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SOME EFFECTS OF HIGH LEVELS OF GAMMA IRRADIATION ON THE LIPIDS OF WHEAT[1]

K. H. TIPPLES[2] AND F. W. NORRIS[3]

ABSTRACT

Gamma irradiation produced small changes in the lipid fractions of irradiated wheat. Linoleic and linolenic acids decreased slightly, but only significantly so, at the very high irradiation level of 10^7 rads. Unesterified acids appeared to be more susceptible to radiation damage than esterified acids. Carotenoids and the antioxidant tocopherols decreased with increasing irradiation. Nevertheless, on storage of the milled wheat, linoleic and linolenic acids were autoxidized more slowly in the irradiated samples than in the control. Lipids of irradiated wheat had a higher peroxide value than those of unirradiated wheat. On storage of the flour, lipids of unirradiated wheat showed a gradual increase in peroxide value, whereas lipids of irradiated wheat did not show the same extent of increase. Silicic acid fractionation of flour lipids indicated that irradiation caused a decrease in triglyceride, galactolipid, and phospholipid, and an increase in free sterol, monoglyceride, and diglyceride.

During the past two decades interest has been aroused in the possible application of radiation sterilization to wheat and flour. Technological effects of the gamma irradiation of wheat have been comprehensively reviewed by Milner (1), who dealt with storage, milling properties, biochemical properties, dough and breadmaking properties, and the influence on taste and palatability.

There have been very few investigations of the effects of irradiation on the lipids of wheat. Yen *et al.* (2) reported that splitting of fats did not occur with doses as high as 3.75×10^6 rads. On the other hand, fat hydrolysis increased markedly in stored damp grain when molds developed on samples not sufficiently irradiated to produce sterilization.

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[2]Present address: Grain Research Laboratory, Winnipeg, Manitoba. (This author acknowledges the award of a Spillers Research Scholarship during the course of this work which forms part of a thesis accepted by the University of Birmingham, England, for the Degree of Ph.D., December, 1962.)

[3]Department of Biochemistry, University of Birmingham, England.

The absence of significant splitting of cereal fats with radiation treatment has been confirmed for corn by Romenskii and Chmyr (3), who observed that when this grain was treated at 2.5 megarad, the iodine value also increased significantly.

Gilles *et al.* (4) found an increase in the peroxide value of the lipids of irradiated flour.

Coleby (5) has reviewed the chemical changes produced in lipids by irradiation. He mentioned the notorious difficulties involved in the investigation of lipids and the added drawback in radiation chemical studies that were concerned with only small amounts of chemical change.

The undesirable flavors of irradiated foods show a resemblance to those encountered in oxidative rancidity; and the course of reactions is somewhat similar in both cases, with the formation of hydroperoxides and carbonyl compounds. The removal of oxygen during irradiation and storage prevents some, but not all, of the changes, and antioxidants present in natural lipids can profoundly influence the reactions. Free radical formation is intimately bound up with lipid changes, and their persistence for long periods can cause postirradiation effects. The physical state of the lipids, as determined by temperature, can play an important role during both irradiation and subsequent storage.

Other fat-soluble substances, such as carotenoids and antioxidants, in particular tocopherols, are destroyed on irradiation (6). Bleaching of the carotenoid pigments appears to be smoothly related to dose. The actual extent of destruction varies, and about 50% of the color is usually destroyed by 2×10^6 rads. Lukton and MacKinney (7) state that the destruction of carotenoid pigments is caused by secondary reactions and depends on available free radicals or peroxides formed in the surrounding medium.

The object of the present work was to make a preliminary survey of some of the changes which may occur in the lipids of irradiated wheat. Radiation levels in excess of those likely to be employed for disinfestation of wheat were used. The dose level needed to kill an organism depends mainly on the complexity of the organism; generally the simpler the organism the higher is the dose level needed for destruction. For instance, whereas a dose level of 500 rads would be lethal to man, bacteria and molds may require 1 million rads or more for complete destruction. Cornwell and Bull (8) have published an appraisal of the potentialities and problems involved in the control of insects by gamma irradiation.

