW (the concentration and the mobilities of which clearly distinguish both types of wheats) instead of 42 and 45 gamma gliadins which have almost identical mobilities and concentrations. Screening for quality cannot be performed from HMW subunits.

This result raises the question as to whether gluten quality is caused by gliadin 45 itself or is due to closely linked genes of the $1B_s$ locus: omega 35, LMW glutenin 2, or some other factor with strong functional properties. We are dealing with this question in the following part.

III - FUNCTIONAL PROPERTIES OF SPECIFIC PROTEIC COMPONENTS IN PASTA-MAKING ?

Functional properties of durum wheat proteic components could mean tendancy to interact or to aggregate during pasta processing into a continuous and insoluble network having firm and resilient characteristics and able to entrap the swollen and gelatinized starch granules avoiding surface deterioration and carbohydrates and proteins leaching during cooking (Feillet, 1984, Resmini and Pagani, 1983).

We do not think that comparisons of electrophoregrams (especially SDS-PAGE after reduction by mercaptoethanol) of whole proteins extracts are the best tool to evidence a such tendancy among proteic components. We would think that submitting gluten or pasta to modelized dynamical studies simulating the different steps of technological processes :

. comparison of different milling streams,
. mixing or overmixing of the dough,
. heat treatments : pasta cooking, gluten cooking or thermoforming, high temperature pasta drying.

and investigating the modifications they undergo, is a more appropriate way to obtain strong clues on functional properties of proteic components.

We shall focus hereunder on the effects of the three following heat treatments : pasta cooking, gluten cooking, high temperature pasta heating or drying.

A. Pasta cooking

Spaghetti,processed in our pilot plant from several durum wheat varieties of a large range of cooking quality, were cooked from 1 to 10 mn in boiling water, then frozen and freeze dried. Gliadins were extracted by ethanol 70 % and run in a regular aluminium lactate PAGE (Bushuk and Zillman's system).

It was observed that several gliadin bands lost gradually their solubility in ethanol upon cooking, at first the beta and gamma 42 and 40 fractions, then some alpha. All the slow moving bands (omega n° 20-23-26 and 33-35-38)

remained obviously visible : they dominate the patterns (and are even enhanced) after 10 mn of cooking of pasta. Also, streaks in the gliadin patterns readily desappear upon cooking.

A similar behaviour can be observed from both good cooking (Bidi) and poor cooking quality (Tomclair) wheats. In particular, gliadin 45 seems to be unsolubilized with the same kinetics than gliadin 42. It is especially interesting, however, to notice that the partition between components that are resistant or susceptible to heat denaturation occurs within a genetic linkage group (where recombinations were never observed) : bands 40-42, or 45 are typical gamma gliadins and do aggregate upon heat treatments, bands while 33-35-38 or 35 are typical omega gliadin and are highly resistant. It seems difficult to understand how components coded by a cluster of closely linked genes (and that are supposed to derive from an ancestor gene through point mutations) can have such different functional properties.

Looking now at SDS-PAGE of proteins extracted and reduced with SDS-ME buffer from the same cooked pasta, it can be noticed no difference between semolina and pasta cooked from 1 to 10 mn as if the use of a reducing agent allowed to release the subunits that aggregate during cooking.

Therefore : 1 - Cooking causes heat denaturation in which the most visible effect is a decrease in solubility. It is generally accepted that 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 overcoming the electrostatic repulsion) and to form aggregate or network structure that can be at least partly disrupted by reducing agents or soaps.

2 - As it was already reported by Mc Causland and Wrigley (1976) the different gluten components or subunits do not have the same tendancy to interact or to cross-link through thermal denaturation : alpha and beta gliadins become readily insoluble. So does the streaking material of the patterns. Gamma gliadins 42 and 45 do not seem to behave differently. Sulfur-free omega gliadins, the structure of which is more a random coil type have a very low chemical reactivity and turn out to have a very high heat resistance. Extra uncoiling is however a possible reason why they can bind more dye and give more intense bands.

B - Gluten heating

Gluten samples (1 g wet) were cast into a special moulding cell (previously used by Damidaux and Feillet, 1978) for gluten thermoshaping prior to viscoelastic measurements and immersed in boiling water for different times : 0, 1.5, 10 and 30 mn. Several observations could be done that confirmed the results of Jeanjean et al (1980) :

- Viscoelastic properties were greatly affected : firmness allways increased for all varieties while elastic recovery increased for poor varieties and tend to slightly decrease for the strong ones (Agathe, type 45).

- Large differences were obtained in protein composition : basically, the ratio of ethanol soluble fraction markedly decreased while the ratio

of mercaptoethanol soluble fraction simultaneously increases as if the former were gradually converted into the latter through the formation of new bonds or interchange reactions which could involve S-S bonds and possibly hydrophobic bonds since heating is known to reinforce the strength of hydrophobic bonds.

