Phytochemistry, Vol. 25, No. 4, pp. 811–815, 1986.
Printed in Great Britain.

0031-9422/86 $3.00 + 0.00

CHANGES IN THE LIPID COMPOSITION OF DEVELOPING WHEAT SEEDS

DAVID N. STOKES, TERENCE GALLIARD* and JOHN L. HARWOOD

Department of Biochemistry, University College, Cardiff CF1 1XL, U.K.; *RHM Research, High Wycombe, Bucks HP12 3QR, U.K.

(*Revised received* 20 *September* 1985)

Key Word Index—*Triticum aestivum*; Gramineae; wheat; acyl lipids; fatty acids; developing seed.

Abstract—The accumulation of different acyl lipids in developing wheat seeds was studied. There were initial decreases in the relative amounts of phosphatidylcholine and diacyldigalactosylglycerol and increases in neutral lipid (mainly triacylglycerol). Later developmental stages showed a sharp rise in monoacylphosphatidylcholine which was confined to the endosperm fraction. The component fatty acids of individual lipid classes were analysed and particular distributions and changes noted. In general, the changes in total fatty acids were characterized by a decrease in linolenate and an increase in linoleate, the major fatty acid of the mature wheat grain. Particular features of lipid accumulation in the endosperm and in the pericarp plus testa fractions, which were analysed separately, are discussed.

INTRODUCTION

Most studies on seed lipids have concentrated on commercially important oil-rich seeds such as rape (*Brassica napus*) and soya bean (*Glycine max*), consequently relatively little is known about the accumulation of lipids in cereal seeds. However, lipids have important functions in the developing and germinating wheat grain [1]. They affect many aspects of wheat and flour technology [2] and can provide useful quantities of dietary essential fatty acids [3]. Lipids constitute 2.5–3.5% of the dry weight of wheat flour and it is well established that the major lipid constituents of wheat starch are the monoacylphosphoglycerides [2]. The distribution of acyl lipids in the principal parts of mature wheat kernels has been reported [4] and a microscopic study revealed that triacylglycerol deposits in the starchy endosperm were located in discrete oil droplets which may be spherosomes [2]. However, there is little information on the accumulation of lipids in developing wheat seeds. Thus, because of the importance of wheat lipids in processes such as milling, flour storage and baking, we have studied their accumulation in developing seeds. Preliminary data from some aspects of these studies have been reported [5, 6].

RESULTS AND DISCUSSION

Changes in the weights of individual developing wheat seeds are compared with lipid content at different stages of development in Figure 1. As expected, the alterations in fresh weight of seeds follow three distinct phases. In the first stage (0–20 days after anthesis, DAA) a gradual increase in weight is seen. In the second stage (20 to about 35 DAA) weight accumulation is rapid, whilst in the third stage, water loss accounts for a decrease in fresh weight. The pattern of changes in lipid accumulation is very similar to that in seed dry weight during the period of seed development studied. However, on storage the harvested seeds exhibited some loss of total lipid content (Fig. 1) presumably due to acyl lipid metabolism. The accumulation of wheat grain lipid in three phases is similar to that reported for other seed types [7].

The total fatty acyl composition of developing wheat seeds was examined (Table 1). At the earliest developmental stage analysed, palmitate, linoleate and α-linolenate were the principal acyl moieties. With increasing maturation, the major changes were the relative increase in linoleate and decrease of α-linolenate. It should be born in mind that, although the relative proportion of α-linolenate decreased from 25 to 6% of the total acyl moieties, in actual amounts α-linolenate increased on a per seed basis, because of net lipid synthesis (Fig. 1). However, it is clear that mature wheat seeds, in common with other cereal grains [8], contain linoleate and palmitate as their major acyl moieties. It is also noteworthy that the post-harvest decrease in seed total fatty acids (Fig. 1) applies equally to all types (Table 1).

