Changes in Wheat Lipids during Seed Maturation. I. Physical and Chemical Changes in the Kernel[1]

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ABSTRACT

As the moisture content of two varieties of HRS and two varieties of durum wheat decreased from 70 to 12% (30 days preripe to maturity), increases in kernel weight and size were measured. During this time, the ash content of both the whole wheat and its respective flour decreased linearly whereas the protein content increased erratically. When protein and ash contents were calculated on a per-kernel basis, both showed a consistent increase as the wheat ripened. The total fat content, determined by acid hydrolysis, reached a maximum when the moisture content of the grain was near 65%, after which it declined steadily. The average lipid content of the four varieties, expressed in mg. fat per kernel, increased threefold during maturation. Successive lipid extractions by petroleum ether and water-saturated n-butanol (WSB) were performed on samples of ground whole wheat, flour, and bran from all varieties at each stage of maturity. The proportion of sugars and free amino acids, which were extracted by WSB, decreased with maturity. Infrared analysis of the lipid extract indicated changes in the lipids with maturity.

Earlier maturity studies of wheat have dealt mainly with the effect of premature harvest on grain quality (1,2,3). In general, it was concluded that only minor differences in final quality existed; flour from early-harvested wheat gave larger bread loaf volume, but color and texture improved with maturity. Scott et al. (4) in 1957 found that maximum yield, test weight, and kernel weight occurred when the moisture content of the sample was near 40%. These results supported Woodman and Engledow's earlier report (5) that no food material was translocated into the developing grain after its moisture reached 40%. Both investigators used HRW wheat as their source material.

During wheat maturation, ash content has been found to decrease steadily (3,6). In contrast, protein content declined until the dough stage was reached, after which it increased slightly. However, Harris et al. (1), who studied three varieties of HRS wheat, reported a consistent increase in protein content during seed maturation.

With the modern advance in technology, the emphasis of maturity studies has

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been focused on chemical changes during grain ripening. Some of these investigations, notably those of Coulson and Sim (7), and Scheffer and Lorenz (8), were concerned with changes in protein composition during wheat maturation.

Lipid changes during ripening have been studied by Daftary and Pomeranz (9), who used two varieties of HRW wheat. They found a slight decrease in total fat content with maturity and a steady increase in 1,000-kernel weight. As a result, the amount of fat in each kernel increased steadily during maturation.

Two varieties each of HRS and durum wheats were employed in this study for comparison. Physical changes in the kernel, as well as changes in ash, protein, and lipid content, are the primary concern of this report. A later paper will deal with chemical changes in lipid composition during the maturation process.

MATERIALS AND METHODS

Two varieties of HRS wheat, Justin and Chris, and two varieties of durum, Leeds and Stewart 63, were collected from North Dakota State University field plots located at Fargo at 3- to 5-day intervals during the 1967 crop year. Moistures were taken immediately on 3-g. samples which had been hand-separated from the surrounding material. The remainder of each sample was freeze-dried. Portions of dried whole wheat were ground on a LabConco mill; the Brabender Quadrumat Jr. yielded flour and bran from tempered 200-g. portions of each wheat sample. All fractions were stored in closed containers at 3°C.

Test weight, 1,000-kernel weight, and kernel-size distribution were determined on dried wheat kernels according to the standard methods used at the Department of Cereal Chemistry and Technology, North Dakota State University, Fargo (10). Protein and ash contents of all wheat and flour samples also were determined by standard methods. Duplicate samples of ground whole wheat were subjected to acid hydrolysis (11) to determine total fat content.

Samples of flour, bran, and ground whole wheat from each stage of maturity were extracted with petroleum ether (b.p. 30° to 60°C.) in a Soxhlet apparatus for 12 hr. The solid residue was air-dried and re-extracted at room temperature by shaking for 30 min. with water-saturated n-butanol (WSB). Where possible, the extraction procedures were performed under nitrogen. All lipid extracts were stored at 3°C. under nitrogen. The lipid extracts were evaporated, weighed, and redissolved in petroleum ether and chloroform, respectively, to form solutions of known concentration.

