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RECENT DATA ON THE BIOCHEMICAL BASIS OF DURUM WHEAT QUALITY

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I. INTRODUCTION

Durum wheat (*Triticum durum* Desf.) is the raw material of choice for the manufacture of pasta products because of the superior rheological properties of durum wheat pasta doughs and 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 or soup pasta, 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 especially important that quality of durum wheat meets demands of semolina and pasta-making industries.

The term durum wheat quality generally includes all characteristics of durum wheat and more especially :

- the semolina yield, i.e. the weight of semolina of a given purity that can be processed on wheat basis (Feillet and Abecassis, 1976)
- the ability of semolina to be processed into pasta which is bright yellow in color and which, when cooked, resists desintegration and retains a firm structure (Feillet, 1977).

It is primarily the second group of characteristics that we are dealing with in this chapter since it is the most closely related to biochemical composition of semolina and since poor cooking quality varieties recently raised concerns is some countries, so that the production of high cooking quality varieties is presently a major objective of durum wheat breeders.

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Pasta color is relatively well understood : yellowness, which is favourable, is a function of semolina carotenoid content and lipoxygenase activity and browness, which is unfavourable, is correlated to peroxidase and polyphenoloxidase activities (Kobrehel *et al.*, 1974 ; Laignelet, 1981). On the contrary, explaining varietal differences in cooking quality in terms of simple differences in biochemical composition is an old research objective that has remained unfulfilled and considerable work is still needed in this field.

Therefore, we would like to underline the major results that have been recently reached by several groups in the world concerning the biochemical basis of durum wheat cooking quality. A special attention is given to those obtained in our laboratory by Damidaux, Feillet, Jeanjean, Kobrehel and Laignelet. Before, we think it essential to clearly define what we mean by cooking quality.

II. THE CONCEPT OF INTRINSIC COOKING QUALITY OF A VARIETY

According to Feillet (1980), the word quality does not mean many things by itself and cannot be expressed in terms of specific characteristics. Its significance is different for millers, pasta-processors, consumers, nutritionists or genetists.

From the viewpoint of *millers or pasta processors*, the best method to assess the *quality of a sample* is to put it through a test similar to the one for which it is intended : pasta-making test, cooking test. On the other hand, the *breeders* have a quite different objective and, more especially at early breeding stages, they need quality tests allowing to assess the *intrinsic value of their lines*, i.e. a potential level of quality which is afterwards open to express itself differently according to environmental factors.

At present, many fast and small scale methods for direct estimation of durum wheats cooking quality are of course available. Some consist in measuring mixing requirements of the dough by the farinograph method (Irvine *et al.*, 1961 ; Dexter and Matsuo, 1980) or strength through the use of the micromixograph (Bendelow, 1967) or of the SDS-sedimentation test (Dexter *et al.*, 1980). Some others consist in processing semolina into pasta disks or spaghetti, cooking them and determining their characteristics through the use of an aleurograph (Scotti, 1976), a viscoelastograph (Feillet *et al.*, 1977 a, b), a spaghetti tenderness apparatus (Dexter and Matsuo, 1977 a, b) or an alveograph (Walle et Trentesaux, 1980) However, cooking quality assessment at the breeding stage

remains a critical problem.

As a matter of fact, in opposition to the color concept and particularly to its yellow component, a varietal characteristic, cooking quality of a durum wheat is highly influenced by growing conditions. Table I shows the cooking quality scores (12 = excellent, 0 = very poor) of 24 durum wheat samples made up of three cultivars grown in eight different locations.

TABLE I. Variability of Cooking Quality Score (Viscoelastograph Measurements on Cooked Pasta Disks) of Three Durum Wheat Cultivars Grown in Eight Locations.