There were several notable changes in relative proportions of acyl lipids in developing wheat grains (Table 2). Initial decreases in the relative amounts of phosphatidylcholine and diacyldigalactosylglycerol were accompanied by an increase in neutral lipid (mainly triacylglycerol). During the period studied there were rather small changes in the amounts of other lipids with the exception of a large increase in the amount of monoacylphosphatidylcholine in the final stages of development. Much of the latter lipid is located within the starch granules, where it represents about 85% of the total lipid [3]. The relative increase in the proportion of monoacylphosphatidylcholine in post-harvest seeds (Table 2) is consistent with its presumed metabolically inert nature in ungerminated seeds. In contrast, triacylglycerol, which has been shown in wheat to be located in spherosomes within both embryo and endosperm tissue [9], undergoes a significant decrease after a maximum percentage at 37 days after anthesis (Table 2). The acyl lipid composition of mature wheat seeds (var *Sicco*) in the present study can be compared with previous data for four other wheat varieties [4] in which triacylglycerol and monoacylphosphatidylcholine were also the major constituents, with diacyldigalactosylglycerol and phosphatidylcholine being significant components.

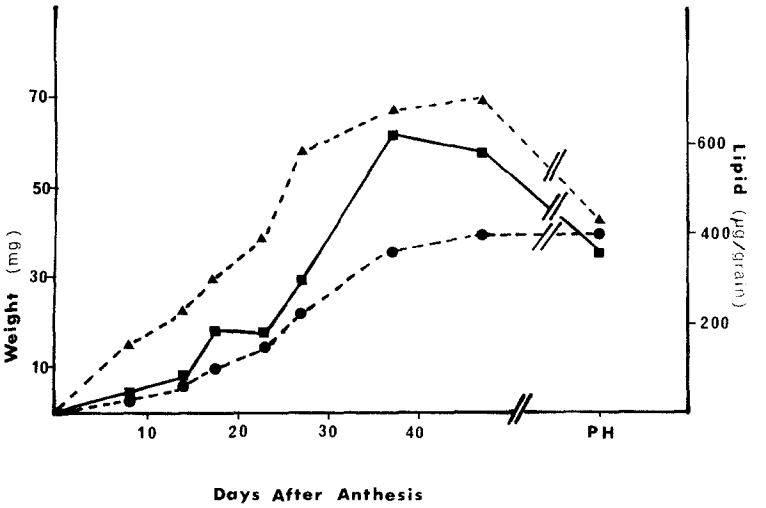

Fig. 1. Changes in fresh weight, dry weight and total acyl lipid (measured as FAME) content of developing wheat grains. Figures represent the average of five determinations of weight. Fatty acid analysis was carried out in triplicate. ▲- - -▲, fresh weight; ●- - -●, dry weight; ■——■, total fatty acid content.

Table 1. Changes in the total fatty acyl composition of developing wheat seeds

Days after anthesis	Fatty acid composition (% total fatty acids) 16:0	18:0	18:1	18:2	18:3
8	28	2	11	34	25
14	28	1	16	36	19
17	23	1	15	41	20
23	29	1	13	44	13
27	26	1	14	49	10
37	24	2	13	54	7
47	27	tr	6	61	6
PH	27	1	9	59	4

Total lipids were extracted from > 0.9 g fresh weight of seeds and fatty acid methyl esters prepared and quantified as described in the Experimental. Abbreviations: 16:0 = palmitic acid; 18:0 = stearic acid; 18:1 = octadecenoic (> 99% oleic) acids; 18:2 = octadecadienoic (> 99% linoleic) acids; 18:3 = octadecatrienoic (> 99% α-linolenic) acids; PH = post-harvest seeds; tr = trace (< 0.5%).

The fatty acyl components of the major seed lipids were analysed and results for three representative developmental stages are shown in Table 3. Initially, membrane lipids such as phosphatidylcholine, phosphatidylethanolamine and diacyldigalactosylglycerol were highly unsaturated and only the neutral lipids had small amounts of α-linolenic acid. This enrichment of α-linolenate is typical of the lipids of most plant membranes [10]. During grain development, all lipid classes showed a relative decrease in α-linolenate content and an increase in linoleate. The fatty acyl composition of all major lipid types in the post-harvest seeds was rather similar (Table 3) with linoleate and palmitate accounting for 80–90% of the total acyl moieties.