Thin-layer chromatography (TLC), on 20 by 20-cm. plates coated with a 0.25-mm. layer of Adsorbosil 5, was used to investigate sugars and free amino acids found in the chloroform solution of the WSB extracts. Plates were developed in chloroform:methanol:water (65:55:8, v./v./v.) solution and were visualized with ninhydrin spray for the amino acids and aniline phthalate for the sugars. In addition, the sugars were separated by paper chromatography (Whatman No. 1 filter paper, water:pyridine:ethyl acetate, 3:4:10, v./v./v., solvent system) and visualized by silver nitrate, or by the anthrone reagent, which is used to detect fructose and fructose-containing oligosaccharides. In both paper and thin-layer chromatography, compounds were identified by comparing R_f values with those of known standards.

For infrared analysis, both petroleum-ether and WSB lipid extracts were

evaporated to dryness under nitrogen and redissolved in spectrophotometric-grade carbon tetrachloride. The double-beam setting of the Beckman IR-10, with carbon tetrachloride as reference, was used to scan the lipid extracts from 4,000 to 300 $cm.^{-1}$

RESULTS AND DISCUSSION

Physical Tests

The original moisture content of the wheat samples was used as the index of maturity throughout this work. Days preripe, a more subjective measure of maturity, are included in Table I, which presents the results of the physical tests on the maturing grain. The test weight of all four varieties increased uniformly from about 40 to over 60 lb. per bu. during the maturation period. Thousand-kernel weight increased in the same manner; the least mature samples had 1,000-kernel

TABLE I. PHYSICAL ANALYSIS OF WHEAT SAMPLES

Variety	Days Preripe	Original Moisture %	Test Weight	1,000-Kernel Weight	Kernel Size Distribution			Flour Yield[a] %
					Large %	Medium %	Small %	
Justin	28	66.7	40.0	11.6	1	74	25	27.6
	25	65.0	45.5	14.6	1	76	23	48.3
	21	51.7	51.5	21.0	4	83	13	56.9
	19	48.3	56.5	22.4	8	79	13	68.0
	16	45.1	52.0	26.7	37	59	4	...[b]
	12	38.3	51.0	31.4	64	34	2	...[b]
	7	22.5	60.0	31.6	32	66	2	63.2
	0	10.2	61.0	32.2	44	54	2	45.0
Chris	28	68.0	41.0	11.7	0	40	60	28.4
	25	61.3	38.0	15.2	0	83	17	34.7
	21	51.4	53.5	19.3	0	90	10	36.2
	19	47.0	56.0	26.6	6	89	5	44.7
	16	46.0	56.0	28.2	39	59	2	48.3
	12	44.0	50.0	30.2	79	20	1	52.8
	7	34.0	61.5	32.6	54	46	0	56.2
	0	13.6	61.0	33.4	65	34	1	50.5
Stewart 63	37	69.7	29.0	6.4	0	1	99	41.6
	34	63.8	35.5	11.8	0	15	85	38.5
	30	61.0	41.5	18.0	2	65	33	53.6
	27	56.0	50.0	25.8	2	86	12	46.3
	24	51.3	45.5	29.6	27	70	3	53.3
	20	46.7	53.0	40.8	59	40	1	56.9
	15	40.2	54.5	41.7	64	35	1	58.7
	8	28.0	63.0	47.4	51	48	1	46.2
	0	12.4	63.5	45.9	57	42	1	46.3
Leeds	37	69.0	41.0	12.4	0	25	75	46.7
	34	66.3	47.5	19.6	0	60	40	49.6
	30	52.7	42.0	26.7	34	62	4	52.3
	27	52.3	50.0	31.6	36	61	3	51.1
	24	46.5	50.5	31.2	60	39	1	44.9
	20	41.1	53.5	40.5	74	24	2	54.3
	15	27.3	61.0	40.3	44	55	1	51.0
	8	25.3	62.5	38.0	36	62	2	48.9
	0	11.8	64.0	41.0	40	58	2	49.8

[a]Based on cleaned, tempered wheat.

[b]Laboratory error; no accurate data are available.

weights of about 11 g., whereas in the ripe samples, the 1,000-kernel weight was over 30 g. for the HRS varieties and over 40 g. for the durum samples. The percent of small kernels in all samples decreased uniformly during ripening. The percent of large kernels increased until the original moisture content of the samples was about 40%, and then decreased slightly until full maturity was attained. Maximum flour yield occurred in samples of near 40% original moisture. This observation also was made by Scott et al. in 1957 (4); however, they found that maximum test weight and 1,000-kernel weight also occurred at 40% original moisture. These characteristics were found in the most mature samples in this work.