Location	Cultivars		
	Agathe	Valdur	Lakota
A	3	1	0
B	4	3	4
C	1	4	0
D	8	4	4
E	9	5	6
F	9	8	4
G	11	8	4
H	12	12	8

Breeders cannot get entire satisfaction from these methods, which are hardly able to account for the respective influences of the genotype and the environment, except by multiplying considerably the number of experimental plots ; what is tedious and time consuming. Therefore, we think it fruitful and even essential to clearly distinguish between :

- *breeding tests*, which should assess what we can call the *intrinsic cooking quality of the varieties*
- *commercial tests*, which should assess the *quality of the sample*, the result of interactions between the intrinsic cooking quality and the growing conditions of the plant.

It turns out that most of the tests that are used by breeders derive from methods which were originally developed to evaluate commercial quality and, until recently, only few studies have been devoted to the development of methods allowing a direct assessment of intrinsic cooking quality of the genotypes. We think furthermore that such methods are required to have the following characteristics :

- independance of the results with regard to the agronomical record of the sample

- high correlation with the varietal ranking that would have resulted from conventional experiments
- potential for analyzing a large series and a small amount of material.

We think that recent progress in the knowledge on the biochemical composition of durum wheat kernel and its genetic control open new fields of investigation in view to understand the biochemical basis of cooking quality and to develop biochemical tests which perfectly meet the above-mentioned characteristics.

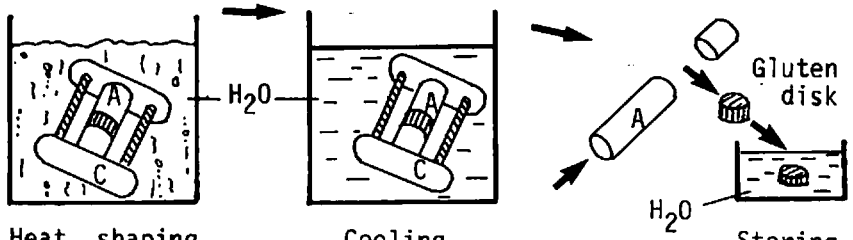
For example, it is largely accepted that cooking quality of durum wheat is associated with the quantity and the quality of its proteins, particularly of its gluten proteins (Dexter and Matsuo, 1977 a, 1978 ; Feillet, 1977 ; Trentesaux, 1979). As the quantity of proteins is influenced to a large extent by environmental factors and the quality only is heritable, we whall underline hereunder the possibilities of developing breeding tests for high intrinsic cooking quality based on gluten quality (i.e. viscoelastic properties) and composition of the two main gluten fractions : gliadins and glutenins.

III. VISCOELASTIC PROPERTIES OF DURUM WHEAT GLUTEN

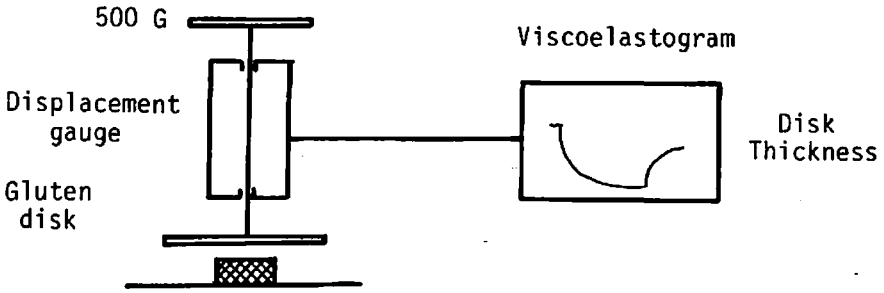
Durum wheats with strong gluten properties, in general, tend to produce pasta with superior cooking characteristics (Irvine, 1971). Gluten strength has been for many years a quality criterion in durum wheat breeding (Matveef, 1966 ; Matsuo, 1974) and, more than ever, gluten strength is a major quality requirement in most traditional pasta-consuming areas (Dexter *et al.*, 1980).

