Changes in Wheat Lipids during Seed Maturation. II. Changes in Lipid Composition[1]

SANDRA K. SKARSAUNE[2]. V. L. YOUNGS[3], and K. A. GILLES[2], Department of Cereal Chemistry and Technology, North Dakota State University, Fargo

ABSTRACT

Quantitative thin-layer chromatography of the extracted lipids of maturing HRS and durum wheats showed that esterification increased as maturation progressed. Triglyceride content increased uniformly, whereas the monoglycerides decreased and the diglycerides remained essentially constant. The sterol esters, which were absent in extracts from the durum varieties, increased steadily in the HRS wheat lipids. Free sterols and free fatty acids decreased in the lipids of all varieties. While the same fatty acids were detected in immature and mature wheats, their proportions changed during ripening. Linoleic acid increased steadily, whereas linolenic and oleic acids decreased. Palmitic and stearic acids showed essentially no change with maturation. Examination of the water-saturated n-butanol extracts of maturing wheat showed that the phospholipids increased during maturation; no consistent changes were detected in the galactolipids.

Changes in lipid composition during seed ripening have been studied in several plant species. Although the information comes from varied sources, similarities in lipid changes during seed maturation were apparent.

Without exception, the content of free fatty acids (FFA) decreases with maturity. In 1941 Evans (1) reported a fivefold decrease in FFA in maturing corn. Similar results were found in flax and safflower, several grains, and HRW wheat (2,3,4). Generally, the content of mono- and diglycerides decreased whereas that of triglyceride increased. However, Daftary and Pomeranz (4) observed no consistent change in the triglyceride level of maturing HRW wheat, although the contents of FFA, mono-, and diglyceride decreased with grain ripening.

Changes in the fatty-acid composition of maturing seeds have been investigated in wheat, oats, barley, safflower, soybean, and alfalfa (3,5,6,7). In general, a proportional decrease in linolenic and oleic acids was found, whereas the content of linoleic acid increased steadily.

The polar lipids have received little attention. No consistent changes in the bound lipids (those extracted with water-saturated n-butanol (WSB)), or in the phospholipids, which accounted for 37.3% of the bound lipid, were reported in

---

[1]Presented at the 54th Annual Meeting, Chicago, Ill., April-May 1969. Co-operative investigations between North Dakota Agricultural Experiment Station and Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, Fargo. Published with the approval of the Director of the Agricultural Experiment Station, North Dakota State University, Fargo, as Journal Series No. 219. Taken in part from a Ph.D. thesis submitted by S. K. Skarsaune to North Dakota State University.

Mention of a trademark name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

[2]Assistant Professor and Professor, respectively, Department of Cereal Chemistry and Technology, North Dakota State University, Fargo.

[3]Research Chemist, Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture and Assistant Professor, Cereal Chemistry and Technology, North Dakota State University, Fargo.

maturing HRW wheat (4). However, Beiss (8) found that the phospholipid content of the sugar beet root dropped from 0.6 to 0.005% of the total lipid during maturation. Galactolipid levels decreased with maturity; they appeared to be intimately associated with photosynthetic activity, which decreases as the seed ripens (9).

A quantitative evaluation of the lipid composition of maturing HRS and durum wheats was the major objective of this research.

METHODS

Samples

The samples of whole wheat, flour, and bran, and their respective lipid extracts, are described in the previous paper (10). In the petroleum-ether extracts, tri-, mono-, and diglycerides, sterols, sterol esters, and FFA were analyzed quantitatively by thin-layer chromatography (TLC). Various phospholipids and galactolipids from the WSB extracts were analyzed by the same method.

Thin-Layer Chromatography

Thin-layer plates (20 by 20 cm.) were prepared with an adjustable thin-layer applicator (Brinkman model S-11) and a slurry of Adsorbosil and water. In most cases a thickness of 0.25 mm. was used; for preparative separations used in purifying standards, plates were coated with a 0.75-mm. layer. The plates were air-dried briefly, heated at 110°C. for 1 hr., and stored in air-tight containers. The plates to be used for polar lipid separations were air-dried about 3 hr. and used immediately.

For quantitative analysis of TLC, spots of known fat content were applied to the plate. The compound under investigation determined the solvent system and the method of visualization (Table I). After visualization, the spots were scanned on a Photovolt densitometer, equipped with a Varicord recorder, response setting of 5. Plates visualized with ninhydrin were scanned immediately since the color fades with time. For these plates, color filter No. 485 was used to increase spot density; with other methods of visualization, no filter was necessary.