Seed maturation was characterized by a steady decline in ash content, which was similar to the observation of Shutt (6) (Table II). The ash content of ripening Chris and Justin wheats was remarkably similar throughout maturation. From initial values of 2.17 and 2.14%, respectively, the whole-wheat ash content dropped to 1.60 and 1.54% at maturity. A similar decrease occurred in the ripening durum wheats.

TABLE II. ASH AND PROTEIN CONTENT OF MATURING WHEAT

Variety	Original Moisture %	Ash[a]		Protein[a]	
		Whole Wheat %	Per Kernel mg.	Whole Wheat %	Per Kernel mg.
Justin	66.7	2.14	0.25	13.4	1.57
	65.0	1.95	0.29	14.5	2.03
	51.7	1.80	0.38	14.6	3.02
	48.3	1.75	0.39	16.1	3.52
	45.1	1.62	0.43	15.1	3.77
	38.3	1.61	0.50	16.5	5.02
	22.5	1.60	0.51	17.1	5.18
	10.2	1.60	0.52	16.5	5.09
Chris	68.0	2.17	0.25	13.3	1.52
	61.3	2.05	0.31	13.6	1.95
	51.4	1.91	0.37	14.2	2.64
	47.0	1.77	0.42	14.2	3.27
	46.0	1.68	0.47	14.8	4.06
	44.0	1.61	0.47	15.5	4.59
	34.0	1.55	0.50	15.9	5.02
	13.6	1.54	0.51	16.0	5.31
Stewart 63	69.7	2.80	0.18	13.5	0.84
	63.8	2.52	0.30	12.7	1.46
	61.0	2.44	0.44	12.2	2.09
	56.0	1.88	0.49	12.1	2.97
	51.3	1.87	0.55	12.3	3.49
	46.7	1.68	0.69	12.9	5.10
	40.2	1.60	0.67	14.1	5.50
	28.0	1.56	0.74	13.8	6.30
	12.4	1.54	0.71	14.4	6.11
Leeds	69.0	2.33	0.29	12.8	1.54
	66.3	2.14	0.42	13.1	2.43
	52.7	1.91	0.51	12.3	3.31
	52.3	1.72	0.54	13.3	4.08
	46.5	1.62	0.50	13.1	4.06
	41.1	1.53	0.62	14.3	5.63
	27.3	1.49	0.60	14.8	5.68
	25.3	1.49	0.57	14.2	5.24
	11.8	1.50	0.61	14.4	5.74

[a]14% moisture basis.

The protein content of the HRS varieties, Chris and Justin, increased with maturity, which confirmed the report of Harris et al. (1) (Table II). The least-mature whole-wheat samples had protein contents of 13.3 and 13.5%, respectively; the mature samples contained 16.0 and 16.5% protein. The protein content of the maturing durum varieties did not follow this pattern of steady increase. From an initially high value, protein content decreased until the samples had an original moisture content of about 55%. After this, protein content increased somewhat erratically to reach its highest value at full maturity.

When ash and protein contents were calculated on a per-kernel basis, both increased steadily with ripening (Table II). An average correlation coefficient of -0.885* resulted from correlating ash per kernel and original moisture; when protein per kernel and original moisture were correlated, an r value of -0.931** resulted.

The fact that test weight, 1,000-kernel weight, and ash per-kernel increase after the sample's original moisture falls below 40% tends to refute Woodman and Engledow's (5) hypothesis that no food material is translocated into the grain after it reaches a moisture of 40%.

Total Fat Content

With the exception of Leeds durum, all varieties showed an initial increase of about 0.2% in total fat content, as determined by acid hydrolysis, at approximately 65.0% original moisture (Table III). The fat content then decreased to reach its lowest value at full maturity in the HRS wheat varieties; in the durum varieties, the lowest fat content occurred just prior to maturity, at about 26% original moisture. The fat content of Leeds durum declined with fair uniformity throughout the maturation period.

