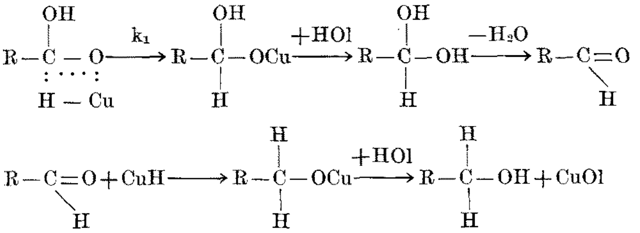
TABLE III

Continuous Hydrogenation of Fatty Acids and Fatty Oils Under the Influence of Cu and Cd Soaps

Hydrogenation of	Cu:Cd in % m	Feed, ml/h	Pressure in atm H_2	Temp, C	Conversion %	Selectivity %
Oleic acid	5:2.5	110	275	260	85	99
Oleic acid	5:2.5	150	230	260	79	99
Oleic acid	5:2.5	110	300	260	88	88
Oleic acid	5:2.5	146	300	260	88	89
Oleic acid	5:2.5	140	275	260	90	90
Capric acid	3:1.5	120	250	260	85	
Olive oil	5:2.5	110	200	260	60	90
Soybean oil	5:2.5	140	250	270	75	75
Linseed oil	5:2.5	180	300	270	70	70

the reaction rate of the acid concentration. The rate determining step could be the following reaction:



$$R{-}C(OH){-}O\cdots H{-}Cu \xrightarrow{k_1} R{-}CH(OH){-}OCu \xrightarrow{+HOl} R{-}CH(OH){-}OH \xrightarrow{-H_2O} R{-}CH{=}O$$

$$R{-}CH{=}O + CuH \longrightarrow R{-}CH_2{-}OCu \xrightarrow{+HOl} R{-}CH_2{-}OH + CuOl$$

Following this scheme we arrive for the rate of the hydrogenation to the following equation:

$$v = k_1\,[CuH \cdot HOl] = k_1K_1\,[CuH]\,[HOl] = k_1K_1K_2\,[Cu]\,[H_2].$$

This equation is in accordance with the first part of the experimentally found equation:

$$v = k\,[Cu]\,[H_2]\,\{1 + K\,[Cd]\,\}$$

For the last part of this equation no explanation can be offered.

The end conversion of the hydrogenation of oleic acid in hendecane as solvent is 67–70%. At higher conversions the reaction mixture becomes heterogeneous. This can be explained as follows: The alcohol, formed by the hydrogenation of the acid, reacts with the remaining acid under formation of an ester. When the conversion is higher than 50% an excess of free alcohol will be present. The excess of alcohol reacts with the soap by which reaction ester, metal oxide and water are formed:

acid + alcohol ⇄ ester + water

alcohol + soap ⇄ ester + water + metal oxide

If it is assumed that the equilibrium constants are independent of the solvent concentration and independent of the conversion, it can be calculated that the point at which the soap will react with the alcohol will shift to a higher conversion when less solvent is present, which is in accordance with the experiments. Moreover, the water concentration in the liquid phase will rise more than linearly with decreasing solvent ratio, thus shifting the equilibrium to the left.

The function of Cd-soap is not understood. It might be, however, that the Cd, e.g., as metal on the wall of the reactor and as soap in solution, stabilizes the CuH against decomposition into Cu and H_2.

ACKNOWLEDGMENT

J. van Mourik, P. J. Seip, J. van der Graaf, P. R. Avis, H. Selles, G. van Gooswilligen and H. W. van der Linden contributed to this paper.

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[Received October 31, 1964—Accepted February 19, 1965]

Lipid Oxidation in Flour Doughs—Observations Involving the Boron Trifluoride Interesterification Reaction

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Abstract

Lipid extracted from unyeasted wheat flour doughs had earlier yielded hitherto unidentified methyl esters using boron trifluoride interesterification when the lipid was isolated from aged or chemically oxidized flours or doughs. The identity of one unknown constituent is shown to be methyl methoxy stearate which could also be an artifact of the interesterification process. Conditions of formation of the artifact have been carefully studied and statistical analysis of the yields of this substance from the lipid of naturally aged flour doughs maintains our view of a reversible lipid oxidation stage being involved in natural and chemical flour maturing in the presence of oxidants in doughmaking.