The areas corresponding to spots were calculated on the Beckman Analytrol. Three different amounts of a standard were applied to each plate. From the areas of these standard spots, a standard curve was drawn, which related quantity (γ) of material and spot density. From this, the γ of the measured compound in the sample was calculated. Standard curves were included on each analyzed plate, and at least three determinations were made of each plate to achieve greater accuracy. This method resulted in a standard deviation of 4.7%, which compares favorably with that reported by Purdy and Truter as cited by Bobbit (14).

Quantitation of the phospho- and glycolipids was done by comparison of areas. Thin-layer separation of polar compounds was difficult, because of the tendency to streak. These compounds were best visualized by use of specific spray reagents. For example, ninhydrin was employed to detect compounds containing an amine group, a diphenylamine spray for detection of glycolipids, and Dragendorff's reagent for visualization of the choline-containing compounds, lecithin (phosphatidyl choline) and lysolecithin (lysophosphatidyl choline). The first two detection methods produced spots which could be analyzed on the densitometer; the color of lecithin

TABLE I. SEPARATION AND DETECTION OF LIPID COMPONENTS

Solvent System	Compounds Separated	Standard Used	Visualization Method
90:10:1.5 (P.E.:E.E.:HOAc)	triglycerides	triolein	50% aqueous H_2SO_4, char
	fatty acids	linoleic acid	
	sterols	beta-sitosterol	50% aq. H_2SO_4[a]
	1,2 diglycerides	1,2 diolein	
95:24 (Benzene:ETOAc)	1,3 diglycerides	1,3 diolein	
10:90:1.5 (P.E.:E.E.:HOAc)[b]	monoglycerides	monoolein	50% aq. H_2SO_4, char
CCl_4	sat'd sterol esters	sitosterol palmitate	50% aq. H_2SO_4, char.
	lecithin	egg lecithin	Dragendorff
65:25:3 ($CHCl_3$:MeOH:H_2O)	lysolecithin	lysolecithin	ninhydrin
	phosphatidyl serine, lysophosphatidyl ethanolamine	phosphatidyl serine	
65:13:3 ($CHCl_3$:MeOH:H_2O)	phosphatidyl ethanolamine	phosphatidyl ethanolamine	ninhydrin
80:10:1 ($CHCl_3$:MeOH:H_2O)	monogalactosyl diglycerides	monogalactosyl diglycerides	diphenylamine[c]
60:35:8 ($CHCl_3$:MeOH:H_2O)[d]	digalactosyl diglycerides	digalactosyl diglycerides	diphenylamine

[a]Sterols could be identified by the color produced when heating with 50% aqueous H_2SO_4.

[b]Reference 11.

[c]Reference 12.

[d]Reference 13.

and lysolecithin with Dragendorff's reagent was not strong enough to measure accurately.

Gas-Liquid Chromatography

Fatty-acid methyl esters for gas-liquid chromatographic analysis were prepared according to the following modification of the procedure of Metcalfe and Schmitz (15). Approximately 0.1 g. of crude fat was placed in a 25-ml. Erlenmeyer flask and 10 ml. of absolute methanol was added. Boron trifluoride was bubbled through for 1 min.; the mixture was refluxed for 1 min. and again treated with boron trifluoride for 15 sec. Petroleum ether, 2 ml. (b.p. 30° to 60°C.), was added to the cooled flask. Water was carefully added to bring the petroleum-ether layer to the neck of the flask. The sample was then ready for gas-liquid chromatographic analysis. If it proved too dilute, the petroleum-ether layer could be concentrated by blowing nitrogen across the mouth of the flask until a more suitable concentration was attained.

All gas-chromatographic analyses were performed on a Beckman GC-2A equipped with a Brown recorder. Operating conditions for fatty acid analysis were as follows:

Column: aluminum, 9 ft. long, 0.25 in. o.d.
Coating: diethylene glycol succinate, 15% by wt.
Support: Gas Chrom Z, 100- to 120-mesh

Carrier gas: helium, 130 ml. per min.
Pressure: inlet, 35 lb. per sq. in.
Temperatures (°C.): column, 199; detector, 205; sampler, 275
Sensitivity: 210 ma.
Chart speed: 0.5 in. per min.

Identification of peaks from gas-liquid chromatographic analysis was made by comparing retention times with those of known standards produced under identical operating conditions. Quantitative determinations were made by triangulation. Peak areas were proportional to the amount of fatty acid present.