Materials and Methods

Two wheats were used: one a hard red spring Canadian Manitoba wheat, moisture content 13.6%, protein content 13.6%; and the other an English soft white winter wheat, variety Minister, moisture content 13.5%, protein content 9.2%. Samples (1–2 lb.) of the wheats were sealed in two thicknesses of polythene and irradiated with a Cobalt-60 source of gamma irradiation. For initial studies one irradiation level of 5×10^6 rads was used, but subsequently additional levels of 10^5, 10^6, and 10^7 rads were employed. The lowest level of 10^5 rads is approximately two to five times the level which would be used for disinfestation purposes. No attempt was made to study the effects of postirradiation storage on the wheat, but a period of at least one week elapsed between irradiation and further examination of the wheats.

The irradiated and nonirradiated control wheats were stored in large glass screw-cap jars until required. All milling of samples to a whole-meal flour was done with a Christie-Norris Junior laboratory mill using a 0.6-mm.-perforation screen.

For flour storage studies, whole-meal flour was stored at room temperature in an atmosphere of air in screw-cap bottles. Flour moisture contents remained reasonably constant over long periods of time under these conditions.

Lipid Extraction. Whole-meal flour samples were shaken with carbon tetrachloride (3 ml. per 2 g. flour) for 2 hr. under nitrogen in the dark. The suspension was filtered, the flour residue suspended in fresh solvent, and the extraction repeated for a further 2 hr. The lipid extracts were combined, dried with anhydrous sodium sulfate overnight, and filtered; the solvent was removed under nitrogen by distillation under reduced pressure at 55°–60°C.

For part of the silicic acid column chromatography work, a more complete extraction of phospholipid material was effected by extracting the flour successively with methanol, chloroform-methanol (1:1 v./v.) and chloroform-methanol (2:1 v./v.). The solvent was evaporated from the combined extracts under reduced pressure at 30°C. under nitrogen and the residue was dissolved in 2:1 chloroform-methanol. Nonlipid material was removed by the washing technique of Folch *et al.* (9).

Preparation of Fatty Acid Methyl Esters. Fatty acids were converted to their more volatile methyl esters for analysis by gas liquid chromatography (GLC). A distinction was made between total fatty acids, i.e., both esterified and nonesterified, and free, nonesterified fatty acids.

Total fatty acids were prepared by the direct interesterification method of Stoffel, Chu, and Ahrens (10), using dry methanolic hydrogen chloride. The methyl esters were sublimed onto a cold finger at 60°C. for 1 hr. under a vacuum of 0.2 mm. of mercury. Methyl esters were rinsed from the cold finger with dry peroxide-free diethyl ether into a sample tube and stored under nitrogen at −20°C. after evaporation of the solvent.

Free fatty acids were methylated directly with diazomethane in diethyl ether and separated from the remainder of the lipid material by microsublimation onto a cold finger as described above. If microsublimation is performed at too high a temperature, sterols and sterol esters may also distill onto the cold finger (11,12). Stoffel *et al.* (10) found that at 60°C. and 0.2 mm. pressure, free cholesterol remained quantitatively in the lipid residue, whereas traces distilled above 85°C. This method was also used as a quantitative measure of the free fatty acid content of the lipid by weighing the methyl esters recovered from the cold finger. A small conversion factor is involved, relating to the difference in molecular weight between the free fatty acid and its methyl ester. Results were comparable to those obtained by normal acidity titrations.

Gas Liquid Chromatography. Quantitative analysis of fatty acid methyl esters was carried out using a Pye Argon Chromatograph. Celite coated with polyethyleneglycol-adipate was used as the column-packing material. Johns-Manville Celite-545 was size-graded and acid-washed as described by Farquhar *et al.* (13). Celite grades of 100- to 120-mesh were used with polyester coatings of 3.5 to 5% at 190°C.

The various component fatty acids were identified by the use of retention volume data of Farquhar *et al.* (13), and standard fatty acid methyl esters were run for comparison. The areas under peaks were directly proportional to the amount of fatty acid present and were measured by triangulation. Samples to be compared were run in rotation, and a total of ten analyses were carried out on each. Percentages of the four main components were calculated, averaged, and analyzed statistically. A "necessary difference" between any two means was calculated for the 5% probability level by the formula:

$$\text{Necessary difference} = t\sqrt{2} \times \text{standard error of mean.}$$

In this study, $t = 2$.

