STUDIES TO DETERMINE THE NATURE OF THE DAMAGE TO THE NUTRITIVE VALUE OF SOME VEGETABLE OILS FROM HEAT TREATMENT[1, 2]

II. INVESTIGATION OF THE NUTRITIOUSNESS OF THE PRODUCTS OF THERMAL POLYMERIZATION OF LINSEED OIL

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FOUR FIGURES

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INTRODUCTION

Heating unsaturated oils to polymerization temperatures has been advocated as a practical method of reducing or eliminating the flavor reversion of shortening produced from such oils (Privett et al., '45). However, if oils treated in this manner are included in the diets of rats, then the rate of gain, the feed intake and the feed efficiency are decreased. These effects may be due to the action of some toxic material (Crampton et al., '51).

Of the oils which have been tested in this laboratory, linseed oil has proved to be the one most susceptible to nutritive damage by heating. Accordingly, linseed oil was selected for further studies on the nature of the material responsible for the toxic effects. The results of these experiments are reported in the present paper.

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Unsaturated fats yield dimeric, trimeric and higher polymeric glycerides when heated at 275°C. in the absence of oxygen. It is rather generally accepted that polymerization does not proceed to any appreciable extent beyond dimerization so far as the fatty acid radicals themselves are concerned (Privett et al., '47). The extent to which such dimerization of the fatty acid radicals may result in the presence of intrapolymeric glycerides remains controversial. Indeed, Bernstein ('48) has dismissed the possibility on theoretical grounds. Adams and Powers ('44), on the other hand, produced considerable experimental evidence in support of the view that such intrapolymers are formed in the early stages of polymerization, and that they may in turn give rise to dimeric and higher polymeric glycerides by transesterification. Experimental evidence obtained in our laboratories supports the contention that thermally polymerized linseed oil may contain considerable amounts of intrapolymeric material (see appendix, table 1). Kass ('47) studied the thermal polymerization of eleostearates, and, as well as observing normal polymerization products, described side reactions leading to production of free fatty acids, aldehdyes, hydrocarbons and acids and esters of reduced molecular weight. He found that some of the latter fragments also polymerize, and produced evidence of cyclization leading to the production of cyclic aliphatic monomers and mono- and polycyclic aromatic compounds. We have essayed to indicate in plate 1 the nature of the substances which are thought to be the main products of thermal polymerization.

At the outset we suspected that polymerized triglycerides might be mainly responsible for the lower nutritive value of heated linseed oil. Consequently we sought to obtain for feeding tests a fraction which would consist essentially of non-polymeric glycerides. Privett et al. ('47) have described the preparation from heated linseed oil of an acetone-soluble segregate which, they reported, was free from "dibasic acids and was, therefore, non-polymeric in nature." Bernstein ('48) has described the use of propanol for the segregation of monomeric from higher polymeric glycerides, such monomeric fractions being regarded by him as free from dimeric acids. In view of these reports, segregates were prepared by the use of acetone and also by the use of propanol. Our chemical data (see appendix, table 2) indicate that propanol was more effective than acetone for recovery of a non-polymeric glyceride fraction, thus supporting the findings of Bernstein in this respect. We have shown, however, that the acetone-soluble segregate of polymerized linseed oil appears to contain appreciable amounts of intrapolymeric material (see appendix, table 1). The propanol segregate does not contain dimeric or higher polymeric glycerides, but we have not examined this fraction for the presence of intrapolymers. Presumably the principal polymerized material, if any, in the propanol segregate is intrapolymeric. The acetone-soluble fraction is probably composed mainly of the molecular types indicated in plate 1, figure 2, while the propanol-soluble fraction is probably composed of those indicated in plate 1, figure 3.

It was also thought possible that the dimeric fatty acid radicals themselves might be implicated in the nutritional defects of polymerized linseed oil. Since solvent segregations offered little promise for the separation of true monomeric glycerides from intrapolymeric glycerides, it was decided to esterify the heated oil and then to separate monomeric esters from dimeric esters by distillation under vacuum. The esterification yielded the fractions indicated in plate 1, figure 4, and the succeeding distillation under vacuum achieved an essentially complete separation of polymeric material (see appendix, table 3). The distillate consisted of monomeric esters, but it may possibly have included some cyclic monomeric material. The undistillable residue consisted essentially of esters of dimeric acids, together with such sterols as were present and presumably some non-volatile products of thermal decomposition which were soluble in acetone.

EXPERIMENTAL PROCEDURE

The materials prepared for feeding comprised fractions of heated linseed oil composed largely of (a) monomeric esters, and (b) dimeric esters, as well as several fractions which contained various mixtures of monomeric and dimeric glycerides and of intrapolymers. In addition to examining biologically the fractions of the oil segregated by solvents (acetone and propanol) and by distillation of the esters of the fatty acids, crude and refined heated oils were compared to discover some indication as to whether or not the non-glyceride constituents of the oil were concerned in growth inhibition.

Polymerization and acetone segregation were carried out by the technique of Privett, McFarlane and Gass ('47). The polymerizations were based on a treatment of 12 hours at 275°C. in an all-glass apparatus while passing through it a current of carbon dioxide. A sufficient flow of carbon dioxide was maintained to keep the oil surging vigorously; attention to this detail resulted in products of excellent color and blandness and with remarkably low acid values (of the order of 0.6 to 1.0% F.F.A.). Propanol segregations were performed according to the directions of Bernstein ('48). Esterifications were carried out by interesterification in the presence of the appropriate anhydrous alcohol, using sodium hydroxide as catalyst; the oil or fraction was alkali-refined to remove F.F.A. prior to esterification. Esters were distilled at about 0.5 mm Hg absolute pressure (see appendix).

The several fractions of linseed oil, indicated in table 1, were fed to albino rats as the source of dietary fat for 28-day periods. The animals ranged from 21 to 28 days of age at the outset of the tests.

The basal diets had the composition described in table 2. Diets were baked for 20 minutes at 375°F. and the resulting biscuit granulated and air-dried for 24 hours. Vitamin B supplements, in the case of diet II; or yeast, in the case of diet III, were then added. Weekly doses of cod liver oil were

administered orally to supply 175 I.U. of vitamin A and 35 I.U. of vitamin D.