Infrared analyses of the lipid extracts were performed as previously described (10).

RESULTS AND DISCUSSION

Changes in Nonpolar (Petroleum Ether-Extracted) Lipids

Sterols and Sterol Esters. Free sterol and saturated sterol ester contents in the petroleum-ether extracts of ripening Chris and Leeds wheat are presented in Table II.

The free sterol content of Chris whole wheat lipid decreased from 62 mg. per g. of extract in the sample with an original moisture of 68%, to 37 mg. per g. in the mature sample, with original moisture of 13.6%. This decrease was fairly typical of all varieties, although the free sterol content in the mature durum wheat lipids was higher than in HRS wheats. The free sterol content of the latter group decreased more regularly than that of the durum. This is shown in Table III, which presents the correlation coefficients between maturity (original moisture) and the lipid content of free sterols.

In the lipids of the HRS varieties, Chris and Justin, the saturated sterol ester content increased regularly as the wheat matured (Table III). In the lipid of whole wheat from the least mature sample of Chris, the content of saturated sterol esters measured as sitosterol palmitate was 7 mg. per g. In the sample of mature Chris whole wheat lipid, the saturated sterol ester content was 31.5 mg. per g. A similar pattern was observed in Justin, although the sterol ester content was a bit higher throughout the maturation process in this variety.

The bran lipid from Chris was an exception to the pattern of increasing sterol ester content; the correlation coefficient relating sterol ester content of Chris bran lipid and maturity was too low for any meaningful conclusion to be drawn.

The increasing sterol ester content of Chris and Justin wheat lipids appeared similar to a previous observation of *Calendula officinalis* (16), where seed formation was characterized by esterification of free sterols. Saturated sterol esters, measured as sitosterol palmitate, were not present at any stage of maturity in the lipids of either of the analyzed durum varieties.

Triglycerides and Fatty Acids. The relation between the lipid content of triglycerides and FFA during maturation of two wheat varieties is presented in Table IV. A marked increase in triglycerides was observed concurrent with a considerable drop in level of FFA. For example, in the lipids from the sample of Chris whole wheat, 68% original moisture, the contents of triglyceride and FFA were 117 and 499 mg. per g., respectively, whereas in the lipids of the mature

TABLE II. STEROL AND STEROL ESTER CONTENT IN LIPIDS OF MATURING WHEAT

Variety	Original Moisture %	Whole Wheat		Flour		Bran	
		Sterol mg./g.[a]	Sat'd Sterol Ester mg./g.	Sterol mg./g.	Sat'd Sterol Ester mg./g.	Sterol mg./g.	Sat'd Sterol Ester mg./g.
Leeds	69.0	67		59		85	
	66.3	52		86		104	
	52.7	31		67		85	
	52.3	58		78		71	
	46.5	43		74		69	
	41.1	43		38		67	
	27.3	49		55		52	
	25.3	44		59		42	
	11.8	48		65		73	
Chris	68.0	62	7	160	27	125	15
	61.3	61	12	163	65	121	20
	51.4	49	15	130	45	55	28
	47.0	56	16	74	118	56	15
	46.0	44	17	50	112	49	27
	44.0	43	23.3	46	133	61	26
	34.0	31	31.1	40	90	37	13
	13.6	37	31.5	29	135	59	24

[a]Amounts are given in mg. per g. of lipid extract. Blanks indicate amounts too small to be measured.

TABLE III. CORRELATION COEFFICIENTS RELATING THE WHEAT LIPID CONTENT OF FREE STEROL AND SATURATED STEROL ESTERS WITH MATURITY

Variety	Whole Wheat		Flour		Bran	
	Free Sterol	Sat'd Sterol Esters	Free Sterol	Sat'd Sterol Esters	Free Sterol	Sat'd Sterol Esters
Justin	0.885*	−0.946**	0.850*	−0.895**	0.899*	−0.847*
Chris	0.833*	−0.884*	0.848*	−0.737*	0.686	−0.180
Stewart 63	0.865*		0.046		0.746*	
Leeds	0.452		0.371		0.706*	

sample (13.6% moisture), triglyceride content was 743 mg. per g. and FFA, 89 mg. per g.

Although Daftary and Pomeranz (4) reported a similar decrease in FFA content, they noted no consistent change in triglyceride level in the lipids of winter wheat.

