

STRUCTURAL BASIS OF WHEAT HARDNESS AND TECHNOLOGICAL CONSEQUENCES*

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A b s t r a c t. The concept of grain hardness still remains to be fully elucidated. It was often mistaken for vitreousness and even for strength of a flour. In fact, hardness essentially depends on genetic origine of wheats and is defined as the more or less friable characteristics of endosperm. Consequently, hardness strongly influences the milling behaviour of wheats as well as the yield in each milling fraction, although the yield in total flour is not associated with kernel hardness. By acting on the degree of disaggregation of particles, granulometry and starch damage, hardness primarily affects flour hydration, especially in low-hydration doughs. However, hardness does not influence flour strength, which remains mainly determined by the composition in storage proteins. Taking into account the world-wide market, it is highly recommended to include hardness in the system of wheat grading.

Key words: wheat hardness, values of flour, milling

INTRODUCTION

The biochemistry of wheat hardness is one of the few subjects that remain, other the years, controversial and enigmatic. Although this statement by Pomeranz [23] still holds, it does not fully illustrate the difficulty experienced by the cereal industry in understanding the problem of wheat hardness. Hardness is a poorly defined term and there is still a degree of confusion between the terms wheat hardness, vitreousness, and even strength of wheat.

Vitreousness and hardness are the two terms used to characterize the texture and structure of the albumen. However, hardness is a mechanical property that does not result directly from vitreousness, which is an optical property. This can be demonstrated by comparing the mechanical properties of a durum wheat with those of a soft even completely vitreous wheat. In fact, vitreousness is closely linked to the growing conditions whereas hardness is a characteristic determined by the plant's genetic make-up. "Hardness" is also often confused with "strength", however, the strength of wheat is not related to its mechanical properties but to the functional breadmaking properties of the flour.

Several textural properties of wheat, as well as its behaviour when milled, depend directly on the hardness of wheat, in particular on how the fracture in the endosperm occurs, fragment size, and sifting behaviour. However, there is no terminology in France and some other European countries to distinguish between wheats in terms of their hardness. Anglo-Saxon terminology is used which has traditionally distinguished between "hard" and "soft" wheats. The absence of vocabulary is indicative

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of how little this characteristic is understood. As far as milling is concerned, distinguishing between soft and hard wheats would be useful.

Apart from making several general points, the aim of this article is to discuss the effect of wheat hardness on milling and on the different values of flour use, to examine current research, and make several recommendation.

THE EFFECT OF GENETIC AND AGRONOMIC FACTORS

Many different methods for measuring wheat hardness have been described, particularly in Anglo-Saxon countries [22]. Two methods are now in general use: the PSI and hardness measured using infrared spectroscopy. PSI involves grinding wheat under controlled conditions and measuring the percentage of the product that has passed through a sieve of 75 μ m [1]. With infrared spectroscopy, hardness is determined from ground wheat using an equation that takes into account 2 wavelengths of 1680 and 2230 nm [2]. These two methods are closely correlated. Figure 1 shows the relationship between the two methods for several French varieties. Whichever method is used, a scale of six to eight classes is used to evaluate hardness.

	PSI (%)
Extra soft	> 37
Very soft	31-36
Soft	26-30
Medium soft	20-25
Medium hard	16-19
Hard	12-15
Very hard	8-11
Extra hard	< 7

Figure 2 shows the effect of genetic and agronomic factors on wheat hardness. Hardness is a characteristic largely determined by genetics. Under the same agronomic conditions, the degree of hardness in the variety Delfi is systematically higher than that of the variety Artaban, which is, in turn, higher than that of Apollo. However, when nitrogen fertilizer applications are increased from 0 to 240 kg/ha, wheat hardness also increases. The response threshold showed that there was no change in hardness for applications of less than 50 units of nitrogen fertilizer and for applications exceeding 180 units, but that there was a clear change between 50 and 180 units of nitrogen fertilizer. This suggests that the structure of albumen in the endosperm changes as protein content increases [28]. This increase which can mean that samples pass into the next class does not undermine varietal classification.

MILLING BEHAVIOUR AND FLOUR YIELDS

Endosperm texture has a strong influence on the initial processing of wheat, particularly on its preparation, its behaviour at milling, and the final product's characteristics.