Peroxide Value. The colorimetric iodometric method of Swoboda and Lea (14) was used.

Fractionation of Flour Lipids. Silicic acid column chromatography was carried out according to Hirsch and Ahrens (15). Mallinckrodt

silicic acid, 100-mesh, labeled "Suitable for chromatographic analysis by the method of Ramsey and Patterson," was used. The column temperature was 25°C. and a stepwise elution system was evolved from the method of Fisher and Broughton (16). Increasing amounts of diethyl ether (1, 4, 8, 25, and 100%) in 60° to 80°C. light petroleum were followed by increasing amounts (1, 10, 25, 50, and 100%) of methanol in diethyl ether. Average flow rates through the column were 30 to 40 ml. per hr. Fractions of 10 ml. were collected and rinsed into tared glass "shells." Solvent was evaporated on a boiling methanol bath under nitrogen and the shells were dried to constant weight in a vacuum desiccator.

Lipid fractions were tentatively identified by comparison with the results of Fisher and Broughton (16). The Liebermann-Burchard reaction was used for identification of sterols, and free fatty acids were identified by their vigorous effervescence with diazomethane in ether. Esterified fatty acids were analyzed by interesterification and GLC as described previously. Phosphorus was determined by the colorimetric method of Dryer *et al.* (17) using N-phenyl-*p*-phenylenediamine. Nitrogen was estimated by the micro-Kjeldahl technique.

Carotenoid Pigments. A measure of total carotenoid pigments was made, using the preparative steps described by Quackenbush *et al.* (18). Flour (20 g.) was extracted with 80 ml. of hexane-acetone-water (15:75:10 by volume) under nitrogen in the dark for 48 hr. with occasional shaking. The carotenoids were isolated in a hexane solution and measured spectrophotometrically at 446 mμ. Results were calculated on the assumption that the absorption at 446 mμ of a 1% carotenoid solution in a 1-cm. cell = 2,500.

Tocopherols. Flour (5 g.) was extracted under nitrogen in the dark for two 1-hr. periods with 60-ml. portions of acetone. Combined filtrates and acetone washings were made up to 150 ml. with acetone. Tocopherols were separated by double chromatography on columns of magnesia and alumina according to Worker (19). The method of Tsen (20), using 4,7-diphenyl-1,10-phenanthroline, was used for estimation of tocopherols.

Results

All irradiated wheat samples had a strong "tallowy" odor which was more intense with higher levels of irradiation and which persisted into the flour when the wheats were milled.

Properties of Carbon Tetrachloride-Extracted Lipid. The carbon tetrachloride-extracted lipid was a mobile, cloudy, yellowish-brown oil whose color darkened appreciably with increased storage of the

milled flour prior to extraction. The total amount of material extractable with carbon tetrachloride did not change significantly with irradiation.

The lipid content (14% moisture basis) of the freshly milled flour samples was as follows:

Variety	*Carbon Tetrachloride Solubles* %
Minister	1.12
Manitoba	1.46

Fatty Acids of Freshly Milled Flours. Wheat flour lipids contained four main fatty acid components, namely: palmitic (C 16:0), oleic (C 18:1), linoleic (C 18:2), and linolenic (C 18:3) acids. In addition, small amounts of four other acids were normally detected under the conditions used for analysis. They were stearic acid (C 18:0), about 1.5%; a C 20:1 acid, about 1.8%; myristic acid (C 14:0), about 0.3%; and a C 15:0 acid, about 0.3%.

As preliminary estimations indicated that irradiation had very little effect on the proportions of the four minor components, subsequent analysis was simplified by determining the proportions of the four main acids only. All quantitative results given, therefore, list fatty acid figures as a percentage of the total of the four main acids.