FEEDING TRIAL RESULTS

Table 1 is a summary of the weight gains and feed intakes of animals fed the various fractions of linseed oil. It is recognized that negative gains are unsatisfactory as quantitative measures of the nutritional values of rations, nor are

TABLE 1

Summary of average daily gains and average feed intakes of animals fed diets containing 20%[1] of various fractions of linseed oil

TEST NO.	OIL FRACTION	TREATMENT AT 275°C.	PRINCIPAL COMPONENTS OF FRACTION	NO. ANIMALS	AVE. DAILY GAINS	AVE. DAILY FEED INTAKE
		hrs.			*gm*	*gm*
1. A	Whole	none	Monomeric glycerides	5	3.0	6.8
B	Whole	12	Monomeric glycerides Intrapolymers Dimeric and polymeric glycerides	5	−3.0	4.6
C	Acetone-soluble	12	Monomeric glycerides Intrapolymers Dimeric glycerides	5	−3.0	5.3
2. A	Acetone-soluble, alkali-refined	12	Monomeric glycerides Intrapolymers Dimeric glycerides	6	−2.1	4.5
B	Acetone-soluble, crude	12	Monomeric glycerides Intrapolymers Dimeric glycerides Non-glyceride constituents	6	−0.7	3.5
3. A	Whole	none	Monomeric glycerides	10	3.8	10.0
B	Acetone-soluble	12	Monomeric glycerides Intrapolymers Dimeric glycerides	10	−1.4	6.0
C	Propanol-soluble	12	Monomeric glycerides Intrapolymers	10	0.2	5.5
4. A	Whole	none	Monomeric glycerides	8	3.4	9.7
B	Whole ethyl esters	none	Monomeric esters	8	2.3	7.5
C	Acetone-soluble ethyl esters	12	Monomeric and dimeric	8	0.8	5.8
D	Distillate of 4C	12	Monomeric esters	8	0.7	4.5
E	Residue of 4C	12	Dimeric esters	8	[2]	3.5

[1] Excepting test 2, where 30% fat was used.
[2] No gain could be calculated, as 6 out of 8 animals died within the first week. The remaining two survived three and 4 weeks, respectively.

statistical analyses of weight losses justified. In these trials such data are nevertheless indicative of the differences in nutritive quality of the diets studied. Where weight losses were encountered (tests 1, 2, and 3), the diets may be judged unwholesome without the benefit of statistics. However, it was possible to make an analysis of the variance of both gain and feed intake in test 4. Differences significant at the 5% point were apparent between A and B, B and C, and B and D. C and D were not different.

TABLE 2

Composition of diets

INGREDIENTS	DIET I (test 2)	DIET II (test 1)	DIET III (tests 3 and 4)
White flour (%)	26.5	47.0	44.0
Casein (%)	15.0	11.5	11.5
Milk powder (%)	20.0	19.0	19.0
Linseed oil (%)	36.0	20.0	20.0
Bone meal (%)	2.0	2.0	2.0
Salt (%)	0.5	0.5	0.5
Yeast (%)	..	..	3.0
Thiamine	[1]	10 p.p.m.	..
Niacin	[1]	230 p.p.m.	..
Riboflavin	[1]	5 p.p.m.	..

[1] Where diet I was fed (test 2), thiamine, niacin and riboflavin were administered orally to provide daily intakes of 0.1 mg, 2.3 mg, and 0.5 mg, respectively, per rat.

It is realized that purified esters are of uncertain feeding value because of their susceptibility to oxidative changes (Quackenbush et al., '42). For this reason, the effects on rat growth of esters vs. the triglycerides were compared. Animals fed esterified unheated oil gained significantly less weight than animals fed the unheated triglyceride; the feed intake of ester-containing diets decreased progressively during storage at room temperatures, indicating decreasing palatability of those diets. However, the utilization of feed for producing weight gain was not significantly different between the ester- and triglyceride-containing diets, nor could any clinical indication of the toxicity of the esters be found. Esters of heated linseed oil showed the same adverse clinical effects as did the heated triglycerides, as will be seen in table 1.

DISCUSSION

The data shown in table 1 confirm that 12 hours' heating at 275°C. impairs the nutritive value of linseed oil.

A comparison of heated, whole oil with the acetone-soluble fraction (test 1) shows both of them to be equally incapable of supporting normal growth in rats. One may conclude, therefore, that acetone does not segregate toxic from non-toxic material.

There was no difference between the crude and refined oils (test 2), suggesting that the deleterious material in polymerized oil is derived from the glyceride component.

The propanol-soluble fraction was not significantly better nutritionally than the acetone-soluble fraction (test 3). It thus follows that the glyceride polymerization is not responsible for growth inhibition by heated linseed oil. Since neither acetone nor propanol is effective in segregating out the toxic material in heated linseed oil, one naturally turns to a consideration of the intrapolymers as the offending material. An interpolymer is a triglyceride within which two fatty acid chains are dimerized, and one might suspect the dimeric fatty acid structure of being the deleterious factor in heated oil. It is the only quantitatively important component of the propanol-soluble fraction of heated oil that is not present in unheated oil. How far this surmise is correct is evident from test 4, in which the "dimeric" fraction separated from the ethyl esters of the acetone segregate of polymerized oil was fed.

This undistillable "dimeric" fraction, which may also contain minor amounts of non-fatty material, proved lethal to rats in approximately 5 days.

The animals fed the distillable fraction (i.e., monomeric esters), although they did not grow, were thrifty in appear-

ance and excreted well-formed feces. Those fed the undistillable residue had oily, matted coats and excreted dark, sticky feces. This condition was also noted, but to a lesser degree, with the rats fed the mixed monomeric plus dimeric esters, and had also been recorded in previous tests in which the acetone-soluble fraction of heated oil had been fed. It appears possible, therefore, that the explanation of the failure to gain in weight on a diet containing the monomeric esters may be the absence of an essential unsaturated fatty acid, presumably because these more reactive acids had become polymerized or isomerized during heating. Thus dimerization may not only remove essential fatty acids, but produce toxic radicals as well. The strong adverse reaction of the rats to the dimeric esters may be the consequence of the absence of a needed growth factor plus the presence of a toxic agent.

