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LIPID COMPOSITION OF BARLEY IN RELATION TO GRAIN SIZE.

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Abstract—With increasing grain size, higher proportions of neutral and phospholipids and a lower glycolipid proportion are found in the lipids extracted from six-rowed winter barleys, counting for at least part of the relationship between grain size and fatty acid composition. A positive correlation between grain size and proportion of non-starch lipid, observed only in the case of two-rowed barleys, explains the less pronounced variation of the fatty acid composition with varying grain sizes for this barley type.

INTRODUCTION

Total fatty acid composition of barley grains is known to be affected by genotype [1, 2] as well as by physiological [3] environmental [1] and agricultural [4] conditions. Since the observation of a significant relationship between barley grain size and fatty acid composition [5] the grain size distribution can be used for the separation of direct and indirect effects due to endogenous and exogenous factors. Indeed, every change in grain size distribution can result in a total fatty acid composition, which is more or less altered (Indirect effect). On the other hand, in studies on the Direct effects of endogenous or exogenous factors on barley fatty acids, one should be aware of the above relationship and analyses accordingly performed on fractions of samples obtained after standardised sieving. This is because a possible combination of direct and indirect variations may be present when samples with different grain size distribution are compared.

Regarding the possible origin of the relationships between grain size and fatty acid composition, a theoretical model based on the proportions of different lipid classes was suggested [5]. The best fit for the experimental results was obtained by a hypothetical model in which grains with greater size contained a lower glycolipid proportion and higher relative amounts of neutral and phospholipids [5].

To the best of our knowledge however, no data on a possible variation of lipid proportions with barley grain size have been reported. Therefore, the total barley lipids extracted from grains of different sizes are in this work fractionated into nonstarch and starch lipids, or alternatively into neutral (NL), glyco- (GL) and phospholipids (PL). All of these lipid fractions have a significantly different fatty acid composition. From the observed changes in lipid proportions with barley grain size and from the average fatty acid composition of each lipid class, the total fatty acid composition is estimated and compared with experimental data. The possession of this data allows a critical evaluation to be made of the theoretical model suggested previously [5].

RESULTS AND DISCUSSION

The results of triplicate fatty acid analysis of six barley varieties (two-rowed and six-rowed), fractionated according to grain size are shown in Table 1. The analysis of variance and Duncan-test were carried out on the data of different barley types. The bigger six-rowed barley grains have significantly higher proportions of palmitic (16:0) stearic (18:0) and oleic (18:1) acid and lower proportions of the poly-unsaturated fatty acids (18:2 and 18:3). These relationships are less significant in the case of two-rowed barleys, which is also in agreement with our previous findings [5]. Thus, the relationship between fatty acid composition and grain size holds for grains from two different growing seasons.

Successive extractions with cold (20°) and hot (100°) water-saturated *n*-butanol (WSB) yield the nonstarch and starch lipids respectively [6]. The remainder of the lipids, quantified as fatty acid methyl esters, are subsequently referred to as residual lipids.

From the separate analysis of the fatty acid composition of non-starch and starch lipids (Table 2A) it is clear that the relationship between grain size and fatty acid composition holds for both these fractions. Most of the increases in palmitic and stearic acid proportions with greater grain size can be ascribed to the starch lipid fraction whereas the rise of the oleic acid proportion is almost entirely due to its variation in the nonstarch lipid fraction. The negative relationship between grain size and proportion of linoleic acid is most significant in the case of the starch lipids.

In a typical experiment, the fatty acid composition of the nonstarch lipid fraction corresponds quite well to the total fatty acid composition. On the other hand, starch lipids contain significantly higher proportions of saturated fatty acids (16:0 and 18:0) and lower relative amounts of oleic, linoleic and linolenic acids. These results for barley starch lipids are in agreement with earlier reports on wheat [7]. It has been shown that starch lipids in wheat contain almost exclusively lysophospholipids, which are believed to exist as amylose-inclusion complexes throughout starch granules [3, 8].

Table 1. Mean total fatty acid (TFA) content and composition (in %TFA) of six barley varieties grown at one location in Belgium (results of triplicate analysis and Duncan-test).