The endosperm and the pericarp plus testa fractions were dissected and analysed separately. Most lipids increased substantially in amounts in both fractions during development. In contrast, the quantities of diacyldigalactosylglycerol, phosphatidylglycerol and phosphatidylcholine in the pericarp plus testa fraction changed little during grain development between 17 and 27 DAA (Table 4). The increases in amounts of neutral lipids (mainly triacylglycerol) in both fractions and of phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol and monoacylphosphatidylcholine in the endosperm fraction were particularly large. Neutral lipids were by far the most important lipids in the pericarp plus testa fraction by 27 DAA. In the endosperm fraction while neutral lipids accounted for half of the total lipids, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine and monoacylphosphatidylcholine each represented 7–18%.

It is interesting to compare the results with those of Tan and Morrison [11] for maize. The maize and wheat plants in these studies took approximately the same time to reach maturity (Fig. 1 and ref. [11]) so that it is possible to compare the data directly. Over the equivalent period of seed development, the pericarp of maize showed an increase in triacylglycerol content with little change in the other lipid classes with the exception of acylsterylglycoside which was reduced. Acylsterylglycoside was not a major component of wheat pericarp lipids. In the endosperm of developing maize, the increases in triacylglycerol, digalactosyldiacylglycerol, phosphatidylcholine and monoacylphosphatidylcholine paralleled those in wheat (Table 4). Points of difference were that the phosphatidylethanolamine content of maize endosperm remained unchanged in the period 9–23 days after pollination and that the level of unesterified fatty acids rose suddenly between 23 and 36 days after pollination [11]. In general, however, there were many similarities between the changes in the lipid contents of the endosperm and the pericarp fractions for the two cereal types (Table 4 and ref. [11]).

When the fatty acids of individual lipid fractions from

Table 2. Changes in the acyl lipid composition of wheat grains during development

Lipid class	Days after anthesis (% total acyl lipids)							
	8	14	17	23	27	37	47	PH
LysoPC	4	5	6	16	9	10	14	29
PC	37	10	12	4	5	4	6	10
PG	13	19	11	10	6	5	4	5
PE	11	8	6	4	7	3	5	9
Neutral	13	32	51	50	64	68	59	30
DGDG	13	8	6	12	2	1	1	4
PI/PS	4	6	1	2	2	3	5	6
Others	5	12	7	2	5	6	6	7

Lipids were extracted from > 1.2 g fresh weight of seeds and analysed as detailed in the Experimental. Results are the average of four determinations corrected to whole figures. Abbreviations: lysoPC = monoacyl phosphatidylcholine; PC = phosphatidylcholine; PG = phosphatidylglycerol; PE = phosphatidylethanolamine; neutrals = neutral acyl lipids (> 94% triacylglycerols); DGDG = diacyldigalactosylglycerol; PI/PS = phosphatidylinositol + phosphatidylserine; PH = post-harvest seeds.

Table 3. The fatty acid composition of major lipid classes at different development times in wheat grains

Lipid	Age (days after anthesis)	Fatty acid composition (% total) 16:0	18:0	18:1	18:2	18:3
Neutral	8	42	6	12	29	11
lipids	23	26	1	16	46	11
	PH	22	tr	12	62	4
DGDG	8	28	n.d.	12	24	36
	23	25	1	8	35	31
	PH	18	1	5	71	5
PE	8	12	1	7	23	57
	23	21	n.d.	9	53	17
	PH	15	n.d.	7	75	3
PC	8	30	n.d.	14	38	22
	23	44	1	10	36	9
	PH	18	n.d.	15	62	5
lysoPC	8	28	2	11	34	25
	23	29	1	13	44	13
	PH	27	1	9	59	4

For abbreviations see the legends to Tables 1 and 2. n.d. = none detected.

the endosperm or pericarp plus testa fractions were analysed (Tables 5 and 6) some differences were seen during seed development. In the pericarp plus testa fraction, all lipids showed a decrease in the relative proportions of α-linolenic acid during the developmental period from 14 to 27 DAA. In most lipid fractions, this decrease in percentage of α-linolenate was balanced by an increase in the relative percentage of linoleate (Table 5). In contrast, the proportions of palmitate and oleate were relatively unaffected by seed age during the developmental period 14–27 DAA. Diacyldigalactosylglycerol and phosphatidylethanolamine contained the largest percentage of linolenic acid at all times, whereas palmitic acid represented about one third of the acyl groups of diacyldigalactosylglycerol, phosphatidylcholine and monoacylphosphatidylcholine. As expected, the proportions of linolenic acid were highest in membrane lipids rather than neutral (storage) lipids, especially at earlier developmental times (Table 5).