Infrared analysis of the lipids extracted by petroleum ether yielded confirmatory evidence for concurrent decreasing FFA and increasing triglyceride contents in ripening wheat (Fig. 1). The absorption band at 1,700 $cm.^{-1}$ was due to the free carbonyl function; it predominated in lipids from unripe samples. In petroleum-ether extracts from ripe samples, the absorption band at 1,740 $cm.^{-1}$ predominated, owing to the carbonyl ester function. This indicated a shift from the free to the esterified carbonyl function with ripening. This observation was

TABLE IV. TRIGLYCERIDE AND FREE FATTY ACID (FFA) CONTENT IN THE LIPIDS OF MATURING WHEAT

Variety	Original Moisture %	Whole Wheat FFA mg./g.[a]	Whole Wheat Triglycerides mg./g.	Flour FFA mg./g.	Flour Triglycerides mg./g.	Bran FFA mg./g.	Bran Triglycerides mg./g.
Leeds	69.0	462	96	321	136	793	284
	66.3	415	201	374	280	564	138
	52.7	380	405	165	307	389	575
	52.3	194	558	209	363	284	497
	46.5	137	554	139	402	253	557
	41.1	78	585	53	231	291	545
	27.3	84	786	72	535	196	553
	25.3	99	719	48	542	228	616
	11.8	10	725	91	553	171	502
Chris	68.0	683	100	253	37	778	113
	61.3	494	245	231	194	460	402
	51.4	488	249	146	264	333	426
	47.0	76	407	137	531	295	520
	46.0	141	389	76	533	175	603
	44.0	62	550	57	578	158	619
	34.0	39	706	62	463	84	525
	13.6	89	743	31	467	109	718

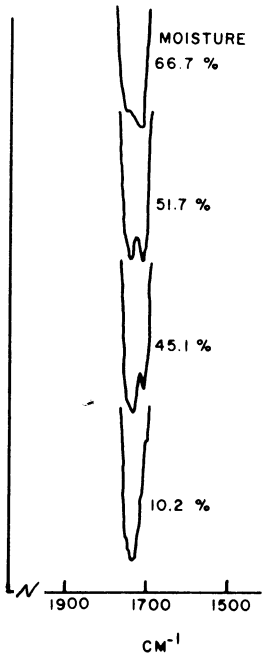
[a]Amounts are given in mg. per g. of lipid extract.

consistent with the observed increase in triglycerides and decrease in FFA as determined by TLC.

While the same fatty acids (palmitic, oleic, stearic, linoleic, and linolenic) composed more than 95% of the total free and esterified fatty acids of each lipid sample, their proportions did not remain constant during ripening. Figure 2 shows the changes which occurred in the fatty acid composition of lipid of Chris whole wheat during maturation. The changes shown in Fig. 2 are typical of the four varieties analyzed.

In lipids from samples of bran and whole wheat, linolenic acid decreased with increasing maturity. For example, in the lipids from immature Chris whole wheat and bran, linolenic acid accounted for 11.2 and 10.4%, respectively, of the total fatty acid. In lipids from mature (13.6% moisture) Chris whole wheat and bran, 3.8 and 4.0% of the total fatty acid was linolenic acid. In lipids from flour samples of corresponding moistures, linolenic acid decreased from 4.8 to 3.1% of the total fatty acid content. This finding agreed with the work of Gray et al. (9), who related photosynthetic activity and linolenic acid content. Since photosynthesis is limited to the green outer covering of the seed, i.e., the bran, it is to be expected that the most notable changes occur in that fraction.

Oleic acid, during seed maturation, also showed a decrease in all samples. Linoleic acid, however, increased by nearly 25% and accounted for nearly 60% of the total fatty acid in the lipids from mature samples. These observations were consistent with those of Lindberg et al. (3), who studied the fatty acids of ripening oats, barley, and wheat. Palmitic and stearic acids showed inconsistent variations with maturity. In all varieties, the linoleic acid content was higher in flour lipid than in the bran lipid. When the fatty acids of mature HRS and durum wheat lipids



