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CHANGES OF LIPID COMPOSITION IN MATURING BARLEY KERNELS

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Key Word Index—*Hordeum vulgare*; Gramineae; barley; seed ripening; fatty acids; protein content.

Abstract—During seed ripening, total fatty acid content (in mg per kernel) of spring barley rises to a maximum 37 days after anthesis (DAA), mainly due to an increase in the free lipid fraction. The structural lipid content increases until 24 DAA and declines further until maturity. Starch lipids appear only from nine to 14 DAA. During the final days of maturation, the contents of free and starch lipids decrease significantly, due to their involvement in metabolic processes of ripening. From these data it was deduced that the date of harvest becomes an additional source of variation for the lipid content and composition of mature barley kernels. With regard to the fatty acid composition of free, structural and starch lipids, a rapid rise in 18:2 during the first 14 DAA was balanced by a steep decrease of 18:3 and by minor changes in the proportions of other fatty acids.

INTRODUCTION

Studies on the variations in the fatty acid composition of mature barley kernels [1–4] have raised some questions concerning the accumulation of lipids during seed maturation. Indeed, if changes in lipid composition take place during the period before harvest, the date of harvest would become an additional source of variation for the lipid composition of mature barley grains.

Another reason for carrying out the present study was the observation of a relationship between barley fatty acid composition and kernel size [3, 4] and the possible similarity between the lipid composition of small mature grains and immature kernels. Little information is available on the composition of lipids and fatty acids of barley at different stages of ripening. Most papers on lipid accumulation during maturation concern wheat and oat because of their role in baking and in supplying metabolizable energy in animal feeds. To clarify the matter, three classifications of total cereal lipids, according to different criteria and described previously [5, 6] are shown in Fig. 1.

As a general rule, the main accumulation of cereal lipids (expressed as wt per kernel) occurs early in seed formation or while the seed still has more than 50% moisture content (wheat [6, 8]; oat [9]; barley [10]). In wheat, the major increase of total lipid content is due to the accumulation of storage lipids, located within spherosomes of the endosperm and germ, and to a sharp rise of monoacylphosphatidylcholine [8], located within the endosperm starch granules as lipid-starch inclusion complexes. The relative proportions of structural lipids (phospho- and glycolipids) decreases throughout maturation. In wheat and oats, changes in total fatty acid composition are characterized by a decrease in the proportions of 18:3 and by a sharp increase of the 18:2 content [8, 9, 11] during the early stages of maturation.

In this study, the variations in the composition of lipids and fatty acids, as well as in the contents of dry matter and protein, were determined during the ripening of different spring barley varieties.

RESULTS AND DISCUSSION

The average fresh weight and dry weight of the ears, as well as their moisture content (in %) and thousand corn weight (TCW) during the maturation of five spring barely varieties (1985–1986 growing season) is shown in Fig. 2.

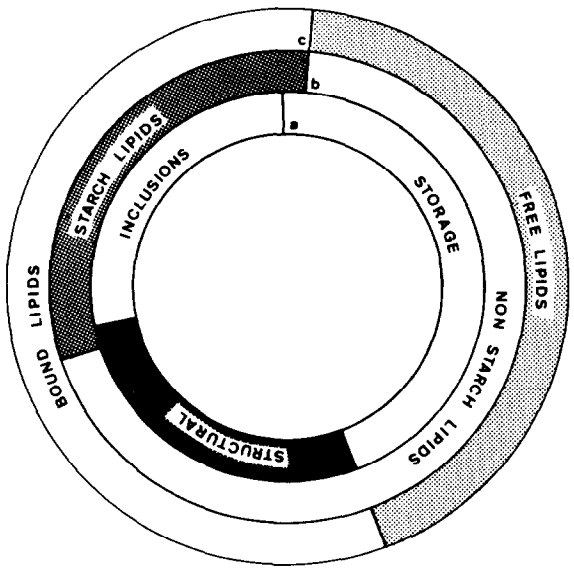

Fig. 1. Classification of total cereal lipids according to several criteria: (a) location and function; (b) extractability by cold and hot water-saturated *n*-butanol; (c) involvement in associations (membrane and organelle structures, lipid-starch inclusion complexes).