CONCLUSIONS

On the basis of these results one might tentatively conclude that the primary cause of the lowered nutritive value of diets containing thermally polymerized linseed oil is the presence of one or more dimeric fatty acid radicals which are in some way inimical to the well-being of the animals. Their deleterious effects could well be aggravated since they are produced at the expense of unsaturated fatty acids, some of which may be those essential to the animal for growth.

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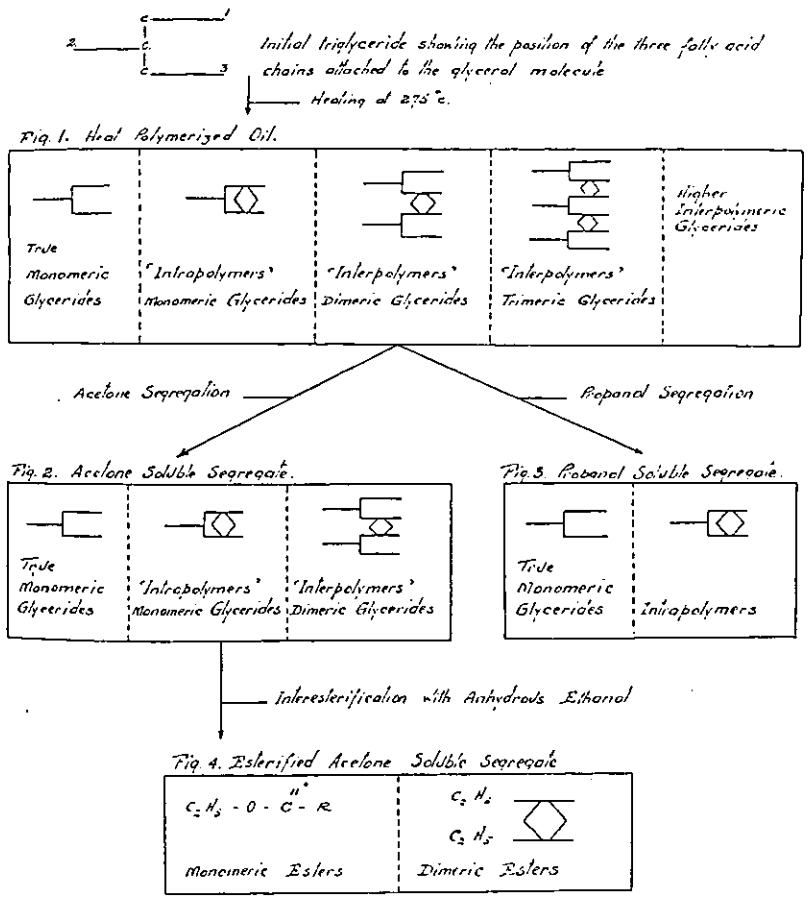
DIAGRAMMATIC REPRESENTATION OF THE MAIN CONSTITUENTS OF LINSEED OIL FOLLOWING THERMAL POLYMERIZATION, SOLVENT SEGREGATION AND ESTERIFICATION.



APPENDIX

Distillation of ethyl esters of the acetone-soluble fraction from polymerized linseed oil

The distillation of the esters was carried out using simple distilling bulbs and receivers and a glycerol bath. The distilling bulb was filled with clean glass wool to reduce bumping and frothing. Distillation temperature limits were established on esters of alkali-refined whole linseed oil, and these were observed closely in all distillations of esters from polymerized oils. The distillation pressures were of the order of 0.5 mm Hg. The distillable esters usually distilled completely between 155° and 175°C. When the vapor temperature had passed its maximum, the bath temperature was taken steadily up to 240°C. If no further rise in vapor temperature occurred, the bath was removed and the residue allowed to cool rapidly in the bulb without breaking the vacuum.

Demonstration of the presence of intrapolymers in the acetone-soluble fraction of polymerized linseed oil

A series of linseed oils were polymerized in a current of CO_2 for varying periods at 275°C. The methyl esters of their acetone-soluble segregates were prepared by interesterification. Cryoscopic determinations of mean molecular weight were carried out on the acetone-soluble fractions themselves and on the methyl ester fractions. The cyclohexane (British Drug Houses) used as cryoscopic solvent was carefully purified by successive treatment with fuming sulfuric acid and barium hydroxide, followed by distillation in a Stedman column. The purified solvent had M.P. 6.4°C., refractive index (25°C.) = 1.4237, values which are close to those recorded for very pure cyclohexane (Glasgow et al., '46). The observed mean molecular weights were corrected for the presence of free fatty acids by the method of Bernstein ('48), which assigns to the free fatty acids a dimeric average mean molecular weight of 558; i.e., assumes that association of the free fatty acids is practically complete. From the cryoscopic mean molecular weights of the acetone-soluble fraction, and assuming the presence of monomeric and dimeric glycerides of theoretical mean molecular weights of 878 and 1756, it was possible to calculate the percentages of monomeric and dimeric glycerides. From these figures, and assuming that polymerization of fatty acid radicals had not proceeded beyond the dimeric stage (Privett et al., '47), a theoretical yield of monomeric and dimeric esters could be calculated. The difference between these values and the observed values for monomeric and dimeric esters obtained by distillation may be reasonably ascribed to the presence of intrapolymers, which would behave as monomeric glycerides in the mean molecular weight determinations. The percentage differences can be converted to a triglyceride basis by multiplying by 3/2. The results are set out in appendix table 1, and provide support for the belief that the acetone-soluble fractions of heated linseed oil contain considerable amounts of dimeric fatty acid in the form of intrapolymers.