TABLE I

FATTY ACID DISTRIBUTION OF THE CARBON TETRACHLORIDE EXTRACTS OF FRESHLY MILLED WHOLE WHEAT

IRRADIATION LEVEL	C 16:0	C 18:1	C 18:2	C 18:3
rads	%	%	%	%
	Total fatty acids — Manitoba wheat			
0	14.7	19.2	61.4	4.8
10^7	15.2	19.6	60.9	4.4
Necessary difference	0.4	0.3	0.5	0.3
	Free fatty acids — Manitoba wheat			
0	17.4	14.1	63.3	5.2
5×10^6	18.5	14.1	62.6	4.9
10^7	19.2	15.7	61.0	4.1
Necessary difference	0.4	0.4	0.6	0.4
	Total fatty acids — Minister wheat			
0	14.7	14.7	64.4	6.2
10^7	15.2	14.7	64.3	5.8
Necessary difference	0.4	0.3	0.4	0.3
	Free fatty acids — Minister wheat			
0	17.4	10.8	64.9	6.9
5×10^6	18.1	10.7	64.6	6.6
10^7	19.1	11.8	62.9	6.2
Necessary difference	0.4	0.4	0.4	0.4

Results of fatty acid analysis of the lipids of freshly milled samples of irradiated wheat are listed in Table I. Results are not given for the irradiation levels of 10^5, 10^6, and 5×10^6 rads where there was no significant difference in the fatty acid pattern. With both types of wheat at 10^7 rads there was a slight decrease in linoleic and linolenic acids and a corresponding apparent increase in oleic and palmitic acids. The free fatty acids seemed to undergo more change on irradiation than the total fatty acids, indicating that the free fatty acids were more susceptible to irradiation change than esterified acids.

The carbon tetrachloride-extracted lipids were analyzed for total fatty acid content by the addition of a known proportion of pure margaric acid (C 17:0) to the lipid before interesterification. Composition of the lipid material was as follows:

Variety	*Total Fatty Acids* %	*Free Fatty Acids* %
Minister	76	6.2
Manitoba	84	6.3

Hence the free fatty acid fraction accounted for 8.1% of the Minister total acids and 7.5% of the Manitoba fatty acids, whereas the free fatty acids seemed to account for 20 to 30% of the total fatty acid irradiation damage.

Effects of Storage on Milled Whole-Meal Flours. Total and free fatty acids were analyzed in samples of milled wheat flours which had been stored at room temperature for considerable periods of time. For this experiment only one level of irradiation, namely, 5×10^6 rads, was used.

The effects of storage on the fatty acids of irradiated Manitoba and Minister wheats is shown in Table II. With longer storage of the flours the fatty acids of unirradiated samples showed a decrease in linoleic and linolenic acids due to autoxidation, with a corresponding apparent increase in oleic and palmitic acids. The effect was slightly more marked with the free fatty acids than with the total acids, indicating that they were more susceptible to oxidation than esterified acids. The irradiated samples showed a similar trend, and differences between control and irradiated results were slight. However, there was a tendency for irradiated samples to oxidize less rapidly than control samples.

The development of free fatty acid in the lipid during flour storage did not appear to be impaired by irradiation. After 680 days' storage of the whole-meal flour, the free fatty acid content of carbon

TABLE II

FATTY ACID DISTRIBUTION OF LIPIDS OF STORED FLOURS FROM IRRADIATED WHEATS

STORAGE	C 16:0		C 18:1		C 18:2		C 18:3	
	Control	5×10^6 Rads	Control	5×10^6 Rads	Control	5×10^6 Rads	Control	5×10^6 Rads
days	%	%	%	%	%	%	%	%
	Total fatty acids — Manitoba wheat							
0	14.7	14.8	19.2	19.2	61.4	61.4	4.8	4.6
536	14.9	15.0	19.9	19.7	60.3	60.5	4.9	4.8
680	16.4	16.0	19.9	19.9	59.6	60.0	4.1	4.2
Necessary difference	0.3		0.3		0.4		0.3	
	Free fatty acids — Manitoba wheat							
0	17.4	18.5	14.1	14.1	63.3	62.6	5.2	4.9
536	14.9	15.2	21.3	19.8	59.4	60.2	4.4	4.8
680	16.9	16.2	21.5	20.2	57.7	59.3	3.9	4.3
Necessary difference	0.3		0.4		0.6		0.3	
	Total fatty acids — Minister wheat							
0	14.7	14.7	14.7	14.5	64.4	64.7	6.2	6.0
536	13.1	14.6	16.1	16.8	64.8	63.1	5.9	5.6
680	16.3	15.8	17.2	15.6	61.8	63.2	4.8	5.3
Necessary difference	0.4		0.3		0.4		0.3	
	Free fatty acids — Minister wheat							
0	17.4	18.1	10.8	10.7	64.9	64.6	6.9	6.6
536	14.3	13.4	18.3	17.1	62.3	63.9	5.1	5.5
680	17.7	16.4	18.9	16.4	58.8	61.4	4.6	5.8
Necessary difference	0.4		0.3		0.4		0.4	