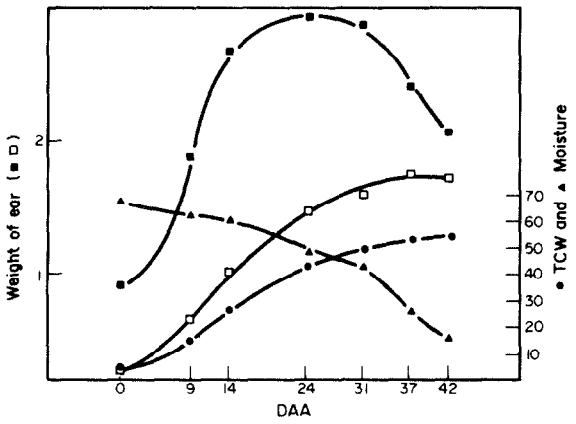

Fig. 2. Changes in fr wt (g), dry wt (g), moisture content (%) of ears and thousand corn weight (TCW) (g/1000 kernels) during the maturation of spring barley. Averages of five varieties, grown at one location (Neerhespen, Belgium) during the 1985–1986 growing season. (■), fr wt; (□), dry wt; (●), TCW; (▲), moisture.

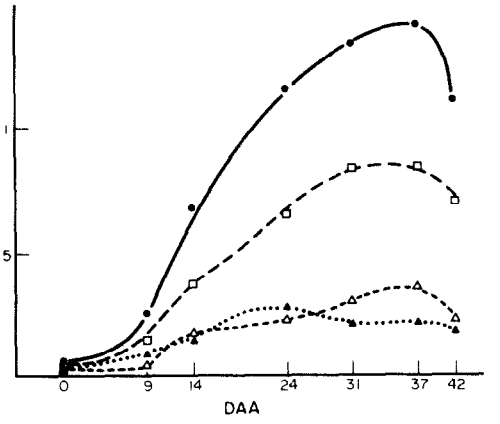

Figure. 3. Variations in the contents (expressed in mg per kernel) of total (●——●), free (□ – – □), structural (▲....▲) and starch (△ ---- △) lipids during the maturation of spring barley. Averages of three varieties, grown at one location (Neerhespen, Belgium) during the 1985–1986 growing season.

Similar to what is found for other seed types [8, 12], the accumulation of dry matter in the ear follows a three stage pattern. After a short first stage of increasing accumulation rate, the second phase is characterized by an almost linear increase of dry matter. During the third stage, dry weight decreases, as well as the absolute figures for fresh weight. In agreement with earlier observations [10], more than 90% of the kernel dry weight is synthesized during the first 30 days after anthesis. Moisture content decreases from 70% (anthesis) to 16% at the time of harvest (42 DAA). The variations of TCW during maturation are of course similar to those found for the dry matter accumulation.

Expressed in mg total fatty acid (TFA)/kernel (Fig. 3) total lipid content increases to a maximum 37 DAA. The decline during the final days of maturation confirms earlier findings for barley [10] and wheat [8] and it was suggested that this might be due to the involvement of lipids in the metabolic processes of ripening [8, 10]. Calculated on a weight per kernel basis, total weights of free and starch lipids increase throughout maturation, the structural lipids reaching a maximum 24 DAA and declining slowly to a value of 0.18 mg per kernel after 42 days. As to the metabolism of lipids during the preharvest period, our data show that principally the contents of free (storage) lipids and those associated in starch-lipid inclusion complexes show a significant decline. The protein content (data not shown) follows a two-stage pattern: there is a linear increase up to 6 mg/kernel 31 DAA, followed by a stage of slow protein accumulation which continues until the time of harvest. In contrast to what is mentioned above for the lipids, apparently no net-protein becomes metabolized towards the end of the maturation, which is also in agreement with earlier findings [10].