Even after 13 hours of heating, the acetone-soluble fraction contained approximately 30% of its total fatty acids in the form of dimeric acids, but only approximately 12% could be accounted for as dimeric glyceride and some 19% of dimeric acid was probably present as intrapolymers. The amount of intrapolymers (soluble in acetone) would be expected to decline as polymerization progresses. It seems quite clear from these results that solvent segregation methods are unlikely to be effective in differentiating between intrapolymers and true monomeric glycerides.

Superiority of propanol over acetone in segregating a non-polymeric glyceride fraction from thermally polymerized linseed oil

The analytical data in table 2 show that both the viscosity and the refractive index of the propanol-soluble fraction were lower than those of the acetone-soluble fraction. The data confirm that propanol is the more effective solvent in segregating a non-polymeric fraction from thermally polymerized linseed oil.

Effectiveness of vacuum distillation in separating monomeric from dimeric ethyl esters of the acetone-soluble segregate of polymerized linseed oil

The analytical data presented in appendix table 3 relate to two samples of methyl esters prepared from acetone-soluble fractions of heated linseed oil. The data show that the "distillable" fraction consisted essentially of methyl esters of monomeric fatty acids, the mean molecular weights being 296 and 295. The "non-distillable" fraction consisted essentially of methyl esters of dimeric fatty acids, the mean molecular weights being 587 and 596.

The fact that refractive indices and iodine values for the recombined samples were practically the same as the values for the charge before distillation is evidence that the heating during distillation had little, if any, effect on the constitution of the esters.

APPENDIX TABLE 1

Calculation of the intrapolymer content of the acetone-soluble fractions of thermally polymerized linseed oil

HOURS HEATED	MEAN MOLECULAR WEIGHT OF GLYCERIDES OF HEATED OIL BY CRYOSCOPY	CALC. % DIMERIC GLYCERIDE	CALC. % DIMERIC METHYL ESTERS	OBSERVED % DIMERIC METHYL ESTER BY DISTILLATION	DIFFERENCE TO BE ASCRIBED TO INTRA-POLYMERS
5	1,010	36.1	8.7	21.7	13.0
9	1,050	29.6	9.7	23.1	13.2
13	1,060	34.4	11.5	30.7	19.2
17	970	19.0	6.3	14.8	8.5

APPENDIX TABLE 2

Analyses of acetone and propanol fractions from heated alkali-refined linseed oil

FRACTION	YIELD	REF. IND 25°C.	VISCOSITY AT 250°C. CP.	IODINE VALUE
	%			
1. Unheated, recovered from acetone		1.4789	50	183
2. Heated, acetone soluble	45	1.4811	180	134
3. Heated, propanol-soluble	28	1.4791	127	135
4. Heated, propanol-insoluble	72	1.4851	890	121

APPENDIX TABLE 3

Properties of methyl esters and fractions obtained in distillation

SAMPLE	FRACTION	PER CENT YIELD	MEAN MOLECULAR WEIGHT	REF. IND. AT 250°C.	IODINE VALUE
1	Charge (acetone-soluble)		334	1.4618	128.0
	Distillate	82.0	296	1.4600	137.2
	Residue	17.5	587	1.4896	105.5
	Recombined sample[1]			1.4647	127.6
2	Charge (acetone-soluble)		336	1.4647	129.0
	Distillate	81.9	295	1.4600	135.6
	Residue	17.6	598	1.4901	102.8
	Recombined sample[1]			1.4647	128.4

[1] Distillate and residue blended in the exact residue of their yields, to approximate original sample.

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STUDIES TO DETERMINE THE NATURE OF THE DAMAGE TO THE NUTRITIVE VALUE OF SOME VEGETABLE OILS FROM HEAT POLYMERIZATION[1, 2]

I. THE RELATION OF AUTOXIDATION TO DECREASE IN THE NUTRITIONAL VALUE OF HEATED LINSEED OIL

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Ingestion of heat polymerized linseed oil was followed by lowered growth rates in rats, this growth depression becoming more severe with increased lengths of heating time of the oils and with increased amounts of oil in the diet (Crampton et al., '51). Our earlier experiments indicated that the low nutritive value of heated linseed oil diets is attributable to the oil itself and not to the effects of the heated oil upon non-lipid diet constituents.

As thermal treatment causes oil to be less stable to oxidation, it has been proposed that biologically deleterious products may accumulate through progressive autoxidation during storage of the oil-containing diets at room temperature. Oven temperatures used during the baking of the diets may also promote oxidative changes.

The extent of autoxidation can be controlled by the addition of an anti-oxidant. A mixture of nordihydroguararetic acid (NDGA) with citric acid has been shown to be an effective

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anti-oxidant for edible fats, increasing stability even in baked products (Mattil et al., '45; Higgins and Black, '45).

The question of vitamin E deficiency in heated oils also seemed worthy of consideration at this time, since polymerization at 275°C. would destroy any of this vitamin inherent in the original oil. The absence of vitamin E, a natural antioxidant, presumably permits a more rapid onset of autoxidation. In addition, a relation of vitamin E to growth, over and above its stabilization of linoleic acid, has been shown in insects by Fraenkel and Blewett ('46). In the feeding of heated lard to rats, Morris and his co-workers ('43) noted a depression of growth and also a paralysis simulating a vitamin E deficiency. Therefore it was possible that in our tests thermal destruction of vitamin E was responsible in part for the adverse effects we had observed to result from feeding heat-polymerized linseed oil.

EXPERIMENTAL

Feeding trials were conducted to examine the effects of baking, of controlled autoxidation, and of vitamin E supplementation of diets containing heat-polymerized linseed oil.

Oil preparation

Polymerization and acetone segregation were performed according to the method of Privett, McFarlane and Gass ('47). Preliminary trials in our laboratories showed no nutritional difference between whole heated oil and its acetone soluble fraction, beyond a decrease in intake of the diets containing the latter. This effect was thought to be due to residual traces of acetone (Langerman, '49). Therefore in the present test, in which acetone segregation of heated oil was involved, the control (unheated) oil was subjected to like treatment with acetone.