tetrachloride extracts had risen to about 40%. Thus, with longer storage periods over half of the total fatty acids were in the free state and the free fatty acid pattern tended to resemble more closely that of the total fatty acids.

TABLE III

PEROXIDE VALUES OF CARBON TETRACHLORIDE EXTRACTS OF STORED WHOLE-MEAL FLOURS FROM IRRADIATED WHEAT

STORAGE	IRRADIATION LEVELS (RADS)				
	Control	10^5	10^6	5×10^6	10^7
days	$\mu M/g.$	$\mu M/g.$	$\mu M/g.$	$\mu M/g.$	$\mu M/g.$
	Minister wheat				
0	1.08	1.30	1.41	1.70	2.09
25	1.49	1.37	1.26	1.48	1.48
124	1.94	1.61	1.64	1.39	1.11
185	3.12	2.69	3.02	1.31	1.07
	Manitoba wheat				
0	1.49	1.85	1.77	1.97	2.27
25	1.55	1.57	1.70	1.75	2.11
124	2.47	2.32	2.30	1.95	2.10
185	3.20	3.21	2.03	1.47	2.69

Peroxide values of the lipids of stored flours were measured for the whole range of irradiation levels, and these followed an interesting pattern as shown in Table III. The peroxide values of freshly milled flour lipids increased with increasing irradiation. On storage, the normal increase in peroxide value shown by unirradiated samples became less with increasing irradiation. In some cases the peroxide value tended to fall off with longer storage. Thus, after 6 months' storage the flours from wheats with higher irradiation dose levels produced lipids with lower peroxide values than the control. This effect was more noticeable for the Minister wheat lipids.

Silicic Acid Fractionation of Flour Lipids. Silicic acid fractionation was carried out on lipids from freshly milled untreated wheat and that irradiated at the highest level of 10^7 rads. Manitoba wheat flours were extracted with carbon tetrachloride, and the Minister

TABLE IV

WEIGHT DISTRIBUTION OF LIPID CLASSES OF CONTROL AND IRRADIATED WHEAT LIPIDS FROM SILICIC ACID CHROMATOGRAPHY

Peak No.	Probable Identity	Elution: Ether in Pet. Ether	Elution: Methanol in Ether	Minister (Methanol, Chloroform/Methanol Extract): Control	Minister (Methanol, Chloroform/Methanol Extract): 10^7 Rads	Manitoba (Carbon Tetrachloride Extract): Control	Manitoba (Carbon Tetrachloride Extract): 10^7 Rads
		%	%	%	%	%	%
A				0.4	0.8	0.6	0.7
B	Sterol esters	1		3.1	3.4	3.6	3.4
—				0.4	0.5	1.2	0.8
C	Triglyceride	4		46.1	43.1	62.7	59.9
—							
D	Free fatty acids			5.1	4.9	8.6	5.7
—				0.7	0.6		
E	Free sterol	8		3.8	6.2	3.6	7.8
—				0.5	0.4		
F	Diglyceride	25		8.3	9.2	8.9	10.3
—				0.5	0.8	0.2	0.2
G	Monoglyceride	100		4.2	5.3	3.3	5.2
H				0.8	1.3		
—			1	0.1	0.2	0.1	0.3
I				2.0	2.1	0.4	0.5
J			10	3.3	3.1	0.8	0.7
K	Galactolipid region			8.4	4.9		
L			25	2.1	3.1	2.4	1.3
M				1.3	2.3		
—				0.5	1.0	0.5	0.5
N				3.1	2.5	0.8	0.9
—	Phospholipid region		50	0.6	0.6	0.4	0.4
—				0.2	0.4		
O				3.3	1.7	0.5	0.8
P			100	0.6	0.9		
Q				0.2	0.4	1.3	0.7
R				0.4	0.3		

flours with the chloroform-methanol system. The weights of lipid recovered from 100 g. of Minister flours (14% m.b.) after Folch-washing were 1.3 g. and 1.39 g. for control and irradiated samples respectively.