- PAGE patterns of ethanol soluble fractions obtained at different cooking times confirm the above mentioned results on pasta cooking (except that gluten heating in a glass mould is less denaturing that pasta cooking so that 30 mn of gluten heating were necessary to have a denaturation equivalent to 10 mn of pasta cooking). Like in pasta cooking experiments, beta, then gamma and alpha fractions became ethanol insoluble, so did the streaking material, while omega were still extractable and dominate the patterns after 30 mn of heating. So, from a very firm and elastic gluten, all fractions except the omega gliadins were probably in a highly aggregated form which is ethanol insoluble or did not enter PAGE gels.

- A more interesting result comes from the study of SDS-PAGE patterns of the isolated and freeze dried ethanol-soluble fractions (ME reduced, but only before running the SDS-PAGE i.e. after being separated from the insoluble residue). Basically, all these initially ethanol soluble subunits tend to lose their solubility during heating except omega subunits. First of all, the high molecular weight fractions did aggregate, then the low molecular weight glutenin, then the alpha, beta and gamma (including 45 or 42) gliadins (Figure 2).

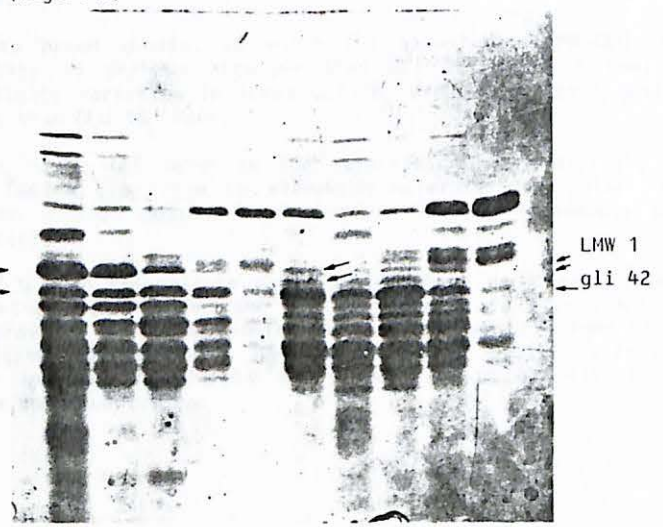

Figure 2 : S.D.S.-P.A.G.E. patterns of ethanol soluble gluten proteins from two varieties : Montferrier (1 to 5) and Calvinor (6 to 10). Proteins were extracted from semolina (1 and 6) and from gluten heated during 0 mn (2 and 7), 1,5 mn (3 and 8), 10 mn (4 and 9), 30 mn (5 and 10).

These results confirm that all these groups of subunits do not have at all the same ability to aggregate upon heating, the same capacity to form a firm and viscoelastic network, the same chemical reactivity, i.e. possibly different functional properties in pasta making. Aggregation capacity therefore seemed to decrease from HMW, to LMW, to alpha-beta-gamma gliadins and to omega gliadins.

C - High temperature pasta heating or drying

Recent pasta technologies using a drying at 70° of 90° may have (more or less according to the varieties that are processed) a strong effect on quality of cooked pasta which consists in an improvement of its rheological characteristics and state of surface.

Preliminary biochemical studies on both 45 and 42 type durum varieties have shown changes that were similar to those in the above mentionned heat treatments :

- decrease in ethanol or acetic extractibility of proteins.
- gradual disappearance (i.e. presumably gradual aggregation) of HMW, LMW, gliadins (except omega) from SDS-PAGE or PAGE patterns.

Since these phenomena occur in a similar way in both types of wheats, the question is to explain why poor varieties can be improved upon such treatments.

It is now generally 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 particules are trapped in a continuous network, promoting high firmness and little stickiness of cooked pasta.

* If (2) prevails, proteins coagulate in discrete regions lacking a continuous framework, giving soft and sticky pasta.

High temperature drying might partially overcome this competition by producing a coagulated protein network in dry pasta without starch swelling.

Accordingly, what we hypothesize is that in potentially strong varieties (type 45) with strong LMW GLU 2, a viscoelastic network is usally formed at the mixing or extrusion stage (before any heat treatment) due to pronounced functional properties of LMW GLU 2.

In contrast, poor varieties (type 42) have a low quality potential and they lack a viscoelastic network, because they have minor LMW-GLU 1 subunits (instead of a strong LMW 2). These poor varieties, to be improved into a firm structure would need a heat treatment which may cause a rapid aggregation of the HMW subunits.