Where oils were artificially stabilized (test II), an antioxidant mixture of NDGA with citric acid was added as 0.05% of the oil. Peroxide values on these oils when freshly prepared

and after 28 days of storage at room temperature were estimated by the method of Skellon and Wills ('48). Peroxide determinations on chloroform extracts of the oil-containing diets were made at intervals throughout the 28-day feeding trial by a modification of the Skellon and Wills method (see appendix).

Biological procedure

The animals used in these trials were albino rats ranging in age from 21 to 28 days at the outset of the test period. They received, ad libitum, the diets described in table 1.

TABLE 1

Composition of diets

INGREDIENTS	DIET A (test II)	DIET B (tests I and III)
White flour (%)	47	54
Milk powder (%)	19	19
Casein (%)	11.5	11.5
Oil (%)	20	10
Bone meal (%)	2	2
Salt (%)	0.5	0.5
Yeast (%)	..	3
Vitamins		
Thiamine	10 p.p.m.	..
Niacin	230 p.p.m.	..
Riboflavin	5 p.p.m.	..

Diets minus vitamins or yeast were baked (except where specified in test I) for 20 minutes at 375°F. and the resulting biscuit granulated and air-dried for 24 hours. Vitamin supplements in diet A, or yeast in the case of diet B, were then added. Weekly doses of cod liver oil were administered orally to supply 175 I.U. of vitamin A and 35 I.U. of vitamin D.

In addition to these vitamins, half of the animals in test III each week received orally alpha-tocopherol dissolved in corn oil. The doses, administered two days following the A and D supplementation, were of such amount as to supply 7 mg of vitamin E to each animal.

RESULTS

When the oils were stabilized, there was no appreciable accumulation of easily reducible peroxides. However, definite signs of progressive peroxidation were shown in the unstabilized oil over the 28-day trial. These facts are evident from the peroxide values given in table 2 and calculated at the beginning and the end of the test period on oil extracted from the feed and also on oil that was not combined with the feed.

It is true that peroxide values are not a full indication of rates of total oxygen absorption and that other diet components may have been attacked by peroxides as rapidly as

TABLE 2

Peroxide values

(Calculated as milligrams of peroxide oxygen per kilogram)

	OIL EXTRACTED FROM FEED		STORED OIL	
	Fresh	After 28 days	Fresh	After 28 days
Unheated oil	99	276	47	1,334
Unheated stabilized oil	26	55	49	172
Heated oil	116	333	0	270
Heated stabilized oil	25	38	0	51

these were formed. In such an event, no accumulation of easily reducible peroxides would have been noted. However, a steady increase in peroxide values in unstabilized oils throughout the test points to the likelihood that extensive peroxide decomposition had not yet begun in the stabilized samples.

The growth rates and feed intakes of the animals in test II, given in table 3, show that the stabilized oil was no better nutritionally than the unstabilized control oil. The equality of the growth responses of animals fed heated oils regardless of anti-oxidant treatment makes it unlikely that autoxidation is responsible for the low nutritive value of polymerized linseed oil.

This is further supported by the data from test I, where the growth responses of the animals show that baking did not influence the growth inhibition exerted by the heated oil. Exposure to oven temperatures would, if anything, encourage

TABLE 3

Effect of baking, of anti-oxidant stabilization, and of vitamin E supplementation on the nutritive value of unheated or heated whole linseed oil or on the acetone soluble fraction of the heated oil

TEST NO.	OIL USED	TREATMENT	OIL IN DIET	NO. ANIMALS	AVE. DAILY GAIN	AVE. DAILY FEED INTAKE	GAINS PER 1,000 CAL.[1]
			%		*gm*	*gm*	*gm*
I	Unheated	Diet baked	10	8	4.3	12.1	72
	Unheated	Diet unbaked	10	8	4.2	12.4	69
	Heated	Diet baked	10	8	3.1	10.6	60
	Heated	Diet unbaked	10	8	2.7	9.7	57
II	Unheated	nil	20	10	3.7	10.1	68
	Unheated	Oil stabilized[2]	20	10	3.8	9.6	73
	Heated (acetone soluble fraction)	nil	20	10	—0.1	6.0	..
	Heated (acetone soluble fraction)	Oil stabilized[2]	20	10	—0.1	5.8	..
III	Heated	nil	10	24	2.4	10.0	49
	Heated	Vitamin E supplementation to diet	10	24	2.3	9.8	48

[1] Gross calories (calculated from heats of combustion): 10% fat diet contained 4.9 Cal./gm; 20% fat diet contained 5.4 Cal./gm.

[2] Protected against autoxidation with NDGA and citric acid.

autoxidation. These results imply that the damaging factor in heated oil is not produced oxidatively.

Supplementation with vitamin E failed to alter the nutritive value of polymerized linseed oil (test III).

CONCLUSIONS

The results of these experiments show that peroxidation of polymerized linseed oil is not concerned in the development of any toxic factor, and also suggest that a lack of vitamin E is not involved in the growth inhibition resulting from the ingestion of heated oil.

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APPENDIX

Modification of the Skellon and Wills procedure for peroxide determinations on oil extracted from diets

A weighed sample of biscuit containing about 1 gm of the oil was shaken up with a 25-ml portion of chloroform and the filtrates were combined. The extract was evaporated to 10 ml by passing over it oxygen-free nitrogen *without heating*, and transferred to a 250-ml flask containing 2 gm sodium bicarbonate. A stopper was fitted, carrying a short piece of glass tubing capped by a rubber Bunsen valve. This served as a one-way pressure relief valve. Fifteen milliliters of glacial acetic acid were used to rinse the last traces of chloroform extract into the reaction flask, and the acid-bicarbonate reaction released CO_2 which swept air out of the flask through the relief valve. When foaming subsided, 1 ml of saturated fresh potassium iodide solution was added, and the tightly stoppered flask was left in the dark for one hour. Fifty milliliters of boiled, cooled water were then added, and the iodine titrated with 0.002 N thiosulfate.

Peroxide values were calculated as milligrams of peroxide oxygen per kilogram of oil, thus:

$$PV = \frac{V \times N \times 16 \times 10^3}{W \times P}$$

where,

V = Volume of thiosulfate used (ml)
N = Normality of thiosulfate
W = Sample weight (gm)
P = Per cent oil in sample.