TABLE III. FAT CONTENT OF MATURING WHOLE WHEAT

Variety	Original Moisture %	Fat[a] %	Fat per Kernel mg.	Variety	Original Moisture %	Fat[a] %	Fat per Kernel mg.
Justin	66.7	2.28	0.26	Stewart 63	69.7	2.70	0.18
	65.0	2.68	0.41		63.8	2.97	0.35
	51.7	2.40	0.51		61.0	2.43	0.45
	48.3	2.50	0.57		56.0	2.31	0.60
	45.1	2.23	0.64		51.3	2.52	0.75
	38.3	2.25	0.73		46.7	2.60	1.06
	22.5	2.08	0.68		40.2	2.35	0.98
	10.2	1.89	0.64		28.0	1.72	0.82
Chris	68.0	2.22	0.27		12.4	2.35	1.08
	61.3	2.43	0.39	Leeds	69.0	3.44	0.45
	51.4	2.36	0.47		66.3	3.34	0.67
	47.0	2.17	0.53		52.7	2.91	0.82
	46.0	2.16	0.63		52.3	2.87	0.96
	44.0	2.19	0.67		46.5	2.91	0.95
	34.0	2.15	0.72		41.1	2.68	1.08
	13.6	1.84	0.62		27.5	2.70	1.09
					25.3	2.25	0.86
					11.8	2.67	1.10

[a]Fat as determined by acid hydrolysis; 14% moisture basis.

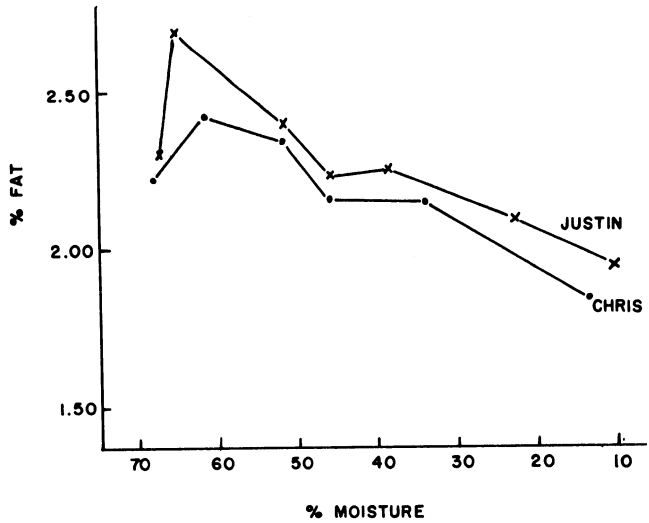

Fig. 1. A graph relating fat content, as determined by acid hydrolysis, with moisture content of maturing Justin and Chris wheats.

The pattern of initial fat increase is illustrated in Fig. 1.

Daftary and Pomeranz (9), in their maturity study of the lipids of HRW wheat, did not observe an initial increase in fat content. However, their least mature samples were 23 days preripe, which would correspond to a moisture content of approximately 50%, based on the varieties analyzed in this investigation. Since the maximum fat content occurs near an original moisture content of 65%, samples having lower moisture would be relatively too mature for the pattern described in this work to be observed.

The fat content, when calculated on a per-kernel basis, increased throughout the maturation period (Table III). In Chris and Justin, the least mature samples had 0.27 and 0.26 mg. fat per kernel, respectively; in the mature samples, the fat content was 0.62 and 0.64 mg. per kernel. The values of fat content on a per-kernel basis for the durum wheats ranged from 0.18 and 0.45 mg. for immature Stewart 63 and Leeds, to 1.08 and 1.10 mg. for the mature samples. The average correlation coefficient relating original moisture and mg. fat per kernel was -0.782*. The increase in mg. fat per kernel during the maturation process confirmed the report by Daftary and Pomeranz (9).

Lipid Extract

The petroleum-ether extracts of the whole-wheat samples showed an initial increase of about 0.26% followed by a fairly steady decline as the samples matured (Table IV). In contrast, the petroleum-ether extracts of the flour samples declined with maturity, with the exception of Stewart 63, where an initial increase was noted. The amount of lipid extracted from the bran by petroleum ether increased until the original moisture of the samples was approximately 50%, after which it declined slightly. Throughout ripening, the petroleum-ether extract of the bran was greater than that of the flour.