Although changes in ethanol solubility and migration into an electrophoretic gel are a rough tool for assessing a level of proteic aggregation,

and although it is still difficult to know if a higher aggregat.. .. imparts more rheological properties than state of surface or vice versa, we would think that a very high level of aggregation of HMW subunits can be obtained by heat treatment only and does no preexist in the native state or even during pasta mixing or extrusion. If this was not the case all varieties would be good since we have shown that the same HMW patterns can be present in both types of varieties.

CONCLUSIONS

1 - Heat treatments give strong clues in differences of proteic components functionality.

2 - It is essential to distinguish between components that are probably genetic markers (low or questionable functionality : gamma 45/42, omega gliadins) from those which could have strong functional properties (capacity to aggregate into a continuous network).

3 - Among the latter components, we hypothesize that some (like HMW-GLU) could impart quality in general and have little specificity, while some (like LMW-GLU) could impart quality and could also explain varietal differences in functionality.

4 - In contrast to bread wheats, in which the association HMW-GLU (GLU $1B_L$ locus) - quality is perhaps stronger than GLI $1B_s$ locus - quality, in durum wheats allelic variation in locus GLI $1B_s$ probably affects quality at a greater extent than GLU $1B_L$ does.

5 - Within GLI $1B_s$ locus (at least in low temperature pasta drying), the LMW-GLU fractions (which give rise to streaking material in regular PAGE) might contribute to a high potential of cooking quality presumably much more than GLI 45 itself.

6 - In the case of a high temperature pasta drying, heat denaturation (when occurs before cooking) might cause the proteic network to strengthen and entrap starch, improving therefore cooking quality. We would assume, however, that heat aggregation involving HMW-GLU is not a specific effect as it seems to be in the case of LMW-GLU which could impart a native gluten strength in 45 type durum varieties.

REFERENCES

Autran, J.C. (1981). In "The quality of Foods and Beverages", (G. Charalambous, and G. Inglett, ed.), 1, p. 257. Academic Press, New York.

Autran, J.C., Damidaux, R., and Jeanjean, M.F. (1982). Getreide, Mehl und Brot, 36, 29.

Bietz, J., and Wall, 1972). Cereal Chem., 49, 416.

Branlard, G., and Rousset, M. (1980). Ann. Amélior. Plantes, 30, 133.

Bushuk, W., and Zillman, R. (1978). Can. J. Plant Sci. 58, 505.

Cottenet, M., Autran, J.C., and Joudrier, P. (1983). C.R. Acad. Sci. Paris 297, III, 149.

Damidaux, R., and Feillet, P. (1978). Ann. Technol. agric. 27, 799.

Damidaux, R., Autran, J.C., Grignac, P., and Feillet, P. (1978). C.R. Acad. Sci. 297, D, 701.

Du Cros, D., Wrigley, C., and Hare, R. (1982). Aust. J. Agric. Res. 33, 429.

Feillet, P. (1979). In "Proc. Symp. I.C.C. : Matières Premières et Pâtes Alimentaires" (G. Fabriani, and C. Lintas, ed.) p. 77, I.N.N., Rome.

Feillet, P. (1950). In "Cereals for Foods and Beverages" (G. Charalambous and G. Inglett, ed.), p. 183. Academic Press, New York.

Feillet, P. (1984). In "Proc. FAO Workshop on Breeding Methodology in Durum Wheat and Triticale" (in press).

Jeanjean, M.F., Damidaux, R., and Feillet, P. (1980). Cereal Chem., 57, 325.

Kobrehel, K., Abecassis, J., Alary, R., and Feillet, P. (1982). In "Proc. Int. Symp. on Durum Wheat : Industry and Scientific Research" (A. Bianchi, ed.), p. 11, I.S.C., Rome.

Kosmolak, F., Dexter, J., Matsuo, R., Leisle, D., and Marchylo, B. (1980). Can. J. Plant Sci. 60, 427.

Mc Causland, J., and Wrigley, C. (1976). J. Sci. Fd Agric. 27, 1197

Payne, P., Corfield, K., and Blackman, J. (1979). Theor. Appl. Genet. 55, 153.

Payne, P., Jackson E., and Holt, L. (1984a). Journal of Cereal Science 2, 73

Payne, P., Holt, L., Jackson, E., and Law, C. (1984b). Phil. Trans. R. Soc. Lond. B 304, 359.

Resmini, P., and Pagani, M. (1983). Food Microstructure, 2,1.

Sozinov, A., Poperella, F., and Stakanova, R. (1974). In "Proc. Symposium Franco-Sovietique : Amélioration Génétique de la Qualité des Blés Durs" p. 47, INRA, Montpellier.

Wasik, R, and Bushuk, W. (1975). Cereal Chem. 52, 328.