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STUDIES TO DETERMINE THE NATURE OF THE DAMAGE TO THE NUTRITIVE VALUE OF SOME VEGETABLE OILS FROM HEAT TREATMENT[1, 2]

III. THE SEGREGATION OF TOXIC AND NON-TOXIC MATERIAL FROM THE ESTERS OF HEAT-POLYMERIZED LINSEED OIL BY DISTILLATION AND BY UREA ADDUCT FORMATION

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ONE FIGURE

(Received for publication July 28, 1952)

INTRODUCTION

The deleterious effects of heating certain vegetable oils at polymerization temperatures on their nutritional value have been described in a previous paper (Crampton et al., '51c). There is some evidence (Crampton et al., '51a) that these deleterious effects are not a consequence of peroxidation. It has also been shown (Crampton et al., '51b) that the deleterious effects may be demonstrated by feeding the ethyl esters of heated linseed oil.

When the whole esters of heated linseed oil were separated by distillation at pressures of the order of 0.5 mm into a distillable fraction and a non-distillable fraction, then the non-distillable fraction was deleterious to experimental ani-

[1] Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Quebec, Canada, Journal Series No. 304.

[2] Issued as paper 277, Canadian Committee on Food Preservation.

mals. Rats receiving this fraction at the level of 20% of the diet failed to grow; had oily, matted coats; excreted dark, sticky feces; and displayed a heavy mortality. Rats receiving the distillable fraction survived the experiment in good condition and excreted well-formed feces. However, they did not grow as well as rats receiving the whole esters of unheated linseed oil. In fact, their growth was no better than that of rats receiving the whole esters of heated oil. At this stage it seemed possible that the major defect of the heated oil arose from the presence of dimeric acid radicals. However, the distillable monomeric ester fraction was also nutritionally inferior to the esters of whole unheated linseed oil. This fact could not be related to differences in susceptibility to peroxidation, for the esters of the whole unheated oil were considerably more susceptible to peroxidation than the distillable esters of the heated oil (unpublished results).

It seemed possible, therefore, that the monomeric ester fraction might contain material other than straight chain esters, and that such material might play a part in determining the deleterious nature of the heated oils.

The formation of such materials by the exposure of the oils to polymerization temperatures carries implications of some importance so far as oils intended for food are concerned. Accordingly, the preliminary work was repeated and extended, and this paper deals with these later experiments.

EXPERIMENTAL

The nutritional test was performed in two replicates, each comprising observations on 60 animals. Six diets were tested. These diets differed only in (a) the nature of the oil component and (b) the proportion of white flour as adjusted to compensate for the use of 10 or 20% levels of oil.

The formula for the diets, in per cent, was as follows: white flour 53.65 or 43.65; skim milk powder 19; casein 11.5; oil preparation 10 or 20; brewers' dried yeast 3; feeding bone-meal 2; iodized salt 0.5; chromium sesquioxide[3] 0.25; and ferric citrate 0.1.

The oil preparations were certain fractions of the ethyl esters of heat-polymerized linseed oil (12 hours at 275°C. in a strong current of carbon dioxide). The preparation of these fractions is described in detail in the Appendix. They will be designated in this paper for convenience as follows:

1. *Straight chain monomers from whole oil:* that portion of the ethyl esters of heated linseed oil which formed urea adducts with ease.
2. *"Cyclized" monomers and dimers from whole oil:* that portion of the esters of heated oil which did *not* form urea adducts.
3. *Straight chain plus "cyclized" monomers from distillate:* that portion of the esters of heated linseed oil which was distillable at pressures around 0.5 mm.
4. *Straight chain monomers from distillate:* that portion of the distillable esters which formed urea adducts.
5. *"Cyclized" monomers from distillate:* that portion of the distillable esters which did *not* form urea adducts.
6. *Dimers by distillation:* the residue after removal of the monomeric esters by distillation.

The probable general chemical relationships of these 6 fractions are conveniently indicated by a flow sheet (fig. 1). It is to be understood that the designations of the fractions and the symbols used in the flow sheet do not imply that the nature of the fractions is established. While it is reasonably certain that fraction 6 consists mainly of esters of dimeric acids (see table 5) it cannot be considered as finally established whether these are linked by single carbon-to-carbon bonds or by a cyclohexene system. Again, whether the non-adduct-forming monomeric material of fractions 2, 3 and 5 is cyclized or branched is not established: the term "cyclized" must be read as denoting a mode of preparation and not as asserting the presence of a cyclic structure. The chemical characteristics of the fractions are reported in table 5: the

[3] For determination of digestibility.

mean molecular weights confirm the validity of the designations so far as the terms monomeric and dimeric are concerned, but do not provide evidence as to the presence or otherwise of cyclic structures. It may be recalled, however, that Paschke and Wheeler ('49) suggested the presence of cyclic monomers in heat-polymerized methyl linoleate.

The hydroxyl contents of the fractions were determined by the method of Ogg, Porter and Willits ('45). The data