Barley type	Size fraction	TFA (mg/g dry wt)	16:0	18:0	18:0	18:2	18:3
6-rowed	2.8	29.5 B*	21.6 A	1.9 A	13.5 A	57.1 C	5.7 C
($n=27$)	2.5	29.8 AB	21.3 AB	1.7 B	13.0 B	57.6 B	6.1 B
	2.2	30.4 A	21.0 B	1.5 C	12.8 C	58.2 A	6.4 A
2-rowed	2.8	27.8 AB	22.3 A	1.0 A	13.4 A	57.2 A	5.9 B
($n=27$)	2.5	27.4 B	22.2 A	1.0 A	12.3 B	58.1 A	6.3 AB
	2.2	28.5 A	22.3 A	1.0 A	12.0 B	57.6 A	6.8 A

*There was no significant difference between the means in the columns coded by the same letter (separate Duncan-test for each barley type) ($\alpha < 0.05$).

Table 2. A. Variation of nonstarch and starch fatty acid composition (as % of TFA) with grain size (average results of triplicate analysis of six barley varieties) B, variation of non-starch, starch and residual lipid proportions (expressed as % of total lipid content) with grain size (separate Duncan-analysis for 6-rowed and 2-rowed barleys)

		Grain size fractions 2.8	2.5	2.2
A. Fatty acid composition				
Non-starch	16:0	20.3 A*	20.3 A	20.4 A
	18:0	1.5 A	1.4 B	1.4 B
	18:1	14.2 A	13.3 B	13.0 C
	18:2	57.8 B	58.6 A	58.4 A
	18:3	6.2 C	6.4 B	6.8 A
Starch	16:0	37.7 A	36.9 A	34.7 B
	18:0	4.7 A	4.3 B	3.6 C
	18:1	9.2 AB	9.0 B	9.4 A
	18:2	43.4 C	45.0 B	46.9 A
	18:3	5.0 B	4.8 B	5.4 A
B. Lipid proportions				
6-rowed	Non-starch	77.2 A	76.1 B	76.0 B
	Starch	20.1 A	20.1 A	19.9 A
	Residual	2.7 B	3.8 A	4.1 A
2-rowed	Non-starch	74.0 A	71.6 B	68.9 C
	Starch	22.0 C	24.6 B	27.4 A
	Residual	3.9 A	3.9 A	3.7 A

*There was no significant difference between the means in the rows coded by the same letter (separate Duncan-test for each fatty acid proportion and lipid proportion) ($\alpha < 0.05$).

In Table 2B the variation of lipid distribution (NS, S and RES) with increasing grain size is shown for six-rowed and two-rowed barleys. In the case of six-rowed barleys, the proportions of NS, S and RES expressed as percentage weight on total lipids vary only slightly with grain size. On the contrary, significant changes are found in the lipid distribution of two-rowed barleys. Grains with greater size, have significantly higher non-starch and lower starch lipid proportions than the smaller ones, the residual lipid fraction remaining much unchanged.

In order to understand the consequences of this typical pattern of changes in the lipid distribution of two-rowed barleys, total fatty acid composition was estimated from the lipid proportions (Table 2B) and the mean fatty acid composition of nonstarch, starch and residual lipids from the data given in Table 2A. The results of these computations are shown in Table 3. Thus, increasing the non-starch lipid fraction, without altering the fatty acid compositions of the respective fractions, is anticipated to result in lower proportions of 16:0 and 18:0 and in higher proportions of 18:1 and 18:2 in the total fatty acid composition (Table 3A).

Table 3. Calculated variations in total fatty acid composition for two-rowed barley kernels with different sizes

	Size fraction	16:0	18:0	18:1	18:2	18:3
A. Calculated*	2.8	24.2	2.1	12.6	55.0	5.9
	2.5	24.7	2.2	12.5	54.7	5.9
	2.2	25.1	2.2	12.4	54.3	5.9
B. Calculated	2.8	25.0	1.9	13.0	54.2	5.7
	2.5	25.2	1.8	11.8	55.0	6.0
	2.2	24.9	1.9	12.0	54.6	6.4

*A. Effects from an alteration of the proportions of nonstarch and starch lipids without variations in fatty acid composition; B. Combined effects of varying non-starch and starch lipid proportions and a changing fatty acid composition with the kernel size.

The best fit for our experimental results is obtained by using a model that combines the effects of varying proportions of nonstarch and starch lipids, with the variation of the fatty acid composition of these fractions (Table 2) respectively. This explains why the original relationship between grain size and fatty acid composition is less pronounced in the case of two-rowed barley types, as shown in Table 3B.