When the fatty acids of individual lipids from the endosperm of developing wheat grains were analysed, a decrease in α-linolenate and a corresponding increase in linoleate was again seen (Table 6). Most acyl lipids contained larger proportions of linoleate and smaller amounts of α-linolenate in the endosperm (Table 6) when compared to the pericarp plus testa fraction (Table 5). Other fatty acids were present in similar amounts in the individual acyl lipids from the two fractions. As pointed out above the increase in total lipid per grain (Fig. 1) means that the relative decline in α-linolenate with development is most probably due to reduced rate of synthesis rather than to any degradation. Furthermore, the greater content of α-linolenate in the pericarp plus testa fraction (especially at early times) probably derives from the photosynthetic capacity of the pericarp at early stages of development.

In general, the changes in total fatty acids of developing wheat seeds are characterized by a decrease in linolenate, the typical photosynthetic membrane fatty acid [10] and an increase in linoleate, the predominent fatty acid in mature wheat seed [8]. The data for total lipid accumulation agree with typical profiles for other maturing seeds [7, 12], showing a rapid rise in fat during a mid-development period. In addition, analysis of the lipid composition of endosperm and of pericarp plus testa fractions show many features in common with the distribution of lipids in maize seeds [11]. Particular features are the importance of triacylglycerol in the pericarp plus testa fraction and the accumulation, in addition, of monoacylphosphatidylcholine in the endosperm fraction. Although some preliminary work has been carried out on the biosynthesis of acyl lipids in developing wheat seeds (cf. [5, 6]), it would be both interesting and important to learn more about the control of lipid formation in such a tissue.

Table 4. Comparison of the lipid composition of the endosperm with that of the pericarp plus testa fraction in developing wheat grains

Age (days after anthesis) Lipid class	Fraction	14	17	23	27
		Lipid mass (μg total fatty acids/grain)			
Neutral	P+T	8.8 (32)	34.5 (48)	48.9 (57)	112.6 (76)
lipids	E	2.6 (36)	22.2 (42)	32.4 (39)	62.8 (49)
DGDG	P+T	1.6 (7)	7.0 (10)	10.0 (12)	6.3 (4)
	E	0.5 (7)	2.8 (5)	5.6 (7)	3.5 (3)
PE	P+T	1.8 (8)	2.9 (4)	4.8 (6)	6.9 (5)
	E	0.5 (7)	3.6 (7)	5.5 (7)	10.3 (8)
PG	P+T	2.2 (10)	9.3 (13)	8.4 (10)	6.0 (4)
	E	1.0 (14)	7.8 (15)	6.6 (8)	8.7 (7)
PC	P+T	5.0 (22)	8.2 (11)	5.1 (6)	4.4 (3)
	E	0.9 (13)	5.3 (10)	8.6 (10)	12.1 (9)
lysoPC	P+T	1.7 (7)	5.4 (7)	6.2 (7)	6.4 (4)
	E	0.2 (3)	5.5 (10)	20.3 (24)	22.4 (18)
PS+PI	P+T	0.6 (3)	0.6 (1)	0.5 (1)	2.9 (2)
	E	0.4 (6)	0.9 (2)	2.6 (3)	3.8 (3)
Other	P+T	1.0 (4)	4.5 (6)	2.1 (2)	2.8 (2)
	E	1.1 (15)	4.5 (9)	2.1 (3)	4.1 (3)

The methods of analysis are described in the Experimental. Results are the average of duplicates with the figures in parentheses representing the percentage composition. P+T = pericarp+testa fraction, E=endosperm fraction, FAME = fatty acid methyl esters. Other abbreviations are defined in the legend to Table 2. See the Experimental for comments regarding the nature of the two grain fractions analysed at different developmental times.