TABLE IV. LIPIDS EXTRACTED FROM MATURING WHEAT[a]

Variety	Original Moisture %	Whole Wheat		Flour		Bran	
		Pet. Ether %	WSB %	Pet. Ether %	WSB %	Pet. Ether %	WSB %
Justin	66.7	1.87	1.00	1.75	1.13	1.07	0.50
	65.0	1.92	0.69	1.45	0.73	1.99	0.72
	51.7	1.99	0.82	1.26	1.11	2.78	0.67
	48.3	1.76	0.72	1.18	1.39	2.57	0.75
	45.1	1.85	0.47	0.83	0.85	1.74	0.36
	38.3	1.65	0.65	1.79	0.78	2.12	0.53
	22.5	1.05	0.88	0.73	0.58	2.44	0.78
	10.2	1.82	0.73	0.77	0.63	1.30	0.39
Chris	68.0	1.29	0.52	1.89	0.64	1.21	0.80
	61.3	1.76	0.53	1.47	0.88	1.62	0.58
	51.4	2.08	0.71	1.13	0.87	2.28	0.50
	47.0	2.09	0.57	0.97	0.70	2.52	0.54
	46.0	2.10	0.36	0.81	0.68	2.94	0.54
	44.0	1.86	0.96	0.83	0.68	2.50	0.74
	34.0	1.79	0.65	0.53	0.64	3.29	0.77
	13.6	1.61	0.52	0.80	0.52	1.92	0.58
Stewart 63	69.7	1.63	1.22	1.12	0.94	0.77	0.71
	63.8	1.93	1.10	2.25	1.11	1.37	0.66
	61.0	2.13	0.57	1.77	1.00	2.16	0.70
	56.0	1.88	0.75	1.31	1.10	2.92	0.68
	51.3	2.06	0.83	0.71	0.61	4.14	0.78
	46.7	2.13	0.84	0.99	0.50	3.56	0.82
	40.2	1.74	0.60	0.65	0.46	3.50	0.94
	28.0	1.58	0.53	0.72	0.38	3.29	0.70
	12.4	1.62	0.49	0.81	0.35	3.43	0.66
Leeds	69.0	2.52	1.06	2.42	1.18	1.65	0.67
	66.3	2.47	1.58	1.90	0.79	2.37	0.34
	52.7	2.69	0.43	1.41	0.87	3.10	0.46
	52.3	2.37	0.38	1.06	0.71	4.07	0.54
	46.5	2.28	0.32	1.03	0.47	3.80	0.49
	41.1	1.89	0.62	1.22	0.59	3.82	0.57
	27.5	1.55	0.27	0.84	0.49	3.59	0.59
	25.3	1.77	0.25	0.89	0.61	3.95	0.68
	11.8	1.75	0.46	0.92	0.55	3.67	0.70

[a]14% moisture basis.

The WSB extracts of whole wheat, flour, and bran showed no consistent changes with maturity. In the whole wheat, the sum of the petroleum-ether and WSB extracts did not equal the total fat obtained by acid hydrolysis, because the latter solvent extracted material in addition to bound lipid. Some sugars and amino acids also were found in the chloroform solution of these WSB extracts.

Nonlipid Material in WSB Extract

Sugars. All the WSB extracts indicated the presence of sugars, with the highest concentration in the flour fraction of immature samples (Fig. 2, b). In all these lipid extracts, glucose and fructose were the major sugar components. Glycerol was clearly present in wheat of more than 45% original moisture; the amount appeared to decrease with maturation. Conversely, the amount of sucrose appeared to increase during the same period (Fig. 2, a).