In the case of wheat preparation, hardness only has a moderate effect on the speed at which

Hardness PSI

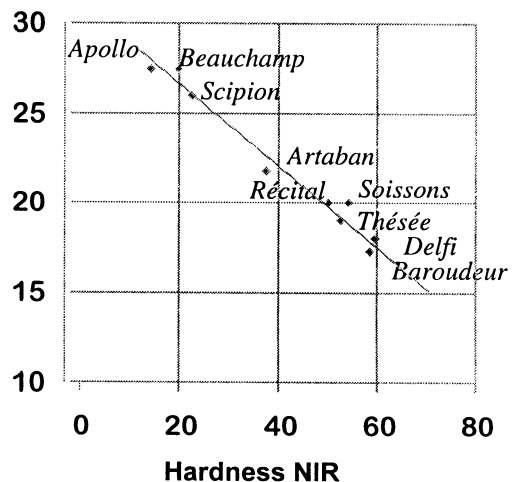


Fig.1. Relationship between PSI and NIR to evaluate grain hardness.

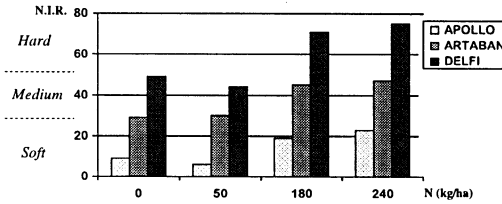


Fig. 2. Effect of genetic and agronomic factors on wheat hardness.

water penetrates the endosperm [17]. This depends more on the wheat's vitreousness and protein content. Protein-rich vitreous wheats have a greater hydration capacity but the speed of hydration is slower [25]. Therefore, protein-rich wheats require a higher water content and a longer resting time. However, when prepared industrially, conditions do not allow for the albumen texture to be modified so that all the endosperm have mechanical properties that are in the same state. Therefore, at milling, wheat behaves differently depending on its hardness.

The first significant difference caused by wheat texture can be seen in how the fracture occurs in the endosperm. When hard wheats are ground, the line of fracture follows the albumen cell walls, whereas with soft wheats, the fracture occurs across the cells [10]. During the last passes, when the splits are close to the aleurone layer, the separation between the kernel and the aleurone layer is clearer for hard wheat, but there is a risk that some of the husks are reduced to smaller fragments. For soft wheats, the fracture occurs across the cells and part of the albumen remains stuck to the husks, which sometimes limits bran purification.

There is a link between hardness and ease of sieving. Hard wheats give a granulometric spectrum of regular-shaped fragments that flow well. Soft wheats have a large number of very small fragments [6]. Below a certain hardness threshold the apparent density diminishes, which reduces fragment mobility and, as a result, reduces sieving quality. The presence of fragments of less than 10 μm is often considered to be the primary cause of a high degree of porosity and poor flowing properties.

Hard wheats require more energy to be milled into flour. According to Kilborn *et al.* [20], the energy required to mill a soft wheat is 12.9 Wh/kg and increases to 34.5 Wh/kg for a hard wheat (CWAD). Even if these values, which relate to laboratory milling, could be reduced in industrial-scale mills, the fact remains that milling hard wheats requires more milling machines and more energy.

The question of the effect of hardness on milling yield remains controversial. Reports from different authors often present contradictory results [7,9,12]. This subject was studied as part of the IRTAC programme on the milling and semolina qualities of wheat [3]. Figure 3 is a summary of the main results obtained. A principal component analysis (PCA) was carried out based on average values from nine varieties using four different nitrogen fertilizer application rates and average values for all the wheats with the same nitrogen fertilizer application rate. The four treatment plots appear on axis 1, which is closely correlated to protein content. The total flour yield is represented by the third bisector. The varieties are spread out along this axis, with hard varieties on the left and soft varieties on the right. The distribution of the different varieties along the axis representing total flour yield shows that hardness has no significant effect on total flour yield. However, there is a large difference in yield between varieties which cannot be explained by hardness or endosperm size. The variety Soissons is set apart from the other varieties, with yields exceeding 81%. On the other hand, the variety Apollo gives an average yield of 77.5%, which is considerably less than yields from the other varieties (average = 79.5%). In addition, the graph brings out the important differences in milling behaviour as a function of wheat hardness. These differences can also be seen in terms of the yield differences between the milling fractions, as in the case of coarse bran and grey short as well as the percentages of ground and fine reduction flour.