Weight distribution curves for the Minister wheat lipid fractionations are shown in Figs. 1 and 2. Table IV shows a full comparison of the control and irradiated wheat lipid data. Percentage recoveries of lipid loaded on the columns were 99.9% for the control and 98.4% for the irradiated sample. Weight distribution curves for the Manitoba wheat lipids are not shown, but were very similar to those for Minister wheat, except that the carbon tetrachloride extracted less phospholipid material. The weight distribution data for Manitoba wheat are also summarized in Table IV.

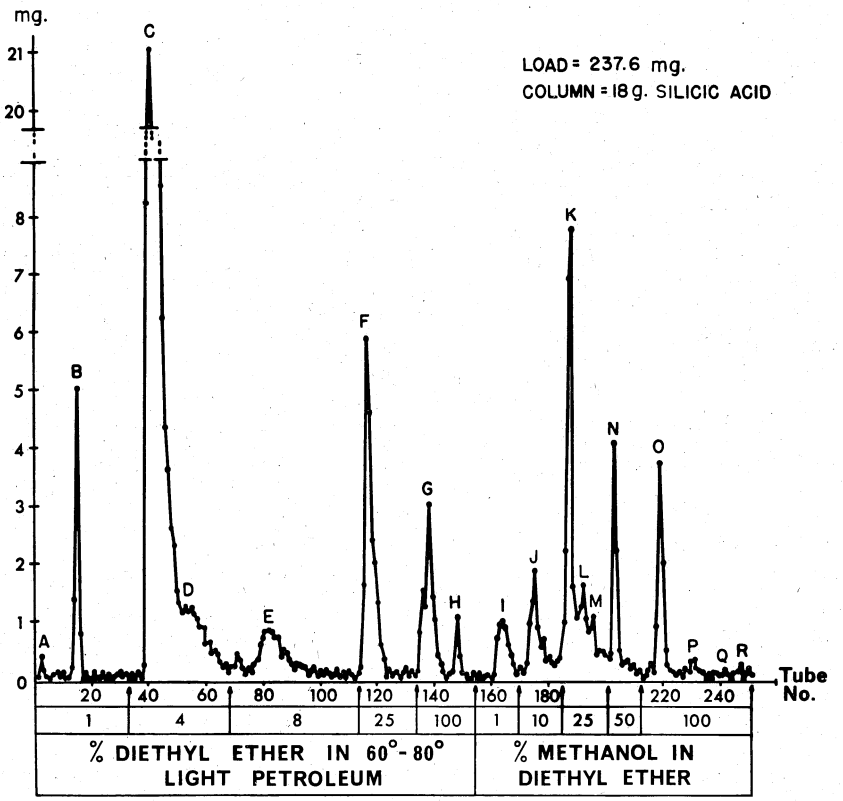

Fig. 1. Silicic acid fractionation of unirradiated Minister wheat lipid (chloroform-methanol extract).