Amino Acids. Glutamic acid, alanine, tryptophan, leucine, and tyrosine, and/or

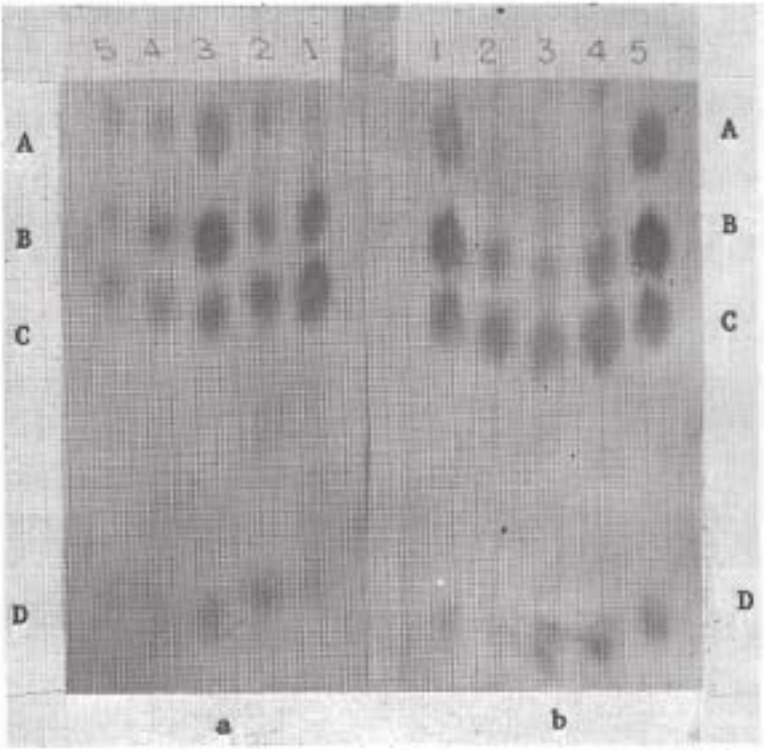

Fig. 2. Paper chromatograms showing some of the sugars present in the WSB extract of maturing Leeds wheat. (a) Spot identification: A, sucrose; B, glucose; C, fructose; D, glycerol; 3, mixture of A, B, C, and D; 5, 4, 2, 1, water-saturated n-butanol extracts of Leeds whole wheat with original moisture content of 11.8, 41.1, 52.7, and 69.0%. (b) 1, mixture of A, B, C, and D; 2, 3, 4, WSB extract of Leeds whole wheat, bran, and flour, respectively. The original moisture content of the wheat was 69%; 5, mixture of A, B, C, and D. Papers developed 12 hr. in ethyl acetate:pyridine:water (10:4:3, v./v./v.) and visualized with silver nitrate.

valine were identified by TLC as components of WSB extracts. Two-dimensional chromatography confirmed this. The amount of free amino acids in the lipid extract decreased as the wheat matured (Fig. 3), although no quantitation was attempted. Scheffer and Lorenz (8) also have reported a decrease in free amino acids during kernel development, and have identified glutamic acid and tryptophan as components of immature wheat kernels.

Infrared Analyses

As the wheat samples matured, the infrared scans of the lipids showed several changes (Fig. 4). A broad absorption band near 3,300 $cm.^{-1}$ developed with maturity. This band was more predominant in HRS than durum wheats. It may represent an overtone of the carbonyl function (1,700 $cm.^{-1}$ X 2 and 1,740 $cm.^{-1}$ X 2), since other groups which absorb in this region are not likely to exist to any extent in the lipids extracted with petroleum ether. Hydrogen bonding among alcohol groups, which are present as components of monoglycerides, diglycerides,