The yield of coarse bran after remilling is much lower for the hard varieties than for the soft varieties. This result corresponds to the

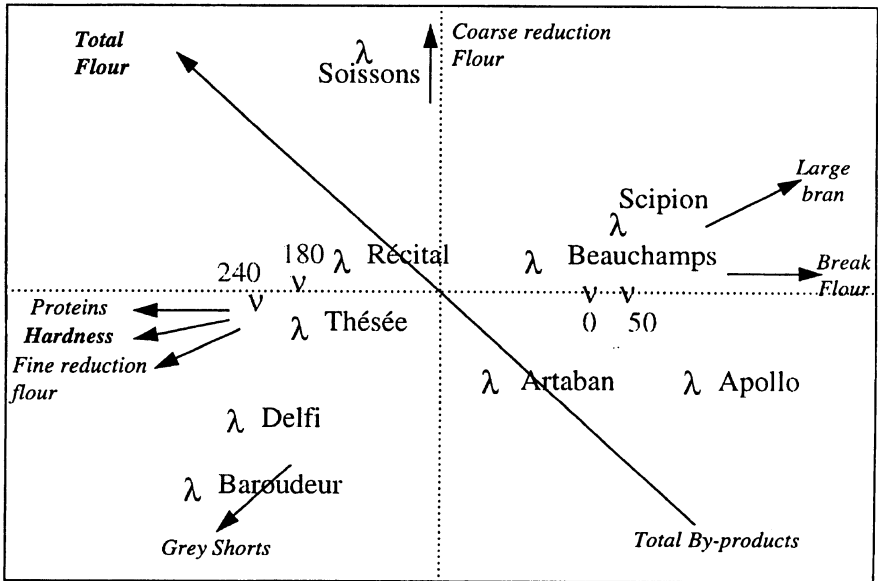


Fig. 3. Principal component analysis of the milling yield factors.

observation that the kernel separates more easily from the husks for hard wheats. However, this means that some of the husks are reduced to fine fragments which then contaminate the coarse and fine middlings. Soissons, a hard variety, behaves in a similar way to soft varieties probably because it has very elastic husks.

The relationship between the production of ground flour and fine reduction flour varies enormously depending on wheat hardness and growing conditions. With soft wheats, the percentage of ground flour and fine reduction flour obtained is virtually the same, whereas for hard wheats, the ground flour represents no more than a quarter of the yields of fine reduction flour.

The percentage of coarse reduction flour in relation to total flour also seems to be a good indicator of milling behaviour. For soft wheats, the yield of this fraction increases with increased nitrogen fertilizer applications, whereas it tends to decrease for hard varieties. This result supports other authors' findings which indicate that milling yield increases with increased nitrogen fertilizer applications for soft wheats, whereas it decreases for hard wheats [27].

To summarize, taking into account previous observations, a good milling wheat has an endosperm that behaves like a hard wheat when milled but has husks that remain elastic. At conversion, it behaves more like a soft wheat and its semolina can be easily reduced to flour. Although wheat hardness has a significant effect on milling behaviour and on the yields of different fractions, total flour yield does not really seem to be affected by hardness.

EFFECT OF HARDNESS ON THE VALUE OF THE DIFFERENT USES OF FLOURS

Can hardness be used as a criterion to determine the different end-uses of wheats and flours? In the USA, the classification of wheats in terms of their hardness is used particularly for flours that are destined for further processing. Thus, Durum wheats are used for pasta, Hard Red Spring wheats for Anglo-Saxon breadmaking, and Hard Red Winter wheats for other uses such as Oriental breadmaking, whereas Soft Red Winter wheats are for biscuit making. The other Anglo-Saxon countries, Canada and Australia, have organized their production and marketing following the USA's model. This model is accepted all over the world

and has become the dominant model in terms of international marketing. However, it cannot easily be applied in a country like France, or to Western Europe in general, where wheats are traditionally soft and have been developed over time to suit the breadmaking techniques used. In addition, attributing a value to the use of a wheat in terms of its hardness adds to the existing confusion that there is between hard wheat and strong wheat. It would be advisable to examine the real effect of wheat hardness on the properties of flour by looking, in particular, at the granulometry, the level of damaged starch, as well as the strength of flour.