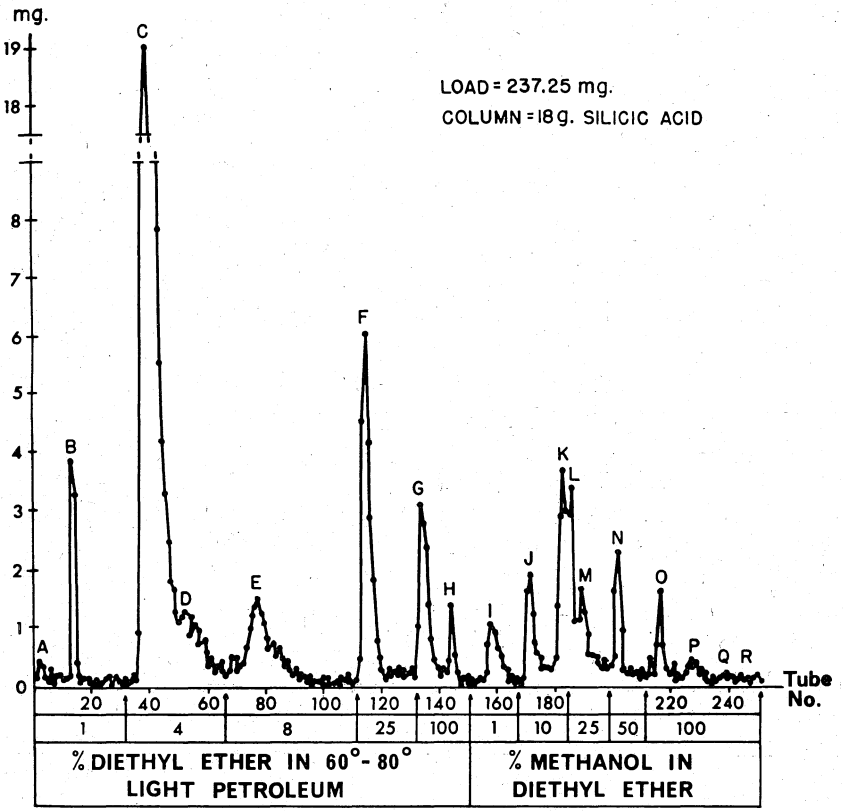

Fig. 2. Silicic acid fractionation of irradiated (10^7 rads) Minister wheat lipid (chloroform-methanol extract).

A tentative identification of peaks was made, based on tests for sterols, free fatty acids, nitrogen, and phosphorus, and also using the assumption that the peaks corresponded to those obtained by Fisher and Broughton (16) with the same elution system. The probable identity of some of the peaks is shown in Table IV.

The very high irradiation level of 10^7 rads produced a decrease in triglycerides, galactolipid, and phospholipid, with a corresponding increase in the amounts of free sterol, diglyceride, and monogylceride. The changes involved were relatively small except for free sterol and phospholipid. Free sterol increased by a factor of more than 1.5 in the irradiated wheat lipids, although the sterol ester contents changed only slightly. Sterol and sterol ester peaks gave a positive Liebermann-Burchard reaction; other peaks gave a negative response. Fatty acids were isolated from the sterol ester peaks (B) by interesterification, but

were not found in the free sterol peaks (E).

In all cases a yellow color was associated with the beginning of peak C and with peaks F and G. Hexane solutions of the colored material showed characteristic carotenoid absorption spectra in the visible range.

GLC analysis of fatty acid methyl esters obtained from silicic acid fractions showed that the content of individual fatty acids varied slightly throughout the various lipid classes. Galactolipid, for example, contained a higher percentage of linoleic acid than other fractions. However, the small differences produced by irradiation were similar for all fractions.

Carotenoid and Tocopherol Contents of Irradiated Wheat. Table V gives the average values of the carotenoid and tocopherol contents of freshly milled flours from irradiated wheat samples. The destruction of carotenoids and tocopherols was smoothly related to irradiation dose. The Manitoba wheat tocopherols appeared to be slightly more susceptible to irradiation damage than the Minister wheat tocopherols.

TABLE V

CAROTENOID AND TOCOPHEROL CONTENT OF FRESHLY MILLED IRRADIATED WHEAT

IRRADIATION LEVEL	TOTAL CAROTENOID		TOTAL TOCOPHEROL	
	Manitoba	Minister	Manitoba	Minister
rads	*μg./100 g. flour*	*μg./100 g. flour*	*mg./100 g. flour*	*mg./100 g. flour*
0	159	178	2.70	2.96
10^5	155	166	2.20	2.73
10^6	144	151	1.67	2.60
5×10^6	85	96	1.10	2.07
10^7	76	74	0.58	1.83

Discussion and Conclusions

As was to be expected from a survey of the literature on the changes of lipids on irradiation, the differences in chemical composition between control and irradiated wheat lipids were small, even at the highest dose level. The level 10^7 rads is some 500 times greater than normal treatment levels that would be used for disinfestation of wheat and flour. However, it is well known that only small amounts of oxidative degradation are necessary to produce profound undesirable changes in odor and flavor, and all irradiated wheat had a strong, unpleasant "tallowy" odor.