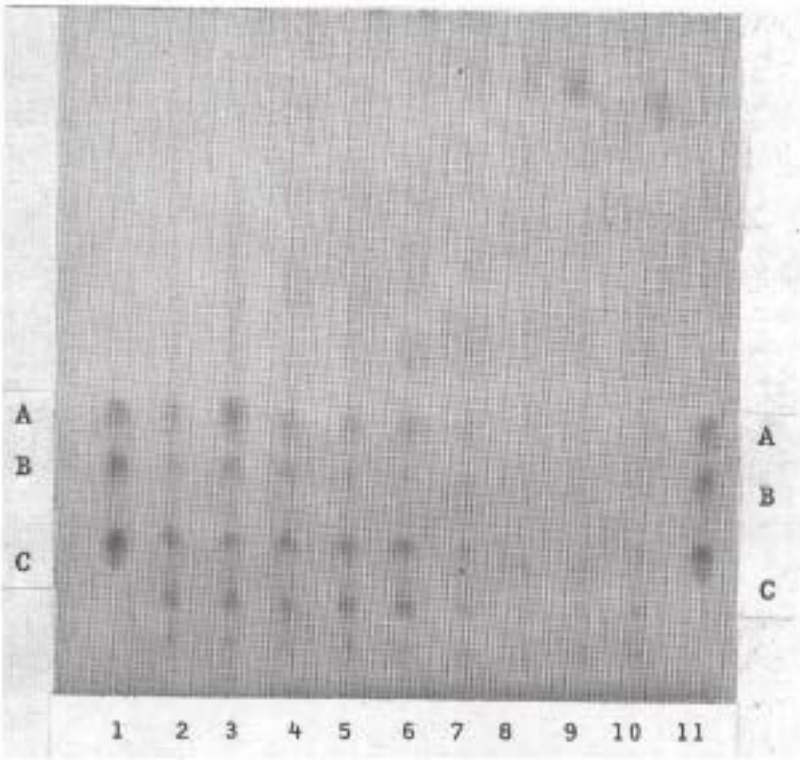

Fig. 3. A thin-layer chromatoplate showing the amino acid content of WSB extracts of maturing Leeds whole wheat. Spot identification: A, tryptophan and leucine; B, tyrosine and valine; C, alanine; 1, mixture of A, B, and C; 2 to 10, extracts of Leeds whole wheat with original moisture contents of 69.0, 66.3, 52.7, 52.3, 46.5, 41.1, 27.3, 25.3, and 11.8%, respectively; 11, mixture of A, B, and C. Plate developed in chloroform:methanol:water (65:55:7, v./v./v.) and visualized with ninhydrin.

glycerol, and sterols, is not likely to cause the absorption at 3,300 $cm.^{-1}$, because this band did not shift to 3,800 $cm.^{-1}$ upon dilution, which would be expected if the absorption were due to hydrogen bonding.

A second change in infrared scans is evident at 1,700 and 1,740 $cm.^{-1}$ The absorption peak at 1,700 $cm.^{-1}$ predominated in unripe samples and decreased with maturity. In contrast, the peak at 1,740 $cm.^{-1}$ increased as the samples ripened. These changes were seen in flour, bran, and whole-wheat samples of both durum and HRS wheats. It has been reported elsewhere (12) that the peak at 1,700 $cm.^{-1}$ is due to acid carbonyl function, and that at 1,740 $cm.^{-1}$ is due to the esterified carbonyl function.

Infrared scans of the wheat lipid extracts also showed changes in the area of 1,300 to 1,450 $cm.^{-1}$ and 1,130 to 1,150 $cm.^{-1}$ The latter change, an increase in absorption with maturity, was more pronounced in the HRS wheats.

The WSB extracts of wheat gave similar infrared scans as the samples ripened. They showed a broad absorption band between 950 to 1,100 $cm.^{-1}$, which may

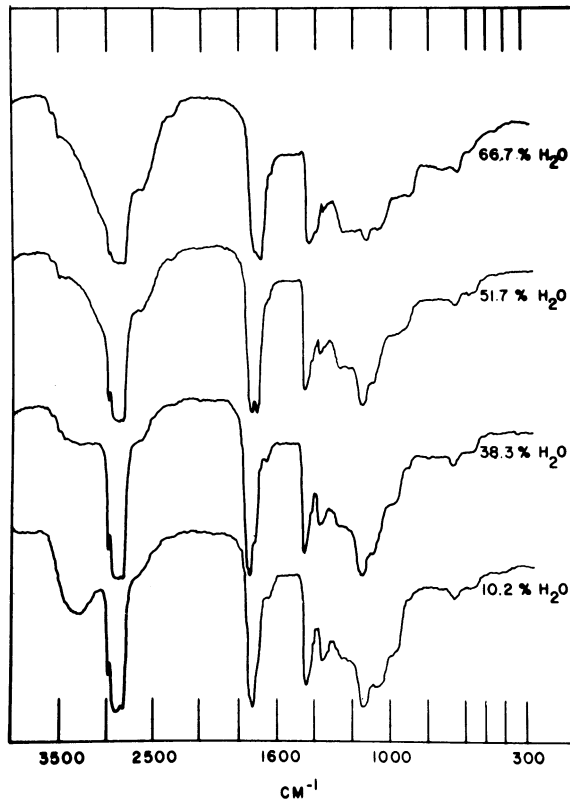

Fig. 4. Infrared spectra of Justin whole-wheat lipids at several stages of maturity. The lipids were extracted by refluxing 12 hr. with petroleum ether (b.p. 30° to 60°C.) in a Soxhlet extractor.

have been due to nitrogenous phospholipids (13). In other respects, these infrared scans resembled those of the petroleum-ether extracts.

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