Since the relation between grain size and fatty acid composition is more clear cut in the case of six-rowed winter barleys, further analytical work was concentrated on three varieties of this barley type.

The relationship between grain size and the neutral- (NL), glyco- (GL) and phospholipid (PL) proportions fractionated according to the grain size is shown in Table 4. All figures are the result of triplicate analysis of three varieties. As anticipated before [5], a significant decrease of the GL-proportion with increasing grain size is found. On the contrary, the NL- and PL-percentages are positively correlated with the grain size. All of these data are in definite support of our working hypothesis.

Table 4. Distribution of neutral, glyco- and phospholipids in the total lipids from barley kernels of varying size

Size fraction	% Neutral lipids	% Glycolipids	% Phospholipids
2.8	74.3 AB*	5.8 C	20.0 A
2.5	74.7 A	6.4 B	19.0 B
2.2	73.7 B	7.3 A	19.0 B

*There was no significant difference between the means in the columns coded by the same letter (separate Duncan-test for each barley type) ($\alpha < 0.05$).

Results of triplicate analysis of three six-rowed varieties and Duncan-test ($\alpha < 0.05$).

Nevertheless, we wish to point out that the interpretation of the relationships between fatty acid composition and grain size should be carried out in a most meticulous way. Indeed, the small analytical variations in lipid proportions contribute to the variations in the total fatty acid composition with grain size. At the same time it must be emphasised that the overall relationship between grain size and fatty acid composition holds more or less for each lipid fraction studied. This possibly implies that the fatty acid pool from which the several lipids are synthesized differs with the grain size as well.

EXPERIMENTAL

Mature seeds of six varieties were obtained from a field experiment by the Provinciaal Onderzoek- en Voorlichtings centrum Rumbeke-Beitem (Belgium) during the 1985–1986 growing season. The barleys were grown at the same location (Veurne, Belgium) and represented six-rowed winter barleys (Chips, Gerbel and Hasso), two-rowed winter barleys (Sonja) and two rowed spring barleys (Triumph and Friponne). Upon their arrival in the laboratory, the samples were stored in the dark at 5°. Each sample was fractionated according to grain width using a Steinecker separator (horizontal set of 3 sieves, aperture width 2.8, 2.5 and 2.2 mm, oscillating horizontally [5]. Grains of width less than 2.2 mm were discarded.

Samples of seeds (*ca* 50 g) were ground with a Tecator Cyclotec 1093 Sample Mill (0.4 mm sieve). Moisture content was determined in triplicate on the flour by heating 5 g for 2 hr at 120°.

Total content and composition of fatty acids, present as acylesters and unesterified fatty acids, were estimated in the flour by the direct methylation micro-method [9] as described previously [1, 4, 5].

The non-starch lipids were extracted with cold (20°) H_2O saturated *n*-butanol (WSB) during 10 min [6] After re-extraction with distilled MeOH to remove interstitial WSB and non-starch lipids, the true starch lipids were extracted with WSB, heated in boiling H_2O, with renewal of the solvent every hour (five successive extractions) [6]. After addition of an int. standard (heptadecanoic acid) the combined extracts were coned by rotary vacuum evapn and derivatised with 2% H_2SO_4 in distilled MeOH during 3 hr at 80°. After successive extraction of non-starch and starch lipids, the residual fatty acids were measured on the same flour by means of the direct methylation procedure (described above), after rinsing twice with MeOH.

Neutral (NL), glyco- (GL) and phospholipids (PL) were separated using prep. TLC on silica gel 60 G (thickness of absorbent layer 0.25 mm). The plates were developed (unidimensionally) with $CHCl_3$–Me_2CO–HOAc–H_2O (10:90:2:3) to 15 cm and consecutively with Et_2O–HOAc (99:1) to 18 cm after drying of the plates. Using this technique [6], PL are found between 0 and 0.5 cm, GL between 0.5 and 15.5 cm and NL between 15.5 and 18 cm. Bands were visualized under UV (254 nm) after spraying the plates with 0.05% 2,7-dichlorofluorescein in 50% EtOH. Lipid fractions were scraped off from the plates, derivatised with 2% H_2SO_4 in MeOH (in the presence of adsorbent) and quantified as FAME using GLC. Proportions of NL, GL and PL were calculated using the FAME conversion factors from ref. [6].

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