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NOTE NOTE

HORDEIN COMPOSITION DIFFERENCES IN VARIOUS ANATOMICAL REGIONS OF THE KERNEL BETWEEN TWO DIFFERENT BARLEY TYPES

COMPOSITION DES HORDÉINES DE DIFFÉRENTES RÉGIONS HISTOLOGIQUES DU GRAIN CHEZ DEUX TYPES D'ORGES

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SUMMARY

Eight samples of flours were obtained by progressive pearling of barley kernels and their composition was determined by sequential extraction and electrophoresis (SDS-PAGE) of proteins.

A gradient was observed within the kernel of winter-types such as 'Sonja': aggregated B-hordein predominate in the outer endosperm, whilst central endosperm was especially rich in aggregated D-hordein. Such a gradient was not observed in 2-row spring-types such as 'Carina'. It was postulated that the distribution of the two classes of protein aggregates may contribute to explain the differences in malting performances between winter and spring barley types.

Key-words: *barley, hordeins, protein aggregates, malting quality.*

RÉSUMÉ

La composition protéique de huit échantillons de farines, obtenues par abrasion progressive de grains d'orge, a été étudiée par extraction séquentielle et par électrophorèse (SDS-PAGE).

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Un gradient de composition a été observé au sein du grain. Pour des types orges d'hiver tels que 'Sonja', les fractions B-hordéines agrégées prédominent dans les régions périphériques de l'albumen, tandis que les D-hordéines sont davantage concentrées dans l'albumen central. Un tel gradient n'a pas été observé dans les types orges de printemps tels que "Carina'. On a fait l'hypothèse que la distribution respective de ces deux classes d'agrégats protéiques au sein du grain pourrait contribuer à expliquer les différences de qualité maltière entre orges d'hiver et orges de printemps.

Mots clés : *orge, hordéines, agrégats protéiques, qualité maltière.*

1 - INTRODUCTION

The amount and composition of barley storage proteins (hordeins) play a major role in malting and brewing (BAXTER, 1980; SHEWRY and MIFLIN, 1983; MOONEN *et al.*, 1987; SKERRITT, 1988). The polypeptidic composition of hordeins, as appears from electrophoresis, gives a fingerprint of genotypes which is helpful for cultivar identification as described by MONTEMBAULT *et al.* (1983) or SMITH *et al.* (1986). However, unlike in wheats, no clear relationship has been established between specific electrophoretic bands and quality potential of barleys (MIFLIN *et al.*, 1983). It has been postulated by SLACK *et al.* (1979) and BAXTER (1981) that hordeins may form a persisting matrix around the starch granules and may restrict the hydrolysis of starch by amylases during mashing. Accordingly, the tendency of some hordein groups (B and D) to form an aggregate (SMITH and SIMPSON, 1983; SMITH and GILL, 1986) or a gel (SMITH and LISTER, 1983) could be especially relevant to the physico-chemical basis of malting quality: a higher tendency to form compact, or hydrophobic (and less soluble) complexes could be associated with an unsatisfactory endosperm degradation, which would result in a lower malting performance (MIFLIN *et al.*, 1983). Subsequent studies have been carried out (SMITH and LISTER, 1983; MARCHYLO *et al.*, 1986; MOONEN *et al.*, 1987; SKERRITT *et al.*, 1987; RASTOGI *et al.*, 1988; WALLACE and LANCE, 1988) to explain genetic differences in malting or brewing quality between barley types and permit more effective quality prediction at the breeding stages. However, no totally convincing evidence based on the physico-chemical or functional properties of hordeins has been given to support pioneer Baxter's suggestion. In this first short communication, we report a) the occurrence of two different aggregative levels in the anatomical layers of barley kernels, that respectively involve B-hordeins and D-hordeins, and b) a different behaviour of winter-type and spring-type cultivars. Currently, we are attempting to confirm these preliminary results by investigating hordein composition from a larger set of 2-row spring, 2-row winter and 6-row winter cultivars with a wide range of malting quality.

2 - EXPERIMENTAL METHODS

The barley genotypes used in this study consisted in the 2-row spring cv. 'Carina' and the 2-row winter cv. 'Sonja'. These cultivars were grown in the same location of the Yvelines departement.

From each cultivar, eight samples of flours roughly representative of successive anatomical layers of the endosperm were obtained by progressive removal of peripherical parts of the kernel using a pearler.

The proteins of these samples were fractionated by using one of the various sequential extractions described by LANDRY (1979), in which five fractions are respectively solubilized by the following solvents: 0.5 M NaCl (soluble proteins), 55% isopropanol (hordein-I), 55% isopropanol + 0.6% ß-mercaptoethanol (hordein-II), 1% acetic acid / 0.6% ß-mercaptoethanol (glutelin I), 0.5% SDS + 0.6% ß-mercaptoethanol (glutelin II), plus an insoluble residue.