As far as the granulometry of flours is concerned, the large differences between flours seem to depend on whether the flours are made from soft or hard wheat. As shown in Fig. 4, the soft varieties have a bimodal distribution, with the first mode at about 25 μm . This mode probably corresponds to endosperm with separate starch particles, whereas flours from hard wheats only have one mode at around 125 μm which corresponds more to cellular aggregates. As shown in Fig. 5, the flour's degree of separation depends, to a great extent, on the hardness of the wheat. Hence, for soft wheats, about 50% of total flour is smaller than 50 μm , whereas for hard wheats only 25% is smaller than 50 μm . These differences stem from varietal differences, as the variety Soissons, which has semolina that appears to separate easily during reduction, is not unusual and behaves just like other hard varieties. In addition, it is important to note that nitrogen fertilizer application has hardly any effect on the granulometry of total flour.

The effect of endosperm texture on the amount of starch damaged during milling is well known. Jones [19] examined two factors for damaged starch production: a "surface factor" which takes into account the abrasion of starch granules resulting from the cylinder surfaces and other fragments and an "internal factor" due to the forces exerted within the fragments by cylinder pressure. According to this author, the differences in the amount of damaged starch between hard and soft wheats is predominantly

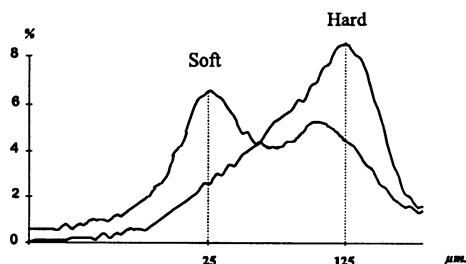


Fig. 4. Effect of hardness on the granulometry of flours.

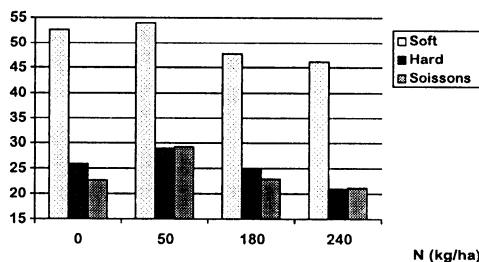


Fig. 5. Effect of hardness on the percentage of fine flour (<50 μm).

due to the internal factor. Greer and Stewart [15] and numerous authors since, have observed an increase in the amount of damaged starch with hard wheat flours. The results obtained in the IRTAC programme confirm these findings and show that the amount of damaged starch, like the granulometry of flours, is only slightly influenced by nitrogen fertilizer applications.

Therefore, the hardness of wheats appears to be an irreducible character that gives flours certain properties. However, the question remains to determine whether these properties are capable of modifying the strength of flours. Results from research carried out at INRA in Clermont Ferrand by G. Branlard [8] on more than 300 genotypes covering the whole range of hardness indicate that hard wheats have a W index, on average 80 points higher than those obtained from soft wheats. In so far as the value for W was taken to be one of the main criterion for registering varieties, it seems that breeders were tempted to use hardness as an indication of the strength of wheat. In fact, it is extremely difficult to control the heritability of the W index. On the other hand, it is known that

endosperm hardness depends on *Ha*, an important gene found on the short arm of the chromosome 5D. This explains why breeders have used the hardness criterion and its correlation with W index in their efforts to improve the strength of French wheats. The direct consequence of this research has led to a spectacular evolution in the hardness of wheats over the past few years, and now soft wheats represent only 20% of the varieties registered in the French catalogue compared with 75% at the start of the 1960s.

However, the real effect of hardness on the alveogram should be examined. From Fig. 6, it can be seen that the increase in W, observed as wheat hardness increases, is mainly due to an increase in the value of the pressure P. It is probable that this increase in pressure is more likely to result from a greater amount of damaged starch in hard wheat flours than from differences in the rheological properties of the proteins. In addition, there appears to be no link between hardness and elasticity measured by the value L on the alveogram. The increase in hardness, therefore, leads to an increase in the relationship P/L, and the consequences this has for the rheological properties of dough are well known.