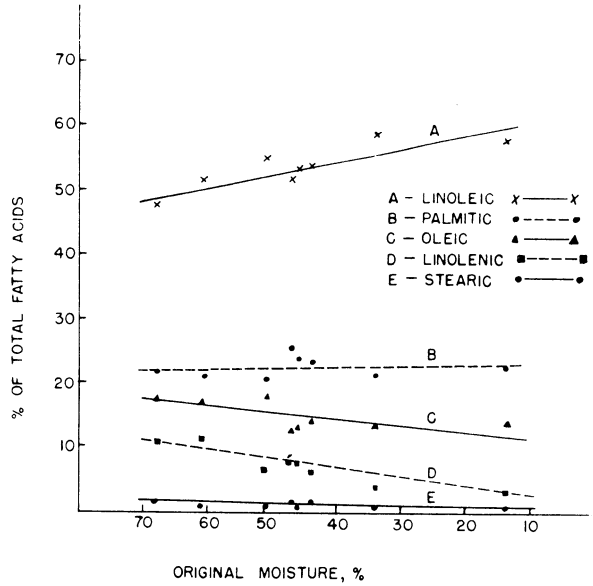


Fig. 1 (left). Infrared spectra of lipids of Justin whole wheat, showing a shift of the peak at 1,700 to 1,740 cm.$^{-1}$ with maturity.

Fig. 2 (right). Changes in fatty-acid composition of lipid of Chris whole wheat during maturation.

were compared, it was observed that the former had more palmitic and less oleic acid than the latter.

Mono- and Diglycerides. A difference was evident in the change of diglyceride contents from the lipids of ripening HRS and durum wheats (Table V). The diglycerides of the HRS wheat lipids changed irregularly; no consistent variation was apparent. In the durum lipids, the diglycerides, especially when the 1,2 and 1,3 isomers were considered together, decreased steadily with maturation. As the original moisture of Leeds whole wheat decreased from 66.7 to 11.8%, the sum of the diglycerides present in the lipids declined from 231 to 92 mg. per g.

The amount of 1,2-diglyceride was almost twice that of the 1,3 isomer in the lipids of the mature samples of each variety. At maturity (13.6% moisture), the 1,2-diglyceride content of Chris whole wheat lipid was 78 mg. per g., whereas the 1,3-diglyceride accounted for 43 mg. per g. The HRS wheat lipids contained more of both diglycerides than the durum lipids.

A difference between the HRS and durum wheat lipids also appeared in the pattern of the monoglyceride content during maturation. The monoglycerides of HRS wheat lipid showed no consistent variation, while those from the durum varieties decreased steadily during ripening. The lipid sample from Stewart 63 whole wheat, which had an original moisture content of 69.7%, had a

TABLE V. MONO- AND DIGLYCERIDE CONTENT IN PETROLEUM-ETHER EXTRACTS OF MATURING WHEAT

Leeds				Chris			
Original Moisture %	Mono-glycerides mg./g.	1,2 Di-glycerides mg./g.	1,3 Di-glycerides mg./g.	Original Moisture %	Mono-glycerides mg./g.	1,2 Di-glycerides mg./g.	1,3 Di-glycerides mg./g.
69.0	28	47	68	68.0	49	67	38
66.3	31	115	116	61.3	33	106	63
52.7	18	112	109	51.4	26	111	57
52.3	14	109	105	47.0	21	99	47
46.5	13	95	103	46.0	40	87	60
41.1	13	89	96	44.0	14	102	51
27.3	7	67	60	34.0	8	67	31
25.3	4	64	42	13.6	19	78	43
11.8	...[a]	56	36				

[a]Amount too small to be measured.

monoglyceride content of 23 mg. per g. The lipid from the mature wheat (12.4% moisture) contained 3 mg. per g. of monoglycerides. Decreasing monoglyceride content in a variety of maturing seeds also has been reported (17).

Changes in Polar (WSB-Extracted) Lipids

Phosphatidyl Ethanolamine. The spot identified as phosphatidyl ethanolamine also contained phosphatidic acid, according to Daftary and Pomeranz (4). However, we found that when the plates were visualized with ninhydrin, only the phosphatidyl ethanolamine was detected; ninhydrin is specific for amine groups, and phosphatidic acid contains no such groups. For this reason, ninhydrin was used to detect phosphatidyl ethanolamine in this work.

Phosphatidyl ethanolamine did not occur in lipid extracts from wheat samples with greater than 45% original moisture (Table VI). The phosphatidyl ethanolamine content increased slightly until full maturity was reached; this was more obvious in durum wheat lipids than in those of HRS wheat. At maturity, roughly the same amount of phosphatidyl ethanolamine was present in the flour and bran extracts of Justin wheat. At the same stage of maturity in the other three varieties, about twice the amount was found in the bran extract as in the flour.