Table 5. Changes in the fatty acid compositions of major acyl lipids of the pericarp and testa fraction of developing wheat grains

Lipid	Age (days after anthesis)	Fatty acid composition (% total) 16:0	18:0	18:1	18:2	18:3
Neutral	14	19	2	16	45	18
lipids	17	19	1	22	38	20
	23	23	n.d.	18	49	10
	27	23	1	18	48	10
DGDG	14	28	n.d.	10	26	36
	17	28	n.d.	14	25	33
	23	26	n.d.	9	27	38
	27	39	1	6	31	23
PC	14	35	1	12	26	26
	17	32	1	12	28	27
	23	35	1	13	37	14
	27	36	1	9	43	11
lysoPC	14	41	1	18	28	12
	17	27	1	24	35	13
	23	35	3	18	30	14
	27	38	1	7	42	12
PE	14	18	1	16	30	35
	17	19	n.d.	13	27	41
	23	20	n.d.	14	39	27
	27	29	n.d.	6	47	18

For abbreviations see the legends to Tables 1 and 2.

Table 6. Changes in the fatty acid composition of major acyl lipids of the endosperm of developing wheat grains

Lipid	Age (days after anthesis)	Fatty acid composition (% total) 16:0	18:0	18:1	18:2	18:3
Neutral	14	11	2	18	49	20
lipids	17	24	1	9	50	16
	23	20	1	15	51	13
	27	22	1	7	62	8
DGDG	14	32	2	14	36	16
	17	34	n.d.	5	45	16
	23	34	1	6	45	14
	27	44	1	6	44	5
PC	14	29	n.d.	6	50	15
	17	31	1	7	45	16
	23	29	1	9	55	6
	27	34	1	8	53	4
lysoPC	14	42	1	6	39	12
	17	40	n.d.	4	45	11
	23	39	2	6	47	6
	27	44	n.d.	5	47	4
PE	14	23	1	6	51	19
	17	21	n.d.	8	54	17
	23	22	1	4	64	9
	27	28	1	6	61	4

For abbreviations see the legends to Tables 1 and 2.

EXPERIMENTAL

Materials. Two varieties of Spring wheat (*Triticum aestivum*)—cv. Sicco and cv. Timmo—were field grown or cultivated under laboratory conditions with continuous illumination. Growth under the latter conditions was rapid, with plants ready for harvest within 4–5 months of germination. However, laboratory-grown plants showed an increased tiller length and decreased fertility compared to field-grown wheat. For these reasons laboratory-grown material was used in preliminary experiments but the analyses reported in the paper are for field-grown (cv. Sicco) wheat harvested in the summers of 1979 and 1980.

Grain was dissected into two fractions; the endosperm and the pericarp + testa fractions. The composition of each fraction varied slightly as a consequence of changes occurring during development. The major compositional difference was due to the behaviour of the aleurone layer. In grains aged 17 days after anthesis (DAA) the aleurone layer separated with the endosperm fraction. Over the following 6 days, grain differentiation resulted in the aleurone layer separating with the pericarp + testa fractions of 23 DAA grain. In these experiments, the germ (embryo + scutellum) was not removed separately, but remained with the pericarp + testa fraction.

Extraction and analysis of lipids. Grains were extracted by prolonged solvent treatment. This procedure was fundamentally that described in ref. [13] whereby after treatment with boiling H_2O-satd *n*-BuOH inactivated grains were homogenized and further extracted $\times 4$ with H_2O-satd *n*-BuOH over a period of 4 hr at 100°. Grain fractions were inactivated immediately after dissection by boiling in *n*-BuOH. This prevented lipolysis and fatty acid oxidation. Lipid samples were stored in $CHCl_3$–MeOH (2:1) at −20° under N_2 before analysis. Fatty acid analysis of the solvent-extracted tissue after acid hydrolysis yielded only small amounts of the total fatty acids, indicating that solvent extraction had removed essentially all of the acyl lipid.