However, most of the attempts made by previous authors in order to explain differences between varieties in cooking quality in terms of physical properties of gluten failed more or less. All these works, restricted to the study of raw gluten, came up against the difficulty of making a well-defined shape gluten test-piece. In the present work, we could overcome this difficulty by thermoshaping the gluten sample, resulting in a well-defined and reproducible gluten disk (Damidaux and Feillet, 1978).

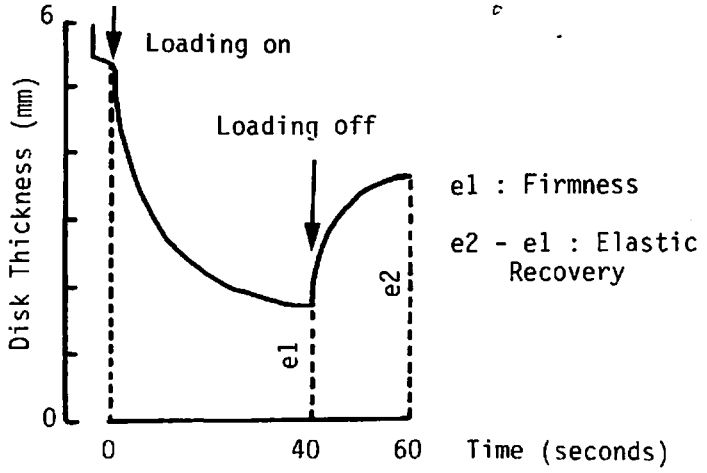
After extraction through manual lixiviation, 1 gram of gluten was put into a moulding cell (Figure 1a). Pistons were placed on either side of the gluten ball and held by a clamping frame. The cell was immersed for 90 sec in boiling water, then for 120 sec in 20°C water. The resulting gluten disk was taken out of the cell and put into water for about 1 min.



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



1b. Evaluation of gluten viscoelasticity.



1c. Cooked gluten disk viscoelastogram.

FIGURE 1. Measurement of Gluten Viscoelastic Properties.

The viscoelastic properties of the gluten were then determined in an original manner by a viscoelastograph[1]. This apparatus (Figure 1b) follows the strain of a solid in terms of applied stress and of time (Feillet *et al.*, 1977 a, b). The gluten disk was taken out of the water and put on a sample plate ; a constant load was applied for 40 sec and then removed. The time dependance of the thickness variation of the gluten disk was scanned before and after loading off (Figure 1c). The gluten absolute elastic recovery ($e_2 - e_1$) was computed from the value of e_1 (thickness immediatly before loading off) and e_2 (final thickness, 20 sec after loading off).

Elastic recovery was determined for a large number of samples of different varietal and agronomical origins (Damidaux and Feillet, 1978). Within all samples, absolute elastic recovery values ranged from 0.3 to 2.1 nm. In a given variety, absolute elastic recovery varies within narrow limits around an average value that tends to decrease as the wheat protein content increases. The lower this average value is, the more important are the fluctuations. A variety can be characterized by this average value of its gluten viscoelasticity. Figure 2 shows that the 122 varieties that were analyzed segregated into two classes around the mean value 0.6 and 1.8 mm and that *there is a genetic dependancy of viscoelastic properties of cooked durum wheat gluten.*

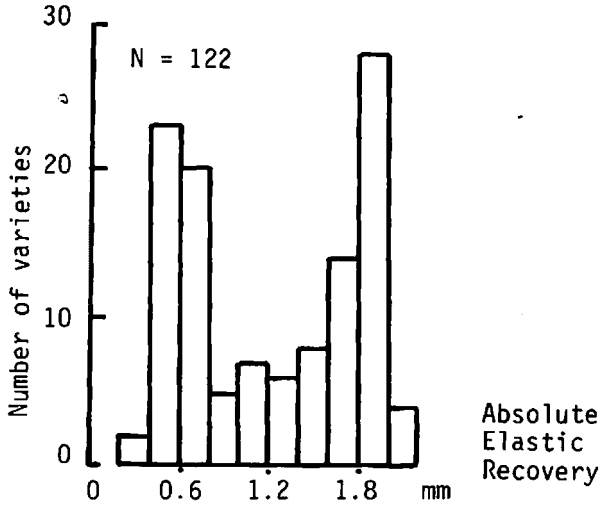


FIGURE 2. Distribution of absolute elastic recovery of gluten in samples of durum wheat.