TABLE VI. COMPARISON OF PHOSPHATIDYL ETHANOLAMINE CONTENT IN WATER-SATURATED n-BUTANOL EXTRACTS OF MATURING WHEAT[a]

Chris				Leeds			
Original Moisture %	Flour	Bran	Whole Wheat	Original Moisture %	Flour	Bran	Whole Wheat
44.0	...[b]	0.10	0.15	41.1	0.15	0.25	0.15
34.0	...[b]	0.20	0.30	27.3	0.25	0.45	0.50
13.6	0.15	0.35	0.75	25.3	0.30	0.85	1.00
				11.8	0.45	1.00	0.45

[a]Compared to mature Leeds bran phosphatidyl ethanolamine content; amount of phosphatidyl ethanolamine in samples of greater moisture content was too small to be measured.

[b]Amount too small to be measured.

TABLE VII. COMPARISON OF LYSOPHOSPHATIDYL ETHANOLAMINE AND PHOSPHATIDYL SERINE CONTENTS IN WATER-SATURATED n-BUTANOL EXTRACTS OF MATURING WHEATS[a]

Chris				Leeds			
Original Moisture %	Flour	Bran	Whole Wheat	Original Moisture %	Flour	Bran	Whole Wheat
44.0	0.13	0.33	...[b]	41.1	0.33	0.40	0.13
34.0	0.20	0.73	...[b]	27.3	0.47	0.20	0.67
13.6	0.40	0.40	0.47	25.3	0.53	0.73	1.47
				11.8	0.47	1.00	0.47

[a]Compared to mature Leeds bran content of lysophosphatidyl ethanolamine and phosphatidyl serine; the title compounds were not present in samples having original moistures greater than those listed.

[b]Amount too small to be measured.

Lysophosphatidyl Ethanolamine and Phosphatidyl Serine. No solvent system gave satisfactory separation of these two compounds. Since both give a positive ninhydrin reaction, they were measured as one spot.

Approximately the same observations made about phosphatidyl ethanolamine were applicable here. Table VII presents the results of semiquantitative analysis. Lysophosphatidyl ethanolamine and phosphatidyl serine did not appear in lipids extracted from samples with greater than 45% original moisture. The amount in the lipid extract increased until maturity, although less regularly than phosphatidyl ethanolamine. At maturity, however, about the same amount of lysophosphatidyl ethanolamine and phosphatidyl serine was present in the flour and the bran extracts of Justin, Chris, and Stewart 63 wheats. In Leeds, the content of these compounds in the flour extract was roughly half the content in lipid extract of bran.

Lecithin. The spots resulting from the Dragendorff analysis of lecithin and lysolecithin were too faint to be measured with the densitometer. Therefore, a 50% aqueous solution of sulfuric acid was used for visualization. Unfortunately, lysolecithin and some sugars have approximately the same R_f values, and charring with sulfuric acid effectively masked the lysolecithin. It could not be measured with any accuracy.

Extracts of immature whole wheats streaked badly, despite the precautions taken, and measuring lecithin in these extracts was considered impractical. However, lecithin in bran and flour lipids could be measured, and the results are presented in Table VIII. The amount of lecithin in the extracts of both flour and bran increases with maturity. Considering this trend, one would expect the lecithin content of whole-wheat lipid to increase during ripening. In the HRS wheats, lecithin in the flour extract was greater than that in bran; the distribution was reversed in the lipids of Leeds and Stewart 63, the durum varieties.

Glycolipids. Repeated thin-layer analyses failed to detect galactolipids in the lipids of several samples of immature whole wheat (Tables IX and X), although the compounds were definitely present in the corresponding flour and bran. The WSB extract of immature germ also showed the presence of mono- and digalactosyl diglycerides. The reason for this discrepancy is unknown.

TABLE VIII. COMPARISON OF LECITHIN CONTENT IN WATER-SATURATED n-BUTANOL EXTRACTS OF MATURING WHEAT[a]

Chris			Leeds		
Original Moisture %	Flour	Bran	Original Moisture %	Flour	Bran
68.0	0.60	0.38	69.0	0.56	0.44
61.3	0.65	0.45	66.3	0.57	0.38
51.4	0.47	0.56	52.7	0.56	0.50
47.0	0.41	0.47	52.3	0.46	0.56
46.0	0.63	0.47	46.5	0.44	0.77
44.0	0.46	0.62	41.1	0.53	0.77
34.0	0.68	0.94	27.3	0.53	0.77
13.6	0.77	0.74	25.3	0.52	0.84
			11.8	0.52	1.00

[a]Compared to lecithin content of mature Leeds bran.