Lipids were partitioned from impurities by the method of ref. [15]. This procedure was found to extract quantitatively the most polar of the major lipids, monoacylphosphatidylcholine, and had been noted previously to be suitable for the others [10]. Acyl lipids were routinely separated by TLC on silica gel G plates with $CHCl_3$–MeOH–HOAc–H_2O (170:30:20:7) as solvent. Neutral lipids were also separated by TLC using Et_2O–petrol–HOAc (10:90:1) as solvent. Non-esterified fatty acids were minor components of this fraction in all samples (cf. Table 2 legend). Lipid bands were located under UV light after spraying with 0.05% aq. Rhodamine 6G or 0.1% 8-anilino-4-sulphonic acid in MeOH.

The bands were identified routinely by comparison with authentic markers but, in addition, all the major lipids were completely identified as previously described [14]. Routine quantitation of individual lipids was by transmethylation of the compounds with 2.5% H_2SO_4–MeOH and measurement of the fatty acid methyl esters by GC [14]. An internal standard of methyl pentadecanoate was used. In addition, the analyses were checked by quantitation of diacyldigalactosylglycerol by galactose estimation and of phosphoglycerides by phosphorus analysis [16]. These methods gave the same results as fatty acid analysis. The identity of individual fatty acids was checked by $AgNO_3$-TLC and by chemical degradation [17].

Acknowledgements—The financial support of the S.E.R.C. and Rank, Hovis, McDougall (CASE award to D.S.S.) is gratefully acknowledged.

REFERENCES

1. Morrison, W. R. (1979) in *Recent Advances in the Biochemistry of Cereals* (Laidman, D. L. and Wynn Jones, R. G., eds) pp. 313–335. Academic Press, London.
2. Morrison, W. R. (1978) in *Advances in Cereal Science and Technology* (Pomeranz, Y., ed.) Vol. 2, pp. 221–348. Am. Assoc. Cereal Chem., St. Paul, MN.
3. Morrison, W. R. (1983) in *Lipids in Cereal Technology* (Barnes, P. J., ed.) pp. 11–32. Academic Press, London.
4. Hargin, K. D. and Morrison, W. R. (1980) *J. Sci. Food Agric.* **31**, 877.
5. Stokes, D. N., Galliard, T. and Harwood, J. L. (1980) *Biochem. Soc. Trans.* **8**, 533.
6. Stokes, D. N., Galliard, T. and Harwood, J. L. (1980) in *Biogenesis and Function of Plant Lipids* (Mazliak, P., Beneveniste, P., Costes, C. and Douce, R., eds) pp. 223–226. Elsevier, Amsterdam.
7. Appelqvist, L.-A. (1975) in *Recent Advances in the Chemistry and Biochemistry of Plant Lipids* (Galliard, T. and Mercer, E. I., eds) pp. 247–286. Academic Press, London.
8. Barnes, P. J. (ed.) (1983) *Lipids in Cereal Technology.* Academic Press, London.
9. Hargin, K. D., Morrison, W. R. and Fulcher, R. G. (1980) *Cereal Chem.* **57**, 320.
10. Harwood, J. L. (1980) in *Biochemistry of Plants* (Stumpf, P. K. and Conn, E. E., eds) Vol. 4, pp. 1–55. Academic Press, New York.
11. Tan, S. L. and Morrison, W. R. (1979) *J. Am. Oil Chem. Soc.* **56**, 759.
12. Gurr, M. I. (1980) in *Biochemistry of Plants* (Stumpf, P. K. and Conn, E. E., eds) Vol. 4, pp. 205–248. Academic Press, New York.
13. Morrison, W. R., Mann, D. L., Soon, W. and Coventry, A. M. (1975) *J. Sci. Food Agric.* **26**, 507.
14. Bolton, P. and Harwood, J. L. (1977) *Biochem. J.* **168**, 261.
15. Garbus, J., de Luca, H. F., Loomans, M. E. and Strong, M. F. (1963) *J. Biol. Chem.* **238**, 59.
16. Kates, M. (1972) *Techniques in Lipidology.* Elsevier, Amsterdam.
17. Bolton, P., Wharfe, J. and Harwood, J. L. (1978) *Biochem. J.* **174**, 67.