In short, wheat hardness, which is a genetically-determined characteristic, has an important effect on the degree of particle separation and on the amount of damaged starch in the flour. The result is a marked modification in the hydration properties of the flour, particularly in the case of doughs that contain little water, such as biscuit doughs. Nonetheless, it is important to avoid confusing

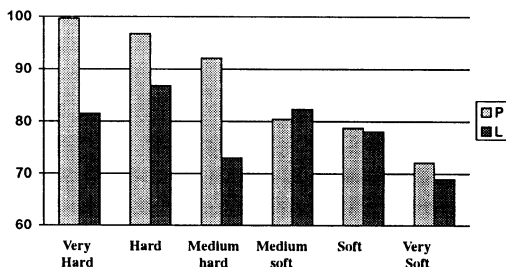


Fig. 6. Relationship between hardness and some alveogram indices.

wheat hardness with flour strength. The latter is, in fact, largely determined by rheological properties and by the composition of the flour's reserve proteins.

CURRENT RESEARCH

The aim of current research is to explain the physicochemical bases of wheat hardness. Four theories have been put forward:

Adhesion between the starch granule and the protein matrix

This hypothesis is the result of work carried out by Barlow *et al.* [4]. After having demonstrated that there was no difference in hardness between the proteins and starches of hard and soft wheats, they put forward the hypothesis that endosperm hardness was dependent on the strength of the bond between starch granules and proteins. This hypothesis suggests that there is a factor that controls the bond between starches and proteins. However, later research by Simmonds *et al.* [24] failed to shed light on this "cement" which would have explained the qualitative differences between wheats.

Continuity of the protein matrix

According to Stenvert and Kingswood [26], there is no need to resort to the adhesion theory to explain wheat hardness. It is a question of there being a noncontinuous protein matrix around the starch granules which significantly reduces the mechanical resistance of endosperm. Although quite plausible, this theory, which gives priority to the physical interactions between starch and proteins, puts more emphasis on the effect of environmental factors to the detriment of genetic factors.

Electrical charges of immature albumen proteins

This hypothesis, put forward by Doekes [11], is the least well known. According to this theory, the cause of the differences in hardness depends on the electrical charges of the proteins in the immature albumen. If the net charge of proteins is high, they will stick together and the endosperm will become soft. On the other hand, if the net charge is low, there will be no such repulsion and the endosperm will remain hard.

If this hypothesis is acceptable for proteins in solution, its validity has yet to be proved for endosperm where the water content drops rapidly from 40 to 12% during maturation [18].

Friabilin

The last hypothesis was proposed by Greenwell and Schofield [14]. They demonstrated the presence of a protein with a low molecular weight (15 kDa) which remains attached to the surface of starch granules when they are purified. This protein is not the cement that Simmonds *et al.* were searching for. On the contrary, it could be a protein with "anti-adhesive" properties (teflon protein) that supposedly weakens the link between the starch and the proteins and gives the endosperm its friable characteristic, hence its name friabilin. This hypothesis seemed very attractive because friabilin is lacking in hard wheats and hard wheats contain less of it than soft wheats. However, this hypothesis has not been confirmed. In fact, Greenwell [13], when using a monoclonal antibody to measure the overall content of friabilin in albumen, observed that there were similar quantities of friabilin in hard and soft wheats.

Even though none of the above four theories have been validated, it is clear that the answer to the question of hardness is to be found at the molecular interface between the starch granule and the protein matrix. It is a matter of understanding how this interface can be the site of a chemical difference that causes hardness properties in the albumen. What is not known is whether the nature of the link involves an adhesion factor that would therefore be more pronounced in hard wheats, or conversely, a repulsion factor that would prevent starch-protein adhesion.

In fact, understanding the starch-protein interface raises a large number of questions that each need to be approached in a specific way.

From the *physicochemical* point of view, little is known about the surface of starch granules. It is described as being like a "hairy billiard ball" [21] from which emerge chains of amylose and amylopectin, but the nature of the relationships between the starch granule and

other constituents is unknown. It could be a question of hydrogen bonds, which are easily broken when there is an excess of water, as is the case in the starch industry. However, hydrophobic interactions cannot be excluded because proteins are capable of bonding to the lipids that stick to the surface of starch granules when they are purified. In addition, the ionic bonds could also be linked to the sieving difficulties encountered with milled products from soft wheats.