Table IX summarizes the analysis of monogalactosyl diglycerides. The monogalactosyl diglyceride content was about twice as great in the extract of flour as that of bran. In flour lipid, the content increased irregularly with seed maturation. This was in contrast to bran, where the content increased until the original moisture content of the wheat sample fell below 50%; the monogalactosyl diglyceride content in the lipid extract then decreased.

Although no consistent variation in digalactosyl diglyceride content could be observed with maturity, a difference between HRS and durum wheat lipids was apparent (Table X). In HRS varieties, the amount of digalactosyl diglyceride in the flour lipid was consistently greater than the content in the bran extract. The same was true in the lipids of the least mature samples of the durum wheats, but after the original moisture content of the samples fell below 50%, the situation was reversed; digalactosyl diglycerides were greater in the bran lipid than in the flour lipid.

TABLE IX. COMPARISON OF MONOGALACTOSYL DIGLYCERIDE CONTENT IN WATER-SATURATED n-BUTANOL EXTRACTS OF MATURING WHEAT[a]

Chris				Leeds			
Original Moisture %	Flour	Bran	Whole Wheat	Original Moisture %	Flour	Bran	Whole Wheat
68.0	0.36	0.40		69.0		0.26	0.44
61.3	1.00	0.74		66.3	0.04	0.60	0.40
51.4	0.94	0.94		52.7	0.06	0.64	0.74
47.0	0.66	1.00		52.3	0.10	0.50	0.94
46.0	0.76	1.30		46.5	0.16	0.60	1.00
44.0	0.54	0.60		41.1	0.26	0.36	0.54
34.0	0.60	0.44	0.60	27.3	0.36	0.44	0.70
13.6	0.66	0.36	0.80	25.3	0.94	0.30	0.56
				11.8	0.76	0.24	0.50

[a]Compared to monogalactosyl diglyceride content of the Leeds whole wheat sample of 46.5% moisture. Blanks indicate amounts too small to be measured.

TABLE X. COMPARISON OF DIGALACTOSYL DIGLYCERIDE CONTENT IN WATER-SATURATED n-BUTANOL EXTRACTS OF MATURING WHEAT[a]

Chris				Leeds			
Original Moisture %	Flour	Bran	Whole Wheat	Original Moisture %	Flour	Bran	Whole Wheat
68.0		0.16		69.0		0.40	0.22
61.3	0.53	0.28		66.3	0.71	0.35	
51.4	0.47	0.19	0.16	52.7	1.00	0.21	
47.0	0.43	0.25	0.16	52.3	0.64	0.27	
46.0	0.29	0.38	0.30	46.5	0.22	0.30	
44.0	0.35	0.24	0.18	41.1	0.10	0.27	
34.0	0.37	0.22	0.34	27.3	0.06	0.37	
13.6	0.29	0.14	0.24	25.3	0.01	0.24	
				11.8	0.13	0.22	0.25

[a]Compared to the digalactosyl diglyceride content of Leeds flour sample of 52.7% moisture. Blanks indicate amounts too small to be measured.

TABLE XI. AVERAGE CORRELATION COEFFICIENTS RELATING ORIGINAL MOISTURE AND SOME COMPONENTS OF LIPID EXTRACTS FROM MATURING WHEAT

	Hard Red Spring			Durum		
	Whole Wheat	Flour	Bran	Whole Wheat	Flour	Bran
Free sterols	0.859*	0.849*	0.793*	0.659*	0.209	0.726*
Saturated sterol esters	−0.937**	−0.816*	−0.514	...[a]	...[a]	...[a]
Free fatty acids	0.817*	0.877*	0.796*	0.850**	0.850**	0.796*
Triglycerides	−0.915**	−0.768*	−0.839*	−0.907**	−0.858**	−0.688*
Diglycerides[b]	0.276	−0.205	−0.263	0.621	0.114	0.137
Monoglycerides	0.581	...[c]	...[c]	0.926**	...[c]	...[c]

[a]Saturated sterol esters, measured as sitosterol palmitate, are not present in durum wheat.

[b]Sum of 1,2- and 1,3-diglycerides.

[c]Data not available.