Expressed in terms of relative amounts (% on dry matter), (Table 1) the protein content rises until 31 DAA and then remains constant at *ca* 12%. Like the total lipid content, the percentage of storage lipids increases during maturation, while the amount of structural lipids decreases. The latter can be explained by the fact that after *ca* two weeks, further kernel growth occurs by cell expansion and accumulation of other cell components overrules the synthesis of membrane structures.

A sharp rise in the proportion of starch lipids occurs between nine and fourteen DAA. This fact strongly suggests that starch lipid accumulation occurs simultaneously with the deposition of starch in the endosperm.

Table 1. Variation of moisture, protein and lipid (total, free, structure and starch lipids) contents (expressed as % on dry wt) of barley kernels during maturation

DAA	Moisture	Protein	Total	Free	Structure	Starch
0	69.5	n.d.*	1.2	n.d.	n.d.	n.d.
9	64.2	n.d.	1.7	0.9	0.6	0.2
14	61.9	10.2	2.6	1.4	0.6	0.6
24	49.8	10.6	2.7	1.5	0.7	0.5
31	44.1	11.6	2.7	1.7	0.4	0.6
37	26.6	11.5	2.7	1.6	0.4	0.7
42	16.2	11.7	2.1	1.3	0.3	0.4

*n.d.: not determined.

In spring wheat endosperm [12], cell divisions cease and starch deposition begins at *ca* day 14.

Changes in total fatty acid composition of developing barley kernels (Fig. 4) are very similar to those described earlier for wheat [8] and oat [9]. During the first two weeks after anthesis, a steep rise in the proportion of 18:2 is balanced by a decrease in that of 18:3. However, when these data are expressed in terms of mg per kernel, a net increase is found in the amount of each fatty acid, the main increase occurring in the case of 18:2.

Changes of the average fatty acid composition of free, structural and starch lipids during seed ripening are shown in Table 2. The differences in fatty acid composition between these lipid classes, known for mature cereal seeds can be recognized at each stage of ripening. Free lipids are rich in 18:1 and 18:3, while structural lipids contain on the average more 16:0 and 18:2. Starch lipids, consisting of more than 80% of lysophosphatidylcholine [5], are characterized by a high content of 16:0 and 18:0 and a relatively low percentage of 18:2 and 18:3.

During maturation, free lipids show a slow decrease in the proportion of 16:0 whilst those of the structural and starch lipids have a minimum at 24 and 14 DAA, respectively. As to the 18:0, 18:2 and 18:3 proportions of the three lipid fractions the variations during maturation are very similar, suggesting that these are a reflection of changes in the fatty acid pool from which the different lipids are synthesized during seed ripening.

This study has shown that during the pre-harvest period the contents of free and starch lipids decrease significantly, due to a possible involvement of these lipid classes in the metabolic processes of ripening. Structural lipids on the contrary, are much less affected during this stage of maturity. The principal changes in fatty acid composition take place during the first two weeks after anthesis, as described above. The conclusion to be drawn here is that the date of harvest and the growth conditions during the pre-harvest period, represent additional sources of variation for the lipid content and composition of mature barley grains.

A comparison of the general patterns of lipid changes during seed maturation to the variations observed between lipids of mature barley seeds with different sizes

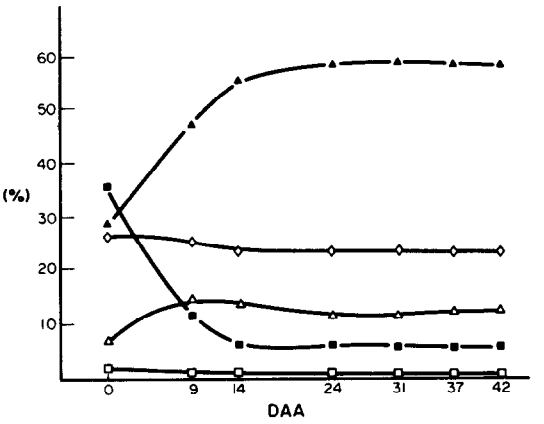

Fig. 4. Changes in total fatty acid composition (in % wt) during the ripening of spring barley kernels. Averages of four varieties, grown at one location (Neerhespen, Belgium) during the 1985–1986 growing season. (◇), 16:0; (□), 18:0; (△), 18:1; (▲), 18:2; (■), 18:3.