The electrophoretic composition of each protein fraction was determined by the SDS-PAGE procedure developed by MONTEMBAULT (1982) and the ratio of the main bands or groups of bands was estimated by densitometric scanning according to AUTRAN *et al.* (1987). A special attention was paid to major storage fractions referred to as B-(sulfur-rich), C-(sulfur-poor) and D-(high molecular weight) species of hordeins (SHEWRY and MIFLIN, 1983).

The distinction between free (monomeric) and aggregated forms of hordeins was based on the determination of ethanol - soluble and alcohol + ME - soluble fractions, respectively, although we could not rule out that some B-hordeins might be present as aggregates which were soluble in aqueous isopropanol.

3 - RESULTS AND DISCUSSION

Table 1 illustrates the main results from two typical flour fractions (one central and one peripherical - the latter is likely to represent the subaleurone region) and from two barleys belonging respectively to winter (cv. 'Sonja') and spring (cv. 'Carina') types.

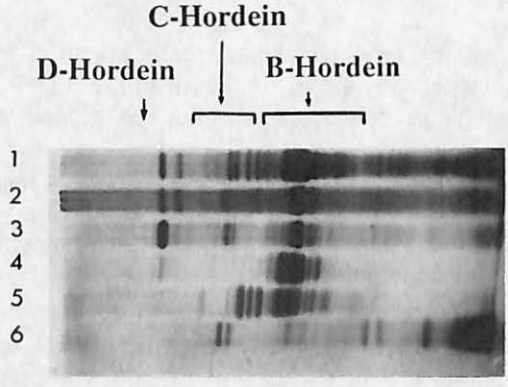
In cv. 'Sonja', peripherical fractions were characterized by a much higher protein content, a higher ratio of salt-soluble, alcohol + ME -soluble (hordein-II) and residual fractions while central fractions contained more alcohol-soluble (hordein-I) and SDS + ME-soluble fractions *(table Ia)*.

Hordein-I contained both B and C bands while hordein-II essentially contained B bands *(fig. 1)*. Unlike C bands that were easily extractable by isopropanol without reducing agent, B bands were present both in hordein-I and hordein-II fractions, and were likely therefore to occur in a wide range of molecular complexes, from "free" or "monomeric" forms (alcohol-soluble) to aggregated forms (soluble upon reduction only).

Table 1
Protein distribution among solubility groups and major hordein electrophoretic bands

Protein fraction		cv. "Sonja"		cv. "Carina"	
		Central endosperm	Peripherical endosperm	Central endosperm	Peripherical endosperm
Ia (in % of total proteins)					
Total proteins (in % d.b.)		8.8	18.0	6.8	19.8
NaCl-soluble		21.8	27.8	23.5	28.3
Isopropanol-soluble	(1)	17.3	10.7	18.9	10.8
Isopropanol + ME-soluble	(2)	10.1	16.5	16.5	13.3
Acetic acid + ME-soluble		12.1	8.3	13.1	6.2
SDS + ME-soluble		30.4	19.6	28.0	13.7
Residue		8.3	17.1	11.3	27.6
Ib (in % of total proteins)					
Total hordeins (B+C+D)		34.6	29.2	40.0	27.1
Total B-hordeins		21.1	21.8	28.7	20.0
Aggreg. B-hordeins		9.7	15.5	15.8	12.9
"Free" B-hordeins		11.4	6.3	12.9	7.1
C-hordeins		5.5	4.1	4.8	3.2
D-hordein		8.0	3.3	6.5	3.9
Ratio: Aggreg. B/"Free" B		0.9	2.5	1.2	1.8
Ic (in % of total hordeins)					
Aggreg. B-hordeins		28.0	53.1	39.5	47.6
"Free" B-hordeins		33.0	21.6	32.2	26.2
C-hordeins		15.9	14.0	12.0	11.8
D-hordein		23.1	11.3	16.3	14.4

(1) Hordein-I. (2) Hordein-II.



(1) Total proteins
(2) SDS + ME-soluble
(3) Acetic acid + ME-soluble
(4) Isopropanol + ME-soluble (hordein-II)
(5) Isopropanol-soluble (hordein-I)
(6) NaCl-soluble

Figure 1
SDS-PAGE patterns of the different solubility fractions from central endosperm of cv. "Sonja"

Interestingly, the D band although reported as an hordein type, was more especially present in the acetic acid + ME solvent (glutelin-I) and was also likely to be highly aggregated.

In agreement with RAHMAN *et al.* (1982), the proteins of peripherical regions, that were likely to be synthesized the latest, contain less C hordeins and a slightly higher ratio of B to C hordeins (a ratio that was described as very sensitive to environmental effects (GRIFFITH, 1987)). Other interesting results came from a detailed study of B and D fractions *(table Ib)*. Peripherical and central fractions contained similar amounts of total B-hordeins, but the former contained much more aggregated forms than the latter (ratios: aggregated/"free" = 2.5 and 0.9 respectively). On the other hand, the highly aggregated D-hordein band behaved more as a centre-specific fraction since its concentrations in outer and inner endosperm were respectively 3.3% and 8.0%, i.e. much more than previously reported by GRIFFITHS (1987).