Introduction

FLOUR TREATMENT involves oxidative reactions which can affect either the protein or lipid of flour. At the 6th I.S.F. Congress in London, 1962, we presented (1) the results of work on the lipids of flour dough in which unexpected changes were observed in the gas-liquid chromatograms of fatty acid ester mixtures prepared from dough lipids by the boron trifluoride/ methanol interesterification reaction (2). Two unidentified compounds, designated x and y, were formed at the expense of the unsaturated fatty acids of the dough lipid, their appearance apparently being enhanced if an oxidant, potassium iodate, was added during dough mixing (Fig. 1). Subsequently we examined factors influencing the appearance of these compounds in ester mixtures and identified x and y

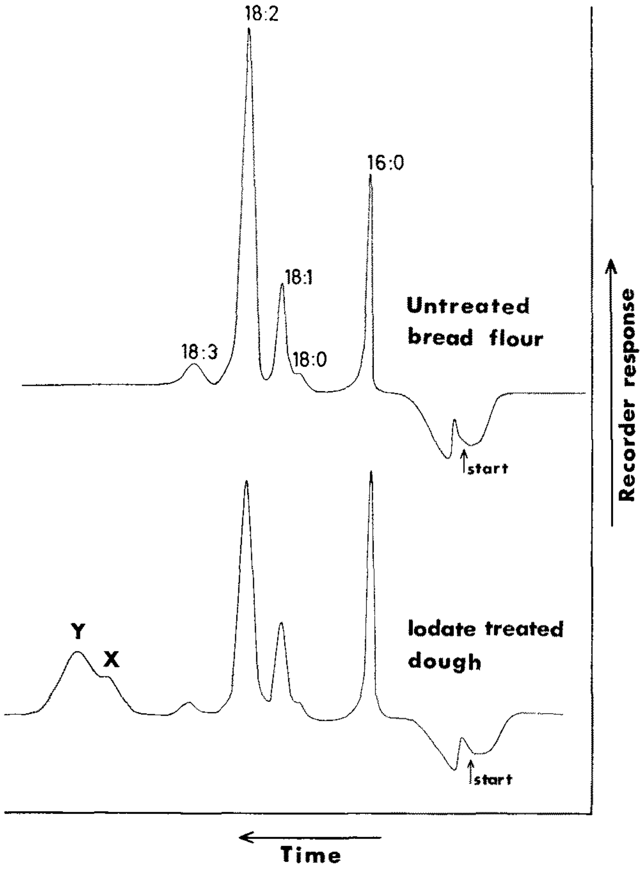

FIG. 1. Comparison of the GLC analysis of the lipid of untreated flour with that of dough lipid after treatment with potassium iodate at 80 ppm.

as esters of methoxy substituted fatty acids. Recently we concluded, coincident with the publication by Lough (3), that the BF_3 reagent may produce such methoxy substituted esters as artifacts during the interesterification reaction. However, as will be seen later, some of our observations are difficult to reconcile with artifact formation alone, although in view of this observation it was concluded that the BF_3 interesterification is too nonspecific a method to be used with confidence in the future. The results we obtained and the inferences we made at the time will be described and finally reconsidered in the light of a statistical evaluation of the variation in x and y formation in esters prepared under carefully controlled conditions.

Experimental

In our early work (1) the presence of air as well as chemical oxidants (iodate and chlorine dioxide) appeared to enhance the yield of x and y in the ester mixtures. Lipid from dough prepared in a mixer open to air, or sampled near the surface of a resting dough piece with free access to air gave ester mixtures richer in these compounds than samples having a limited access to oxygen. A dough which had given a large proportion of x and y esters (wtih a proportional loss of unsaturated fatty acids) might on subsequent sampling, or after baking, give a lipid yielding on analysis a full complement of unsaturated acids with a negligible x and y content.