[1]Tripette et Renaud - Chopin, France.

Well-known cultivars with either high or low cooking qualities were checked for their gluten viscoelastic properties (Table II). Gluten in all cultivars known for good cooking quality had absolute elastic recovery values above 1.6. Glutens in low or medium cooking quality cultivars had absolute elastic recovery values below 1.0 (except for Lakota, which can be regarded as a medium quality cultivar). Therefore, *there is a relationship of gluten elastic recovery value and intrinsic cooking quality of durum wheat cultivars.*

It must be emphasized that the latter relationship does involve intrinsic cooking quality of the varieties since it was demonstrated after averaging many data gathered on this varieties during several years. Accordingly, it essentially concerns the breeders. If a similar investigation is undertaken from a restricted number of samples (i.e. one or two

TABLE II. Intrinsic Cooking Quality and Gluten Viscoelasticity[1].

Variety	Intrinsic Cooking Quality	
	High	Low
Agathe	1.79	.
Bidi 17	1.70	.
Blondur	1.91	.
Brumaire	1.90	.
Diabolo	1.77	.
Edmore	1.71	.
Mondur	1.91	.
Montferrier	1.90	.
Trinakria	1.81	.
Valdur	1.82	.
Durtal	.	0.79
Lakota	.	1.28
Poinville	.	0.72
Randur	.	0.75
Rikita	.	0.47
Tomclair	.	0.59
Valsacco	.	0.59
Wells	.	0.71

[1]Absolute elastic recovery ($e_2 - e_1$ in millimeters) of thermomolded gluten (average of samples of different origins).

locations per variety instead of an average of many data) the relationship may not be as clear-cut as it is found here above. Therefore, viscoelastic properties of cooked gluten can be used as a breeding tool for high intrinsic cooking quality but may not be as effective for an assessment of cooking quality or overcooking quality of a sample at commercial level.

On the other hand, gluten viscoelasticity can be used as a reference tool in investigations on biochemical basis of intrinsic cooking quality, particularly in assying functional properties of isolated gluten proteic fractions. In connection with this, it must be reported a certain number of attempts to explain varietal differences in dough or gluten strength or in gluten viscoelasticity. For instance, the distribution of different gluten fractions was emphasized by Dexter and Matsuo (1978) : F_4 fraction, a glutenin type, imparted excellent cooking quality whereas F_6 fraction, a gliadin type, imparted poor cooking quality and shortened the mixing time. Further studies, by Dexter and Matsuo (1980), based on the gluten breaking strength apparatus (Matsuo, 1978) evidenced the responsability of the residual proteic fraction in variations of gluten strength. However the distribution of gluten fractions, for instance the glutenins to gliadins ratio, might not be a guarantee of superior gluten quality since reverse trends were also observed (Dexter and Matsuo, 1977 c). It is the reason why we thought it necessary to go further into investigations in taking advantage of the recent progress in the fractionation methodology of gliadins and high molecular weight glutenins subunits.