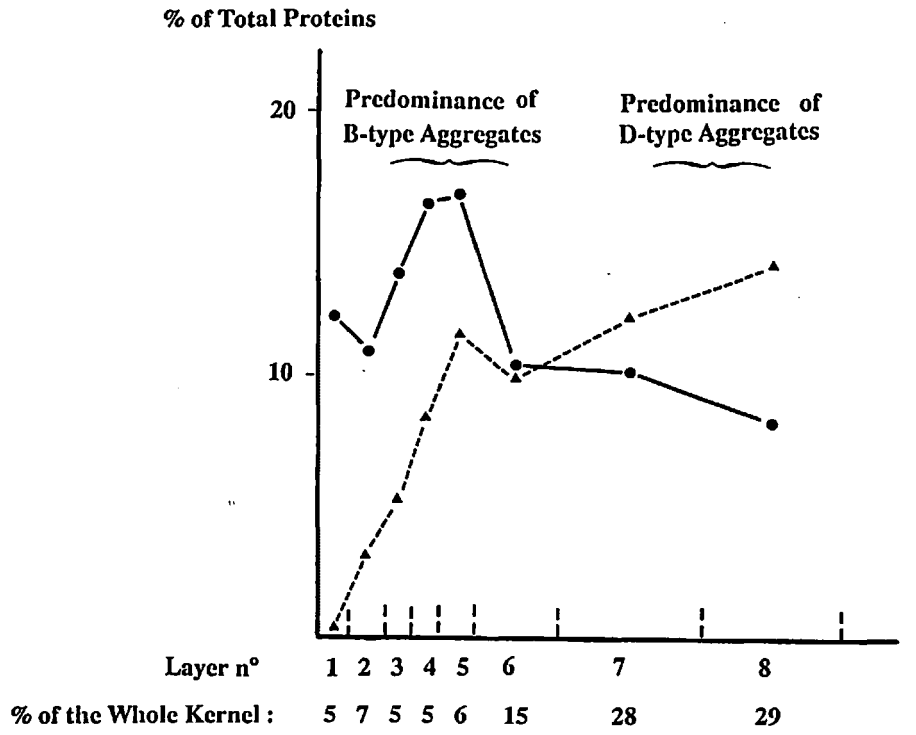


Figure 2
Nitrogen solubility distribution (in % of total proteins) in eight successive anatomical layers of the endosperm (cv. "Sonja")

Layers are numbered from 1 (outer endosperm) to 8 (central endosperm). The width of each interval corresponds to the ratio of the corresponding layer in total endosperm.

—●—●— *Isopropanol + ME-soluble fraction*
---▲---▲--- *Acetic acid + ME-soluble fraction*

Considering winter cultivars, such as cv. 'Sonja', and on the basis of the percent of total hordeins *(table 1c)*, hordeins of peripherical regions consist of as much as 53.1% of aggregated B and only 11.3% of D, while hordeins of central regions consist of 28.0% of aggregated B and 23.1% of D. From the solubility curves *(fig. 2)* of the hordein-II and glutelin-I extracted from the eight different layers of the kernel, it can be noticed that two regions containing aggregative fractions are clearly present: an outer one (possibly corresponding to the subaleurone layer) involving predominantly B-hordein aggregates and a central one, involving a high proportion of D-hordein aggregates.

Considering cv. 'Carina', or other 2-row spring-type cultivars, a different distribution of B and D aggregates is noticed: the peripherical endosperm contains less hordein-II fraction and less aggregated B-hordein bands than the central endosperm, and the D-hordein does not predominate in the central endosperm. The respective gradients of B-hordeins towards the outer region of the kernel, and of D-hordein towards the inner region were much flatter than in the winter cvs.

4 - CONCLUSION

Few significant results could be noticed in previous investigations on physico-chemical aspects of barley quality when considering total hordeins, ratio B/C, or hord-II/hord-I on whole kernel. In this study, the comparison of inner and outer parts of the endosperm emphasizes the differences and gives new insights on the basis of genetic differences in malting quality. To the extent that this approach permits, and subject to further studies from other sets of cultivars, grown in various environments and during several years, we hypothesize that the respective balance between monomeric or aggregated forms of B- and D-hordeins is an important factor of malting quality and that winter-type barleys may be characterized by a higher tendency of their B-hordeins to aggregate at the latest stages of grain development, compared to spring-type barleys. A higher ratio of B-type aggregates in the outer regions, impairing a rapid modification of the kernel could contribute to explain the lower malting performances generally observed in winter-type barleys.

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