TABLE I

Carbon Numbers on Two Stationary Phases

Fatty acid (as Me-ester)	Apiezon L	Peg. A
Palmitic acid C_{16}	16.00	16.00
Stearic acid C_{18}	18.00	18.00
Oleic acid $C_{18}^{1=}$	17.68	18.23
Linoleic acid $C_{18}^{2=}$	17.51	18.87
Linolenic acid $C_{18}^{3=}$	17.07	19.59
Arachidic acid C_{20}	20.00	20.00
Unknown x	19.07	20.58
Unknown y	18.74	20.77

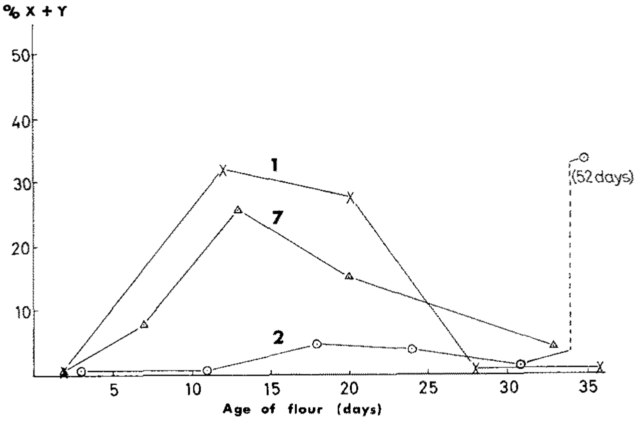

FIG. 2. Untreated flour and dough. Variation in yield of x and y with age of flour. Results with flours from three different wheat consignments. (All Manitoba No. 2 wheat.) Consignment A, x—x; consignment B, ⊙—⊙; consignment C, △—△. (Numerals indicate hour sample).

On the basis of these results it was tentatively suggested that the unidentified compounds were formed from intermediate oxidation products of the unsaturated acids during the BF_3 interesterification. The x and y precursors appeared to be transient in the dough and bread reverting ultimately to the unchanged fatty acids of the parent flour lipid.

Later investigation showed that other factors contributed to the formation of the postulated precursors in the dough lipid apart from the presence of oxygen and oxidant in the dough. Varying yields of x and y were obtained from doughs mixed under identical conditions from different flours, or from the same flour on different occasions. These variable results suggested that grist differences and age of flour might be factors influencing the dough lipid reactions. Varying effects of aging on treated and untreated commercial flours have been reported earlier by Bennett and Coppock (4).

A series of experiments was undertaken to examine the behaviour during storage of Bühler milled flours from different consignments of Manitoba No. 2 wheat, a wheat class used in the preparation of bread flours. The results obtained from untreated doughs are shown

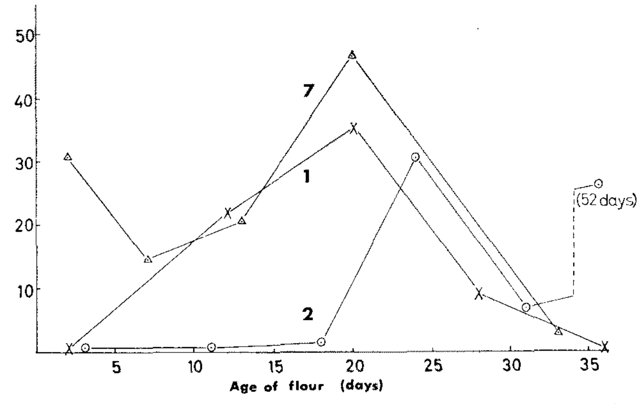

FIG. 3. As Fig. 2, but treated with potassium iodate (80 ppm) during dough mixing.

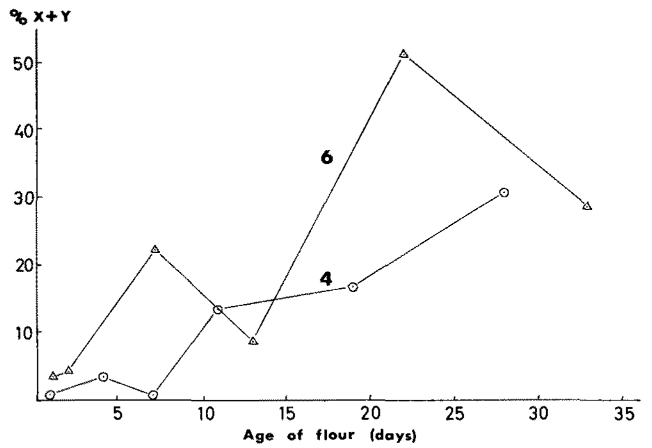

FIG. 4. As Fig. 2, flour treated with chlorine dioxide (28 ppm) immediately after milling.

in Figure 2 and confirmed that the yield of x and y appeared to change with the age of the flour, reaching a maximum after 2 to 3 weeks' storage. While flours 1 and 7 from Manitoban wheats A and C were similar, flour 2 from Manitoban wheat B was slow to develop an appreciable x and y yield.