SUMMARY AND CONCLUSIONS

Table XI summarizes a statistical treatment of the data from analysis of the lipids extracted by petroleum ether. Esterification clearly increases with maturation. In general, the lipid content of free sterols, FFA, and monoglycerides decreases significantly as the samples ripen; the lipid content of the saturated sterol esters and triglycerides increases markedly during wheat maturation. The amount of diglyceride in the lipid extract shows no significant change with maturity.

Grain maturation was marked by a decrease in the proportion of linolenic acid in the total fatty acid content. Oleic acid also decreased, while linoleic acid increased as the wheat ripened. The other major fatty acids, palmitic and stearic, showed no consistent change with maturity.

No consistent pattern emerged from analysis of the WSB extracts of maturing

wheat. The phospholipids, phosphatidyl ethanolamine, lysophosphatidyl ethanolamine, and phosphatidyl serine, were not observed in extracts from samples which had original moisture greater than 45%. This seems peculiar, as these compounds are normal constituents of cellular membranes and, therefore, should be present at all stages of maturity. Perhaps their concentration in the lipids from the immature samples was below the limit of sensitivity of the methods employed. Lecithin, which was measured in bran and flour lipids, increased with maturity. The content of mono- and digalactosyl diglycerides showed inconsistent changes with maturity.

Literature Cited

1. EVANS, J. N. Changes in the biochemical composition of the corn kernel during development. Cereal Chem. 18:468 (1941).
2. McKILLICAN, MARY E., and SIMS, R. P. A. Lipid changes in maturing oil-bearing plants. III. Changes in flax and safflower oils. J. Am. Oil Chemists' Soc. 40: 108 (1963).
3. LINDBERG, P., TENHAUNPAA, E., NILSSON, G., and WASS, L. The fatty acid composition of ripening grain. Acta Agr. Scand. 14: 297 (1964).
4. DAFTARY, R., and POMERANZ, Y. Changes in lipid composition in maturing wheat. J. Food Sci. 30: 577 (1965).
5. HILL, A., and KNOWLES, P. Fatty acid composition of the oil of developing seeds of different varieties of safflower. Crop Sci. 8: 275 (1968).
6. SIMMONS, R. O., and QUACKENBUSH, F. U. Comparative rates of formation of fatty acids in the soybean seed during its development. J. Am. Oil Chemists' Soc. 31: 601 (1954).
7. KLOPFENSTEIN, W. E., and SHIGLEY, J. W. Changes in fatty acid composition of sulfolipid and phospholipid during maturation of alfalfa. J. Lipid Res. 8: 350 (1967).
8. BEISS, U. Lipids of sugar beet. II. The lipid content of sugar beet root. Zucker 20: 611 (1967).
9. GRAY, I. K., RUMSBY, M. G., and HAWKE, J. C. The variations in linolenic acid and galactolipid levels in Gramineae species with age of tissue and light environment. Phytochemistry 6: 107 (1967).
10. SKARSAUNE, SANDRA K., YOUNGS, V. L., and GILLES, K. A. Changes in wheat lipids during seed maturation. I. Physical and chemical changes in the kernel. Cereal Chem. 47: 522 (1970).
11. PRIVETT, O. S., BLANK, M. S., and LUNDBERG, W. O. Determination of mono-, di-, and triglycerides by molecular distillation and thin-layer chromatography. J. Am. Oil Chemists' Soc. 38: 312 (1961).
12. WAGNER, H., HÖRHAMMER, L., and WOLFF, P. Thin-layer chromatography of phosphatides and glycolipids. Biochem. Z. 334: 175 (1964).
13. CARTER, H., OHNO, K., NOJIMA, S., LIPTON, C., and STANACEV, N. Wheat flour lipids. II. Isolation and characterization of glycolipids of wheat flour and other plant sources. J. Lipid Res. 2: 215 (1961).
14. BOBBIT, J. M. Thin-layer chromatography, p. 86. Reinhold: New York (1963).
15. METCALFE, L. D., and SCHMITZ, A. A. A rapid preparation of fatty acid esters. Anal. Chem. 33: 363 (1961).
16. KASPRZYK, F., PYREK, J., and LUROWSKA, G. The variations of free and bound sterols in *Calendula officinalis* during growth. Acta Biochim. Polon. 15: 149 (1968). (Chem. Abstr. 69: 25116e (1968).)
17. FRANZKE, C., and STOLZ, P. Ueber das Vorkommen von Mono- und Diglyceriden in Fette reifender Pflanzensamen. Fette Seifen Anstrichmittel 68: 591 (1966).

[Received August 4, 1969. Accepted February 9, 1970]