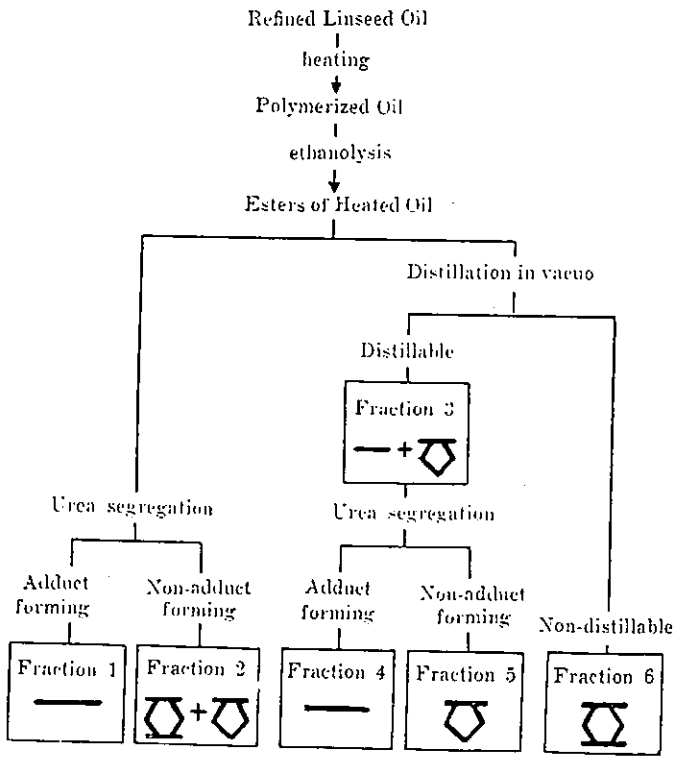

Fig. 1 Flow sheet illustrating preparation of fractions of esters of heated linseed oil used in feeding trials. (For details of preparation, see Appendix.)

are reported in table 5. So far as the monomeric fractions 1, 4 and 5 are concerned, the values correspond to not more than 8, 5 and 15%, respectively, of monohydroxy C18 acids. The available evidence therefore supports the view that hydroxy acids did not constitute a major proportion of the non-adduct-forming monomeric material.

The feeding trials lasted 28 days. Live weight changes and feed consumption were recorded. The apparent digestibilities were determined by the Cr_2O_3 index technique (Schürch et al., '50). Digestible calories were calculated from the gross calorific values of the diets, the feed intake and the simultaneously determined digestibility coefficients. Live

TABLE 1

Comparison of the effect of 10% versus 20% of fractions of the esters of heat-polymerized vegetable oil in the diet of rats (60 rats at each level)

OIL IN DIET	RATS SURVIVING 28 DAYS OF TEST	AVERAGE GAIN IN 28 DAYS	AVERAGE FEED IN 28 DAYS	DIGESTIBILITY OF FEED	GAIN ADJUSTED TO EQUAL INTAKE OF DIGEST. CALORIES
%	%	gm	gm	%	gm
10	83	73	262	91	67
20	63	27	177	83	52
Least sig. diff. ($P = 0.05$)	..	11	17	..	8

weight gains were adjusted by simple regression to equal intake of digestible calories to provide a measure of the relative efficiency of the diets. Least significant differences between means, where shown, were calculated from the expression

$$\text{L.S.D.} = \sqrt{n} \times \sqrt{2} \times t_{.05} \frac{\sigma}{}$$

In the preliminary tests, the use of diets containing 20% of certain ester fractions similar to those used in the present experiments had resulted in heavy mortality. Consequently, half of the rats in each of the lots in the tests now reported received a diet containing 10% oil. The differences between the responses of the rats receiving these two levels of oil are summarized in table 1.

Efficacy of protection of the fractions and diets against autoxidation

Since peroxidized fats and fatty acid esters may be toxic to rats, it is necessary to ensure that the results of feeding such materials are not vitiated by the effects of peroxidation. It has been shown that the deleterious effects of heated linseed oil are not related to the degree of susceptibility of the heated oils or of the diets containing the oils to peroxidation (Crampton et al., '51a). In the present experiments the fractions used in the diets were prepared immediately

TABLE 2

Peroxide values of oils extracted from the mixed feeds at two weeks and 4 weeks after preparation. Feeds stored in refrigerator

FRACTION CONTAINED IN THE DIET	PEROXIDE VALUE, MG PEROXIDE OXYGEN PER KG OIL	
	After 2 weeks	After 4 weeks
Fraction 1	41	98
Fraction 2	38	88
Fraction 3	42	90
Fraction 4	46	102
Fraction 5	38	92
Fraction 6	33	75

before feeding, and were protected by addition of an antioxidant and storage in the refrigerator in the dark. The stability of the oils in the diets was tested at the end of the second week of the second replicate and again at the end of the 4th week. Peroxide values of the lipid components of the feed were determined by the method of Skellon and Wills ('48) as described by Crampton et al. ('51a). Peroxidation was relatively slight in all fractions (table 2). From unpublished observations it is believed that peroxidation would not have begun to interfere with the nutritional well-being of the rats until the peroxide value of the oil extracted from the feed attained values of the order of 1,000 mg per kilogram of oil.

It may be concluded, therefore, that the results of the present feeding experiments were due to the effects of constituents other than products of peroxidation.

RESULTS

With diets containing 20% oil

The results of feeding these 6 oil fractions in diets in which they constituted 20% by weight are summarized in table 3. The first salient feature of the results relates to the *mortality*. It is clear that the presence of the "cyclized" monomers in the diet was associated with toxicity, though the mode of action of this material remains obscure. Rats consuming fraction 5, which contained the highest concentration of this material, survived only 5 to 10 days in spite of a greatly reduced feed intake (and hence low intake of toxic material).

A comparison of the results for diet 2, containing a mixture of approximately three parts dimeric esters to one part "cyclized" monomer, with the results for diet 6, shows clearly the much more harmful nature of the monomeric material. The deaths in group 6 were associated with partial starvation and excessive diarrhoea, whereas the animals of group 2 displayed neither diarrhoea, digestive disturbances, nor overt clinical symptoms suggestive of the cause of death.

The animals receiving fractions consisting mainly of straight chain monomers — i.e., groups 1 and 4 — all survived the test and remained in good health. Their gains were comparable to those secured in previous tests with esters of whole unheated linseed oil.

The *gains* of the rats receiving the various fractions also reflected the toxic effects of the "cyclized" monomeric material and the adverse effects of the dimeric esters. The dimeric ester fraction was associated with low digestibility, and this was doubtless the cause of the diarrhetic condition of the rats receiving this material.