The effect of oxidising agents on the results is shown in the following figures, potassium iodate alone (Fig. 3) having little effect on the general trends observed although slightly higher yields of x and y were obtained over-all. Chlorine dioxide treatment of flours from wheats B and C is shown in Figure 4 where, in spite of a general increase in x and y yields, wheat B again produced a less "active" flour than wheat C. A similar effect with age in flour 6 (wheat C) rising to a maximum at 3 weeks was also seen. Additional treatment with iodate (Fig. 5) increased the yields only slightly, but a high yield was obtained at 2 days with flour 6, which corresponds to a similar early rise on iodate treatment of flour 7 (Fig. 3) from the same wheat. Generally, the use of oxidising agents increased the yield of x and y compared with results from untreated doughs.

In the light of later findings by Lough (2) and ourselves on the production of methoxy derivatives as artifacts during the BF_3 methylation, we realised that these results are likely to have been influenced by artifact formation to an extent difficult to assess. However, it is hard to dismiss completely the repeated rise in x and y yield with flour aging, or dough oxidation and to account for the consistent slow development of x and y from flours from one of the three wheat samples examined. A statistical treatment of x and y yields from various dough lipids will be considered later.

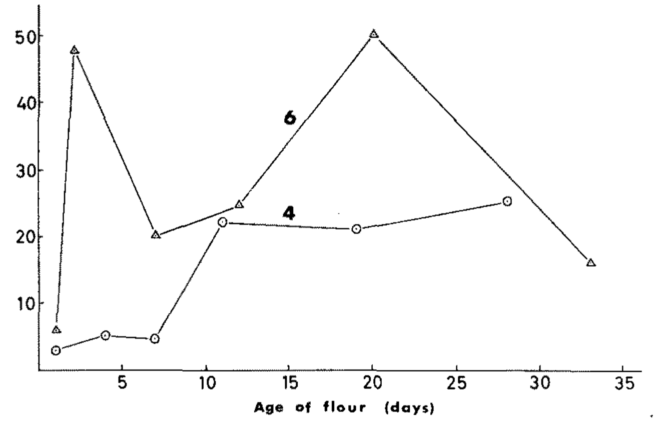

FIG. 5. As Fig. 4, but treated with potassium iodate (80 ppm) during dough mixing.

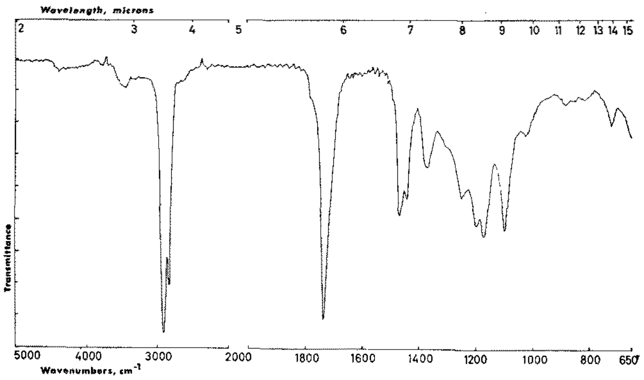

FIG. 6. IR spectrum of pure compound x: methyl methoxystearate.

Pooled ester mixtures rich in compounds x and y were used to study the chemical constitution of these compounds. Their behaviour on different columns (shown in Table I), together with bromination and hydrogenation results, showed that compound y differed from x only by the presence of a double bond in the molecule of compound y. Hydrogenation was used to convert y to x. The large amount of stearic acid formed from the other unsaturated acids present was removed by low temp fractional crystallisation, pure compound x being obtained by preparative gas-liquid chromatography (GLC) of the cold soluble fraction for use in subsequent structure determinations.