IV. GLIADIN ELECTROPHORETIC PATTERNS

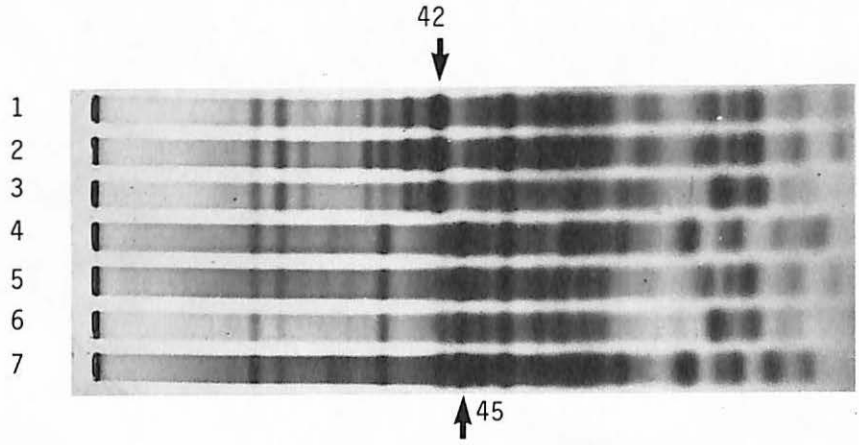
Durum wheat gliadins can be fractionated in about 20 individual components through starch gel or polyacrylamide gel electrophoresis. Patterns are not modified by environmental factors (Feillet and Bourdet, 1967) and provide adequate distinction of cultivars (Autran, 1975 ; Wrigley and Shepherd, 1974). In cataloguing wheat cultivars Doekes (1970), Sozinov *et al.* (1974), Autran and Bourdet (1975), Zillman and Bushuk, (1979) suggested that there might be a relationship between the presence of certain gliadin bands and wheat quality.

Relationships between durum wheat gliadin patterns and gluten viscoelasticity were studied in our laboratory (Damidaux *et al.*, 1978 ; Damidaux, 1979 ; Feillet, 1979). 122 varieties of different genetical origines were examined. After extraction by 70 % ethanol, gliadins were fractionated by polyacrylamide gel electrophoresis according to Bushuk and Zillman (1978). Components mobilities (from 0 to 100) were

established by reference to a 51 durum wheat gliadin band so as to remain in agreement with the common wheat gliadin nomenclature (Autran *et al.*, 1979).

By giving a special attention to the omega (mobilities 20-40) and gamma (mobilities 41-51) regions - and without prejudicing the identity of electrophoretic components showing outwardly the same mobilities - it was possible to classify durum wheat varieties into two main groups (Figure 3).

One was characterized by the presence of a strong band 45 and the absence of a band in the 38-42 region, the other by the absence of a band 45 and the presence of a strong band 42. Sixty-eight varieties belonged to the 45 type, fifty to the 42 type and four to neither (presence of a band 41 or 44).



Varieties: 1 - Lakota; 2 - Wells; 3 - Tomclair; 4 - Montferrier; 5 - Valdur; 6 - Agathe; 7 - Bidi 17.

FIGURE 3. Electrophoregrams of durum wheat varieties (polyacrylamide gel/aluminum lactate buffer, pH 3.1).

One of the most interesting results of this study was the *excellent agreement between the gliadin electrophoretic patterns of the durum varieties and their gluten viscoelastic properties* (Figure 4). In 61 of the 68 varieties (90 %) of the 45 gliadin type, the elastic recovery of gluten was above 1.2 mm. In 49 of the 50 varieties (98 %) of the 42 gliadin type, the elastic recovery was below 1.2 mm.

This result, which was corroborated by Kosmolak *et al.* (1980), raises many questions about the nature of the linkage between the gliadin electrophoregrams and the viscoelastic properties of gluten. Are genes coding for gluten strength

close to genes coding for specific gliadin bands (genetic marking) ? Or, more possibly, do gliadin components have specific characteristics which would act on gluten viscoelasticity (functional relationship) ? This latter hypothesis is supported by recent findings of Jeanjean *et al.* (1980) that some gliadin fractions can participate to the formation of an insoluble protein network which give high viscoelasticity to the gluten upon heating whereas others cannot. It is also supported by recent results concerning the chemical composition of gliadin 45 - higher surface hydrophobicity than gliadin 42 (Godon and Popineau, 1980) and high sulphur content compared to other gliadins (Wrigley *et al.*, 1980) - which are likely to explain a better contribution to insoluble aggregates and to viscoelastic properties of gluten.