From the *biological* point of view, differences in hardness between hard and soft wheats are quick to appear in the developing endosperm, although the exact moment when this starch-protein adhesion takes place is not clear [5]. In addition, the starch-protein interface is not as simple as is generally suggested. In fact, the possible role of remnants of endoplasmic reticulum, vesicles, and membranes that are visible until the fusion of protein bodies and that subsequently get stuck between the growing starch granules and protein matrix, could be investigated.

From the *mechanical* point of view, how can one explain the fact that the fracture always occurs along the cell walls in hard wheats, and across the cell walls and between the starch and the proteins in soft wheats, whatever the wheat's protein content?

Lastly, from the *genetic* point of view, the fact that there is a coincidence between the location of the hardness gene, the friabilin gene, and a factor regulating free polar lipids on the short arm of chromosome 5D could suggest that lipids have a bonding role in the hardness phenomenon.

Many specific questions remain unanswered. In order to answer them, a multidisciplinary approach seems necessary. A general approach could be to re-examine the old idea of interstitial protein described by Hess [16] using new methodologies that integrate: - physicochemistry and genetics, by developing methods for analysing each endosperm using monoclonal antibodies, immunocytochemistry, and isogenic lines in order to understand the nature of the starch-protein association;

- microspectrometry and spectral imaging to determine the chemical composition of small areas of albumen in order to understand the differences in chemical composition at the fracture's interface between hard and soft wheats;
- freeze-fracture electron microscopy to obtain new microstructural information on the developing albumen and in particular on what becomes of the protein bodies;
- lastly, the study of microfracture mechanics at the cellular level in order to establish a direct relationship between the mechanical properties of cellular aggregates and the available data on hardness for a single endosperm or a population of endosperm.

The integration of all the data into a global model could lead to a better understanding of hardness, and could also improve the efficiency of the fragmentation processes. In fact, these studies on fragmentation do not just concern flours, but also the development of non-food uses for cereals, for which it will no longer be a question of controlling fragmentation operations to the nearest 0.1 mm, which is the case now, but at the micron level in order to obtain fractions that are clearly defined histologically and have a purer biochemical composition.

CONCLUSIONS

Three key points can be drawn from this review on the state of the current knowledge of wheat hardness:

- Hardness is an extremely important genetic factor for wheat quality. It determines wheat milling behaviour and directly affects the granulometry of flours and their hydration properties. Nonetheless, this criterion does not determine overall wheat quality. On its own, hardness cannot be used to predict milling yield, strength or rheological properties of flours and doughs.
- In the world market context, Europe will have to adapt its wheat over the next few years to meet world market demands and at the same time continuing to satisfy its internal market. Given the rules that govern the international markets and the characteristics required for

wheat in terms of its end-use, it would be wise to consider hardness as an important criterion for classifying wheats. This would have at least two beneficial effects. Firstly, it would mean better adaptation of wheat to certain end-uses, for example to satisfy the requirements for biscuit-making or the demands of export markets. In addition, milling could be facilitated: instead of trying to find the perfect mixture of wheat to produce an average flour, it would be possible to make up flours with the required properties by milling batches of wheat with different properties.

Finally, further research into understanding the structural bases of hardness and fragmentation could make fragmentation operations more efficient and could help breeders improve the milling value of wheat.