Analysis by GLC has shown that the fatty acids isolated from irradiated wheat contained a reduced proportion of linoleic and

linolenic acids, but the reduction was significant only at the 10^7-rad level. It would appear that linoleic and linolenic acids are somewhat more susceptible to irradiation damage in the free state than when esterified.

Silicic acid fractionation results showed a reduction in triglyceride and a corresponding increase in mono- and diglycerides. This could be due to a splitting-off of one or two fatty acids from triglyceride molecules. However, there was no increase in the amount of free fatty acid recovered. This could mean that the splitting of the ester linkage produced not free fatty acid but a degraded molecule or free radical.

The most striking difference between lipids extracted from unirradiated and heavily irradiated wheat was the increase in the amount of free sterol. Manitoba wheat extracted with carbon tetrachloride, and also Minister wheat extracted with chloroform-methanol, showed this increase: from 3.6 to 7.8% for Manitoba, and from 3.8 to 6.2% for Minister. As the amount of sterol ester extracted did not change appreciably, it is unlikely that the increase in free sterol occurred as a result of sterol ester splitting. Rather, it seems that free sterol occurred partly in a bound form, so that not all of it was extracted by normal solvent extraction. The irradiation could have severed or weakened the linkage by which sterol was bound, thus making the sterol more completely available for solvent extraction.

The most likely explanation of the decrease in phospholipid extracted from irradiated wheat is that the phospholipid was "denatured" and was thus less available for solvent extraction.

The principal effect of irradiation in the presence of oxygen appears to be to induce reactions very similar to those occurring during autoxidation, i.e., hydroperoxide formation leading to carbonyl production (21). The increase in peroxide value noted with wheat lipids is in agreement with this and also with the findings of Gilles (4) with flour lipids.

The complexity of the lipid system is demonstrated by the fact that milled irradiated wheat flours oxidized less rapidly on storage than control flours, as indicated by a slower decrease in EFA destruction and reduced peroxide production. These results were unexpected, as tocopherol destruction would have been thought to decrease keeping-qualities because of reduced antioxidant activity. Astrack *et al.* (22), working with vegetable and fish oils, reported that irradiation of samples containing added antioxidant appeared to confer an increased stability to autoxidation as compared with the unirradiated oil, but stated that the theoretical basis for this effect was far from clear.

The seemingly anomalous storage oxidation results can be explained if we consider an oversimplified representation of a series of oxidation reactions where an unsaturated fatty acid is converted to a nonperoxide end product via a peroxide molecule:

$$\text{UNSATURATED FATTY ACID} \xrightarrow{r_1} \text{PEROXIDE} \xrightarrow{r_2} \text{NONPEROXIDE (e.g., POLYMER)}$$

Reaction rate constants for the two stages are r_1 and r_2. On storage of normal untreated flour, r_1 is greater than r_2 and there is a net increase in detectable peroxide. If irradiation damages the (e.g., enzyme) system catalyzing peroxide formation, then r_1 will be slowed down. If r_2 is unchanged or slowed down to a lesser extent, the net result will be a decrease in the rate of peroxide formation. If, however, r_1 is unchanged and r_2 is increased, possibly by residual free radical reactions, then the net result will again be a decreased rate of peroxide formation, but not in this case due to decreased oxidation of fatty acid. Fatty acid analysis indicated that although irradiation initially caused a slight destruction of unsaturated fatty acids, the rate of destruction of linoleic and linolenic acids on flour storage tended to be slower in irradiated samples. Hence it would appear that the first possibility, namely a slowing down of r_1, took place; and the apparent slower production of peroxide was due to a decreased rate of oxidation of unsaturated fatty acids. It is also conceivable that both possibilities were in effect.

The very small changes in lipid on irradiation were doubtless linked with the formation of unacceptable odors and flavors in the wheats. Research might fruitfully be carried out into the production of flavor components in the irradiated samples.

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