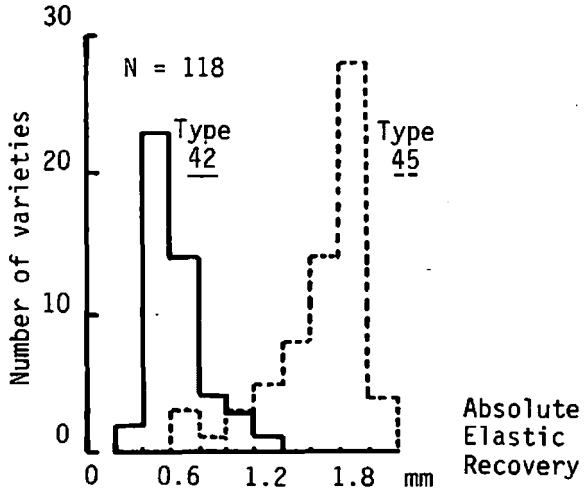


FIGURE 4. Relationship of electrophoretic patterns and viscoelastic properties of durum wheat samples.

Whatever are the basic explanations of these relationships, our work has led us to several practical deductions, however. For instance, the results provide plant breeders with a new tool to screen new high intrinsic cooking quality durum wheat varieties. To assist in this program, we would suggest using the following breeding diagram that we put on Table III.

It must be clearly specified that such 45/42 gliadin types are related to the concept of varietal intrinsic cooking quality based on above-mentioned rheological properties of gluten. Accordingly, others quality factors, like surface deterioration and mushiness of cooked pasta, which we think not to be systematically linked to rheological properties, might not be

taken in account by breeding on 45/42 gliadin bands only. Also, gliadin electrophoresis, a typically varietal characteristic, must not be used for the assessment of cooking quality of durum wheat samples at commercial level.

TABLE III. Proposal of breeding diagram for high intrinsic cooking quality durum wheat varieties.

1. Screen at the F3 or even the F2 generation on half kernels for the presence of the electrophoretic component 45

2. Screen the F5 generation of lines that consistently belong to the 45 type for high elastic recovery of gluten

3. Confirm end-use properties at the later breeding stages by checking the cooking quality through microtest and pilot plant tests and evaluating the effects of growing conditions

4. Simultaneous breeding for high protein content in the kernel and high yellow index of pasta would be advisable

V. GLUTENIN ELECTROPHORETIC PATTERNS

Through reduction and electrophoresis in the presence of sodium dodecyl sulphate (SDS), wheat glutenins generally consist of 15-20 different subunits with molecular weights ranging from 11,000 to 130,000 (Bietz and Wall, 1972) whereas major gliadin fractions are located in the 36,000-44,000 area (Kasarda *et al.*, 1976). Some varietal differences in glutenin patterns were described but there has been for a long time a controversy about relationships of such patterns with technological quality. Huebner (1970) and Orth and Bushuk (1972) found no relationship to baking quality of bread wheats but Payne *et al.* (1979) found a strong correlation between the presence of two glutenin subunits and bread-making quality, at least in the progeny of some crossings. In durum wheats, Wasik and Bushuk (1975) observed that glutenins of varieties with excellent pasta-making quality had more of subunit 6 (MW 53,000) than subunit 5 (MW 60,000). The results that we are reporting hereunder concern therefore further investigations in view to relate intrinsic cooking quality of durum wheats to glutenin patterns based on an improved fractionation

procedure.

Eighty durum wheat varieties were examined. After extraction from wheat kernels and reduction using SDS-Mercapto ethanol-*tris* buffer, proteins were fractionated in vertical SDS-polyacrylamide gel electrophoresis, pH 8.8, according to Payne *et al.* (1979). Whatever was the variety, the patterns consisted of three major groups of components (Figure 5) :

- a high mobility group (I), mainly albumins-globulins
- a intermediate mobility group (II), with heavy gliadin-type bands
- a low mobility group of very sharp bands (III), corresponding to so-called high molecular weight glutenins.