The infrared (IR) spectrum of x is shown in Figure 6; it appeared to be that of a liquid fatty acid ester having a strong absorption at 1100 cm^{-1} probably due to an ether structure. A pure sample of x was compared by nuclear magnetic resonance spectrophotometry with pure methyl stearate prepared under the same conditions in order to obtain further information on the nature of the suspected ether group. The chemical shifts in resonance absorption are listed in Table II and show that compound x differs from methyl stearate by the appearance of absorption at 6.66τ ascribed to protons associated with the ether bond (5,6). The area beneath this absorption peak was the same as that for the methyl ester absorption at 6.28τ and it was concluded that the probable structure for x was methyl methoxystearate. This structure was in accordance with elemental analysis and mol wt determinations on a further sample of pure compound x. The results (Table III) showed good agreement of carbon, hydrogen and oxygen with the calculated values for methyl methoxystearate but the mol wt was low. From the evidence of its carbon number on Apiezon L (19.1, compared with 19.07 for x) and IR spectrum (strong absorption at 1100 cm^{-1}) methyl 12-methoxystearate prepared by Lough from ricin-

TABLE II

NMR Spectra of Compound x and Methyl Stearate

Proton Environment	Chemical shifts, τ (ppm)		
	Approx. values from tables	*Found*	
		Compound x	Methyl stearate
H–C of terminal CH_3	9.1	8.95	9.20
H–C of carbon chain	8.7	8.64	8.70
H–C adjacent to carboxyl	7.9	8.28	8.20
H–C of ester CH_3	6.3	6.28	6.38
H–C of ether CH–O–R	6.7	6.66	—

TABLE III

Elemental Analysis and Mol Wt of x

	Carbon	Hydrogen	Oxygen[a]	Mol Wt
Found	73.6%	11.5%	14.9%	294
Calculated, C_{20}, H_{40}, O_3	73.0%	12.3%	14.7%	328.5

[a] By difference.

oleic acid (3) would appear to be closely similar in composition to compound x prepared from pooled dough oil esters.

During a study of the reactions leading to the formation of the methoxy derivatives during the BF_3 interesterification, the unexpected observation was made that the yield of these derivatives could be varied by a change in the ester separation routine. After refluxing oil samples with the BF_3 reagent (which at the time of this work was being supplied in concentrated form, requiring therefore dilution to 12.5% w/v to be comparable with our earlier work), it had been found advantageous to remove excess methanol to avoid emulsion formation during light petroleum extraction of the reaction mixture. This was accomplished by a short (ca. 10 min) distillation of methanol from the reaction mixture on the steam bath.

It was found that by using a longer distillation time, and thus increasing the quantity of methanol removed, the yield of x and y esters was increased proportionally. The effect is shown in Figure 7 where the volume of methanol removed and the yield of methoxy derivatives are shown plotted against the distillation time. It was clear that variations in the yields of x and y observed previously could have been influenced by artifact formation due to inadvertant variation in distillation time, and a comprehensive investigation of dough lipid behaviour during flour aging was undertaken to test statistically the significance of change in x and y yields under rigidly controlled conditions of ester preparation.

A sample of Manitoba No. 2 wheat similar to that used previously was Bühler milled and sampled at weekly intervals for dough mixing as before. At the same time samples of other Bühler milled flours from a wide selection of available wheats were examined in order to present a large number of results for statistical evaluation. Each extracted dough oil was methylated in quadruplicate and the methanol removed by carefully timed duplicate distillations of either 5 or 10 min duration. Results were thus obtained both near the onset of, and during, artifact formation.

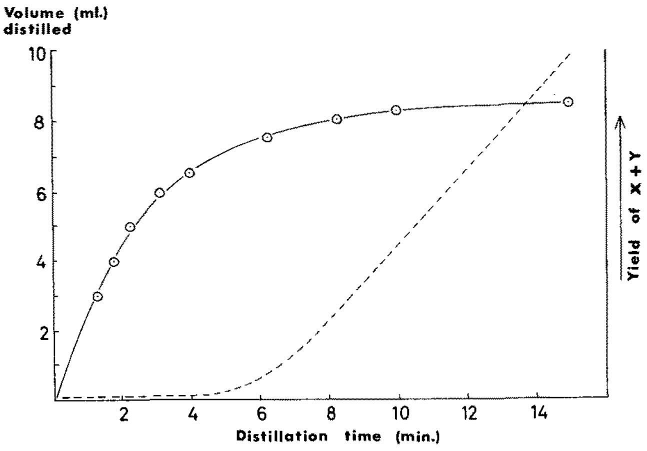

FIG. 7. Change in x and y yield and amt of methanol removed with increased distillation time; ⊙—⊙ methanol; ---- x and y.