REFERENCES

1. **AACC:** Particle size index for wheat hardness. Method 55-30, 1980.
2. **AACC:** Wheat hardness as determined by near infrared reflectance. Method 39-70A, 1989.
3. **Abecassis J., Willm C.:** Influence des facteurs génétiques et agroclimatiques sur la valeur meunière et semoulière des blés. Rapport final de Contrat, convention MESR-IRTAC n° 92 G 0544, 1996.
4. **Barlow K.K., Buttrose M.S., Simmonds D.H., Vesik M.:** The nature of the starch-protein interphase in wheat endosperm. *Cereal Chem.*, 50, 443-454, 1973.
5. **Bechtel D. B., Wilson J. D., Martin C.R.:** Determining endosperm texture of developing hard and soft red winter wheats dried by different methods using the single kernel wheat characterization system. *Cereal Chem.* 73(5), 567-570, 1996.
6. **Berg S.O.:** Is the degree of grittiness of wheat flour mainly a varietal character? *Cereal Chem.*, 24, 274-283, 1947.
7. **Bolling H., Zwinger G.H.:** Über die Relation der Korneigenschaften zum Mahlergebnis III Mitteilung: Der Einfluss der Variabilität der Kornhärte auf das Mahlergebnis. *Getreide Mehl.*, 15, 92-98, 1965.
8. **Branlard G., Felix I., Leblanc A., Koenig J., Bodet C., Marion D., Mahaut B.:** La Dureté des blés sélectionnés en France: Evolution et conséquences (soumis pour publication), 1996.
9. **Chesterfield R.S.:** Segregation of wheat varieties in New South Wales. Pages 100-108 in: Proc. Annu. Conf. 20th (Canberra). R. Aust. Chem. Inst. Cereal Chem. Div. Melbourne, 1970.
10. **Davis A.B., Eustace W.D.:** Scanning Electron Microscop views of material from various stages in the milling of Hard Red Winter, Soft Red Winter, and durum wheat. *Cereal Chem.*, 61, 182-186, 1984.

11. **Doekes G.J.:** Der zusammenhang zwischen elektrischen proteinladungen und der Kornhärte in weizen. Getreide Mehl Brot, 9, 259-263, 1985.
12. **Fowler D.B., De la Roche I.A.:** Wheat quality evaluation. II. Relationship among prediction tests. Can J. Plant Sci., 55, 251-262, 1975.
13. **Greenwell P.:** Programme CEE Eclair: To explore and improve the industrial use for EEC wheats. Newsletter 1, 1991.
14. **Greenwell P., Schofield J.D.:** A starch granule protein associated with endosperm softness in wheat. Cereal Chem., 63, 379-380, 1986.
15. **Greer E.N., Stewart B.A.:** The water absorption of wheat flour. Relative effects of protein and starch. J. Sci. Food. Agric., 10, 248-252, 1959.
16. **Hess K.:** Kolloid, 136, 84, 1954.
17. **Hinton:** Resistance of the testa to entry of water into the wheat kernel. Cereal Chem., 32, 296-306, 1955.
18. **Hoseney C.R.:** Wheat hardness. Cereal Food World, 32, 320-322, 1987.
19. **Jones C.R.:** The production of mechanically damaged starch in milling as a governing factor in the diastasic activity of flour. Cereal Chem., 17, 133-169, 1940.
20. **Kilborn R.H., Black H.C., Dexter J.E., Martin D.G.:** Energy consumption durin flour milling: Description of two measuring systems and the influence of wheat hardness on energy requirements. Cereal Chem., 59, 284-288, 1982.
21. **Lineback D.R., Rasper V.F.:**In «Wheat chemistry and Technology» (Y. Pomeranz Ed.) AACC, St Paul, USA, 277-372, 1988.
22. **Mahaut B.:** Dureté des blés: Méthodes de mesure; situation des blés français. Ind. des Cer. 81, 39 - 44, 1993.
23. **Pomeranz Y., Williams P.C.:** In «Advances in Cereal Science and Technology» (Y. Pomeranz Ed.) AACC, St Paul, USA, Vol 10, 471-548, 1990.
24. **Simmonds D.H., Barlow R.R., Wrigley C.W.:** The biochemical basis of grain hardness in wheat. Cereal Chem., 50, 553-562, 1973.
25. **Stenvert N.L., Kingswood K.:** An autoradiographic demonstration of the penetration of water into wheat during tempering. Cereal Chem., 53, 141-149, 1976.
26. **Stenvert N.L., Kingswood K.:** The influence of the physical texture of the protein matrix on wheat hardness. J.Sci. Food Agric., 28, 11-19, 1977.
27. **Williams P.C., Sobering D.C.:** Influence of variables on wheat hardness. Cereal Food World, 29, 498, 1984.
28. **Willm C.:** Comportement en mouture de 9 variétés de blé tendre: influence de la dureté et de l'apport azoté. Ind. des Cer., 92, 18-29, 1995. 2, 18-29, 1995.