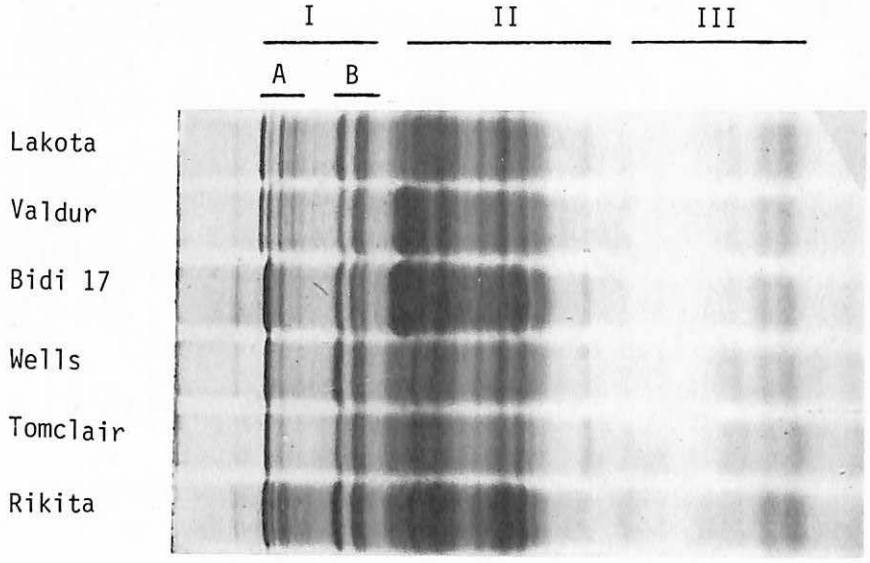


FIGURE 5. Electrophoregrams of Proteins, Extracted and Reduced by SDS-Mercapto ethanol-*tris* Buffer, from Durum Wheat Varieties (SDS-Polyacrylamide gel, pH 8.8).

Giving a special attention to group III, it can be distinguished a A region (MW 85,000-110,000) consisting of 2-5 components and a B region (MW 62,000-70,000) consisting of 2-3 components, according to the variety. In the whole, 18 different components (numbered A_1 to A_{13} and B_1 to B_5) were identified. Proteic polymorphism was larger in the A region (16 different types of varietal patterns) than in the B region (8

types). The schemas of the most widely distributed types are presented in the Figure 6.

Variety	Gliadin type	High Molecular Weight Subunits	
		A Region	B Region
Agathe	45	3 10 13	2 3 4
Bidi 17	45	6 11	2 3 5
Valdur	45	3 6 10 13	2 3 4
Pelissier	45	3 6 10 13	2 2 5
Maristella	45	6 11	2 3 4
Isa 1	45	5 8 12	3 4
Durtal	42	1 5 8 12	1 3 4
Lakota	42	3 10 13	1 3 4
Rikita	42	5 8 12	1 3 4
Tomclair	42	6 11	1 3 4
Valsacco	42	6 11	1 3 5

FIGURE 6. Schemes of the most widely distributed Glutenins Types of Durum Wheat Varieties.

Concerning the major types of the A region, no relationship could be observed between the distribution of the subunits and the elastic recovery of gluten. As a matter of fact, in 22 varieties belonging to the type A 3-10-13, the absolute elastic recovery was above 1.2 mm for 10 but below 1.2 mm for 12 others and in 25 varieties belonging to the type A 6-11, the elastic recovery was above 1.2 mm for 16 but below 1.2 mm for 9 others. However, although it is difficult to draw a conclusion from a fewer number of varieties, a relationship was noticed between some unusual types and intrinsic cooking quality. For instance, all varieties having patterns with subunits A1, or A2, or A5-A8 had poor intrinsic cooking quality, including 3 varieties (Isa 1, Melianopus 69 and Ak Bugda) belonging to the gliadin type 45. In the same way, all varieties

having patterns with both subunits A3-A6 had excellent intrinsic cooking quality.