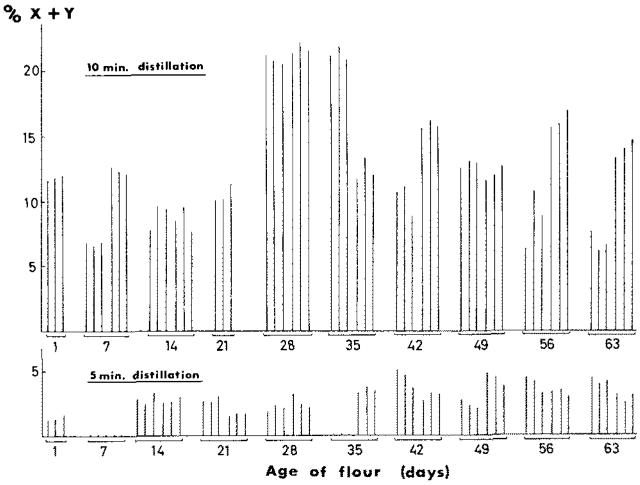

FIG. 8. Variation in yield of x and y with age of flour. Distillation times controlled to either 5 or 10 min duration.

The flour aging experiment was continued for 10 weeks and, together with other flour samples chosen at random, gave some 80 separate interesterifications from which were obtained over 200 GLC measurements of x and y yield. Figure 8 shows a histogram presentation of x and y yield for 5 and 10 min distillations throughout the aging flour experiment. The data for the 5 min distillation showed no statistically significant change during the flour aging experiment. At the 10 min distillation time, the yields were greater over-all than had been observed in the earlier work where zero yields were usually found in the first weeks after milling. The agreement between duplicates was poor and the somewhat insensitive joint test of difference between the means for different flour ages against experimental error did not attain statistical significance. However, a plot of means against age (Fig. 9) showed a trend similar to the experimental observations made in the earlier work but at a higher yield level and showed a maximum at 28 days. The significance of this trend was tested statistically using a parabolic line of best fit. Although a perfect fit was not obtained, the quadratic term in the fitted equation was significant at the 5% level, which can be regarded as evidence that the peak observed cannot be dismissed as spurious. Moreover, analysis of the data from the

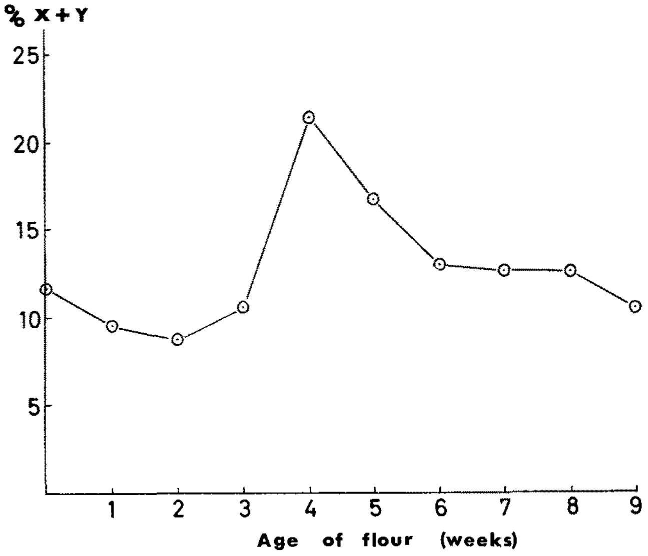

FIG. 9. As Fig. 8, mean values for 10 min distillation plotted against age of flour.

randomly selected flours showed that the differences between flours tested jointly against experimental error were statistically significant at the 5% level, indicating again the presence of some factor in the dough lipid which could influence x and y production over and above simple artifact formation.

Discussion

The observations made in these experiments thus deserve consideration, and the possibility that methoxylation by BF_3 may be enhanced by some oxidation mechanism acting on the dough oil must still be left an open question. If the agreement between our early results is regarded as significant, and if the effect of air and oxidants on x and y yield as the flour ages is taken as evidence of a change in the reactivity of the flour lipid to oxidative reactions, then it is of interest to consider briefly our earlier suggestion of the existence of a reversible lipid oxidation reaction.