Table 2. Changes in fatty acid composition (in % weight of total fatty acid) of free, structural and starch lipids during maturation of barley kernels*

Lipid	DAA	16:0	18:0	18:1	18:2	18:3
Free lipids	0	24.3	3.7	12.6	36.4	22.2
	9	21.9	1.9	18.9	45.3	11.4
	14	20.7	1.4	13.9	55.6	8.1
	24	21.0	1.3	14.8	55.9	6.8
	31	20.5	1.1	13.3	58.2	6.7
	37	20.4	1.1	13.4	58.0	6.7
	42	20.4	1.1	13.6	58.1	6.6
Structural	0	n.d.	n.d.	n.d.	n.d.	n.d.
	9	28.8	2.1	11.2	48.1	9.9
	14	26.7	1.6	7.0	59.6	6.1
	24	21.1	1.5	6.4	61.6	5.7
	31	27.5	1.5	5.6	60.2	5.1
	37	25.5	1.3	8.1	60.9	4.2
	42	24.2	1.3	7.6	62.4	4.1
Starch	0	36.7	11.1	7.7	25.6	18.2
	9	40.6	7.6	11.3	23.5	6.6
	14	34.7	2.5	10.4	48.1	3.9
	24	36.0	2.7	11.0	46.3	3.8
	31	37.7	2.5	8.3	48.2	3.2
	37	41.0	2.6	7.4	48.2	3.7
	42	40.1	2.5	8.2	45.3	3.8

*Averages of three varieties, collected at seven different stages of ripeness (expressed in DAA, days after anthesis) and analysed in triplicate.

shows that the lipid and fatty acid composition of a small mature barley grain are quite different from those of an immature kernel. Major points of similarity are restricted to the higher 18:3 percentage in all lipid classes, the higher neutral and the lower glycolipid proportions as well as the lower relative amount of free (storage) lipids in small mature and immature barley seeds, compared to full-sized mature grains. All other parameters measured in this study vary in a completely different way, so that it can be concluded that, as far as lipid data are concerned, the small mature kernels cannot be regarded as incompletely developed ones.

EXPERIMENTAL

At seven different stages of maturity, ear samples from five spring barley varieties (Atem, Apex, Friponne, Kym and Iban) were obtained from a field experiment by the AVEVE (Neerhespen, Belgium) during the 1985–1986 growing season. Upon their arrival in the laboratory, intact ears were weighed before and after drying for 24 hr at 70°. After threshing manually, the thousand corn wt of the grains was determined. Samples were stored in the dark at 5° until further analysis. 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°. N was determined in duplicate by a macro-Kjeldahl procedure, followed by a dist. into a 1% boric acid soln. The factor 6.25 was used to convert N to protein. Total content and composition of fatty acids, present as acyl esters and unesterified fatty acids, were estimated in the flour by the direct methylation micro-method [13] as described previously [1–4]. The non-starch lipids, i.e. free lipids, were extracted with cold (20°) H_2O *n*-BuOH (WSB) during 10 min [7]. After re-extraction with MeOH to remove interstitial WSB and non-starch lipids, the starch lipids were extracted with WSB, heated in boiling H_2O, with renewal of the solvent every hr (five successive extractions) [7]. After addition of int. st (17:0) the comb exts. were concd by rotary vacuum evapn and derivatized with 2% H_2SO_4–MeOH during 3 hr at 80°.

Neutral (NL), glyco- (GL) and phospholipids (PL), present in the non starch lipid exts, were sepd by prep. TLC on silica gel 60 G (0.25 mm). Plates were developed 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 [7], 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 can be 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, derivatized with 2% H_2SO_4–MeOH (in the presence of adsorbent) and quantified as fatty acid Me esters using GC. Proportions of NL, GL and PL were calcd using the conversion factors from ref. [7]. The NL fraction of the non starch lipids consisted of free lipids, while GL and PL fractions represented the structural lipids (cf. Fig. 1).

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