Concerning the major types of the B region :

- in 21 varieties of the B 1-3-4 type, all had an absolute elastic recovery lower than 1.2 mm and all of them belonged to the gliadin 42 type
- in 24 varieties of the B 2-3-4 or B 2-3-5 types, the absolute elastic recovery was above 1.2 mm for twenty-two and between 1.0 and 1.2 mm for two and all of them belonged to the gliadin 45 type
- in 15 varieties of the B 1-3-5 type, the intrinsic cooking quality was good for eight and poor for seven.

Therefore, in opposition to the A region, the distribution of the B region subunits shows a relationship with intrinsic cooking quality which is in agreement with the gliadin 45/42 distribution : B2 component seems strongly linked to 45 gliadin band whereas B1 seems more associated to 42 gliadin band. Such a result could be explained considering the exact nature of the different high molecular weight subunits (Autran *et al.*, 1981). From the patterns showed by Jeanjean *et al.* (1980), B3, B4 and B5 looked absent in ethanol-soluble fractions and could be typical glutenins like A region components were. On the other hand B1 or B2 (MW 65,000-70,000) could be found in ethanol-soluble fractions and could belong to a gliadin type although generally being classified in high molecular weight fractions. Moreover, we showed (Autran, 1981) after gliadin fractionation and purification that B2 component was analogous to omega gliadin 35, which was strongly linked to gliadin 45. In connection with this, we think that subunits 5 and 6 evidenced by Wasik and Bushuk (1975), were just in the intermediate molecular weight region and could be actually classified in a gliadin type.

VI. CONCLUSION

For many years, attempts to explain differences between durum wheat varieties in cooking quality have been unsuccessful. The discovery of an excellent agreement between the gliadin electrophoretic patterns of the durum wheat varieties and their gluten viscoelastic properties can therefore be regarded as an appreciable progress in the understanding of the biochemical support of technological quality (Damidaux *et al.*, 1980) and the usefulness of polyacrylamide gel electrophoresis in breeding programs is demonstrated in this chapter.

The varietal classification based on gliadins is corroborated and even further splitted up by the study of some high molecular weight fractions but, in general, the association

between electrophoretic composition and cooking quality is stronger in gliadins than in high molecular weight glutenins. There is also some evidence that we are dealing with a cause-and-effect relationship (Wrigley, 1980). For instance, gliadin 45 could have a unique structure that enables strong and elastic proteic network to be formed during pasta process and could be more efficient at doing this than the other gliadins. On the other hand most of the glutenin subunits with highest molecular weight do not seem to have specific properties and could merely act in bulk and by their ratio to other fractions.

It is possible however that cooking quality can be based on a particular ability of proteins to form aggregates or insoluble networks during pasta process and also films that retain integrity of spaghetti during cooking. Therefore, in carrying out glutenin electrophoresis after solubilization and reduction we might be using a wrong method for assessing the chemical basis of quality because we might be loosing the most significant part of the structural information. One can wonder if more dynamical studies aimed to bring to light the exact nature and the strength of functional bonds could not be more helpful than static comparisons of electrophoregrams. For instance, further studies on proteic extractions by increased amounts of soaps (Kobrehel and Matignon, 1980), or on proteic modifications during technological process (Feillet *et al.*, 1977) could be recommended.

On the other hand, we think that pasta cooking quality consists not only of rheological characteristics (firmness, elasticity) but also of physical aspect of cooked pasta (absence of surface deterioration and of mushiness). If the former has been investigated for a long time, the chemical basis of the latter have not been really explored yet and should be considered apart from those of rheological characteristics. In both cases it could be assumed that proteins alone will fail for a total explanation of the observed phenomena and that new insights can be expected from other constituents like carbohydrates and lipids. Since the latter might have together oxydations with gluten proteins (Laignelet, 1979, 1981), a greater attention in the future should be payed on lipids composition and genetics as a further factor to explain varietal differences in quality.

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