While lipids have been found to play a part in the oxidative reactions involving protein $-SH$ groups (7) and have been said to compete with $-SH$ for available oxygen in the dough (8), the importance of lipid peroxides in dough oxidation has been doubted in view of the small quantity and low reactivity of the hydroperoxides found experimentally (9). The existence of a reversible lipid oxidation involving a transient oxidation stage (envisaged as a substrate in the BF_3 methoxylation reaction other than the artifact reaction) would explain the lack of peroxide activity in dough lipid during oxygen transfer between oxidant and protein $-SH$. Even though Privett has obtained evidence of the possible existence of an oxidation stage prior to stable hydroperoxide formation (10), much work remains to be done before the reversible oxidation mechanism we have proposed can be proved or disproved. A method of detection more specific than the BF_3 reaction will be required and the transient nature of such intermediates will add to the experimental difficulties. However, from the limited evidence presented here the implications of such a study can be seen to be of great interest both in and beyond the field of cereal chemistry which is our immediate concern.

ACKNOWLEDGMENTS

R. P. W. Scott performed the NMR determinations; J. C. Dickins, the statistical analysis; and R. J. Davies gave valuable technical assistance. The micro-analytical section of Cambridge University Chemical Laboratories did the elemental analysis and mol wt determination.

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[Received October 26, 1964—Accepted March 26, 1965]

Further Studies of Degossypolized Cottonseed Meal as a Source of Plant Protein in Rabbit Feeds

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Abstract

Degossypolized cottonseed meal was fed to mature female rabbits throughout four successive litters, using a split-plot randomized block design. When used as a replacement for soybean meal, at levels of 4, 7, 10 and 13% of the diet, no significant diet responses were found in the analyses of total litter weaning weight, average individual weaning weight, percentage mortality, number weaned, or feed conversion. However, block and litter effects on litter weight were significant, suggesting genetic differences, and the means for litter weights and mortality suggested an adverse effect at the 10 and 13% levels. Results indicated that at levels of 4 and 7% degossypolized cottonseed meal may serve as a satisfactory substitute for soybean meal in the rabbit diet.

PREVIOUS STUDIES by Casady et al. (1962) showed that degossypolized cottonseed meal, when used as a replacement for soybean oil meal, at levels of 3, 5, 7, and 9% of the rabbit diet, produced no significant diet responses in the analyses of average individual weaning weight per litter, percent mortality in young rabbits, or feed conversion. However, the linear and quadratic responses of total litter weaning weight to additional cottonseed meal were significant. The significant quadratic regression of unadjusted total weaning weight on percentage of cottonseed meal was caused by better growth with the 3 and 5% diets. There were, in addition, indications of a depressing effect on total litter weaning weight at the 7 and 9% levels.

The purpose of this experiment was to obtain more data using levels of cottonseed meal which overlapped

TABLE I

Composition and Chemical Analyses[a] of Rabbit Diets

Ingredient	Diets				
	1 (Control)	2	3	4	5
	(%)	(%)	(%)	(%)	(%)
Sun-cured alfalfa meal	40.0	40.0	40.0	40.0	40.0
Soybean meal (expeller)	18.0	14.0	11.0	8.0	5.0
Linseed meal (expeller)	4.0	4.0	4.0	4.0	4.0
Barley	18.5	18.5	18.5	18.5	18.5
Oats	4.0	4.0	4.0	4.0	4.0
Wheat mixed feed (millrun)	15.0	15.0	15.0	15.0	15.0
Salt (NaCl)	0.5	0.5	0.5	0.5	0.5
Degossypolized cottonseed meal[b]	0.0	4.0	7.0	10.0	13.0
Crude protein	20.49	20.43	20.90	21.08	21.09
Ether extract	3.26	3.24	3.16	3.06	2.69
Crude fiber	14.33	14.61	14.94	15.15	15.66
N.F.E.	46.95	46.82	46.23	45.91	46.06
Ash	6.89	6.86	6.69	6.76	6.55

[a] Determined on an air dry basis.

[b] Free gossypol 0.016%; total gossypol 0.79%. Supplied by San Joaquin Cotton Oil Company, Los Angeles, Calif.

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[1] U.S. Rabbit Experiment Station, and Biometric Services, ARS, Beltsville, Maryland.