The *relative efficiencies* of the diets *per unit of digestible calories eaten* showed that the straight chain monomeric

TABLE 3

The effect of feeding fractions of polymerized linseed oil at 20% level in rat diets (10 rats per lot)

OIL FRACTION FED	PRINCIPAL OIL CONSTITUENTS	NO. OF ANIMALS SURVIVING TEST	AVERAGE GAIN	AVERAGE FEED INTAKE (dry matter)	DIG. OF DRY MATTER	DIG. OF FAT FRACTION	MEAN GAINS ADJUSTED TO EQUAL INTAKE OF DIG. CAL.
			gm	*gm*	%	%	*gm*
1	Straight chain monomers of whole oil	10	101	266	93	94	72
2	"Cyclized" monomers plus dimers of whole oil	2	−22	141	81	77	4
3	Straight chain plus "cyclized" monomers of distillate	9	4	118	94	97	52
4	Straight chain monomers of distillate	10	57	193	94	95	63
5	"Cyclized" monomers of distillate	0	..	..	..	..	..
6	Dimers	7	13	168	55	29	71

esters and the dimeric esters were equally well "metabolized," whereas the presence of "cyclized" monomeric material resulted in a sharp reduction in the utilization of digestible calories.

The *low feed intake* by group 5 made it possible that this might have caused the death of some of the rats receiving the "cyclized" monomers. This point was examined by feeding a supplementary group of 5 rats the basal oil-free diet ad libitum. They were dosed daily by dropper with a quantity of "cyclized" monomer equal to 10% by weight of their measured voluntary intake of the fat-free mixture. The intake of the basal diet under these conditions was low, but it was adequate for maintenance and in one case permitted some gain. Nonetheless, all of these rats died within 16 days, thus confirming that the mortality in group 5 could not be explained on the grounds of low food intake.

It will be noted from the schematic outline (fig. 1) of the preparation of the fractions that fractions 1 and 4 ought to consist mainly of the straight chain adduct-forming material. However, fraction 1 had a lower iodine number and a lower refractive index than fraction 4 (table 5). Moreover, the yield of fraction 1 represented 46%, whereas that of fraction 4 was 49%, of the total esters. These differences in yield and in the characteristics of presumptive straight chain monomeric material according to the route by which it has been prepared, suggest that in the case of fraction 4 the adduct-forming fraction had probably carried with it by entrainment a small proportion of material incapable of itself of forming an adduct: i.e., of "cyclized" monomer.

Fraction 1 was also superior to fraction 4 in nutritional value. This circumstance reinforces the view that fraction 4 contained a small proportion of the non-adduct-forming material ("cyclized" monomer), and that the "cyclized" monomeric ester is either of itself the deleterious component or that the deleterious material accompanies this ester fraction.

TABLE 1

The effect of feeding fractions of polymerized linseed oil at 10% level in rat diets (10 rats per lot)

LOT NUMBER	FRACTION FED	SUBSTANCES VARIABLY SUBSTITUTED FOR	NO. OF DAYS	AVERAGE WEEKLY GAIN	AVERAGE WEEKLY FOOD INTAKE (DRY BASIS)	MOR. OF DRY WATER	DIG. OF FAT FRACTIONS	MEAN GAINS ADJUSTED TO NORMAL INTAKE OF 100 GM.
			gm	*gm*	*gm*	*cc*	%	*gm*
1	Straight chain monomers of whole oil		10	102	300	95	91	72
2	"Cyclized" monomers plus dimers of whole oil		10	39	295	87	82	58
3	Straight chain plus "cyclized" monomers of distillate		10	66	288	94	96	66
4	Straight chain monomers of distillate		10	108	299	91	96	73
5	"Cyclized" monomers of distillate		0	..	..	91	96	..
6	Dimers		10	77	276	87	69	69
Least significant difference (P = 0.05)			..	33	33	..	..	16

With diets containing 10% fat

The results of feeding the diets in which the fat levels were reduced to 10% are shown in table 4. They were in all respects what would have been predicted from the results of feeding the 20% fat levels. The toxic effect of the "cyclized" monomers had been lessened, though it was still clearly in evidence. Gains, feed intake, digestibility and efficiency of utilization from the straight chain monomers were practically identical as between rats in lot 1 with 20% fat and those of lots 4 and 5 with 10% fat. Even at this lower fat level none of the rats receiving the "cyclized" monomeric material (lot 3) survived 28 days; where this "cyclized" monomeric material constituted only 2.5% of the diet intake (lots 2 and 3), the rats were able to complete the test, but with significantly smaller gains.

CONCLUSIONS

1. Heat polymerization of alkali-refined linseed oil results in the formation of monomeric acyl radicals whose esters can be distilled at low pressures but which do not form urea adducts. These esters are highly digestible when eaten but are not utilized for growth. The esters contain a small proportion of hydroxyl groups, but are apparently mainly esters of "cyclized" or branched structure.

2. When a fraction consisting essentially of these esters of "cyclized" monomeric acids comprises as much as 10% of the diet, it renders the diet "toxic." The harmful effect is measurable when this fraction comprises 2.5% of the diet. The effect is ascribed tentatively to the "cyclized" monomeric esters as such, rather than to the small proportion of hydroxy acid present in this and other fractions.

3. Dimeric acyl radicals are also formed on heating linseed oil at polymerization temperatures. A fraction consisting mainly of the esters of the dimeric acids is non-toxic, but this material is largely unabsorbed from the gastrointestinal tract. Its presence results in excreta of the nature and consistency of a mixture of soft feces and fresh varnish.

4. Esters of straight chain monomeric acids (i.e., capable of forming urea adducts) are apparently as nutritious as are esters prepared directly from normal unheated triglyceride oils which, in turn, are but slightly inferior to the triglycerides of normal food.

ACKNOWLEDGMENTS

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APPENDIX

Preparation of ethyl esters of polymerized linseed oil

Commercial alkali-refined linseed oil (Canada Linseed Oil Mills, Montreal, P.Q.) was heated in a stream of CO_2 for 12 hours at 275°C. The oil was heated in batches of about 1,200 gm at a time, and the CO_2 was passed at a rate sufficient to keep the oil surging fairly freely. The resultant polymerized oil was of a clear light straw color.

The polymerized oil was allowed to cool while maintaining the current of CO_2. The polymerized oil was then shaken up with one-third of its volume of absolute ethanol to remove any fatty acids present. The washings were discarded.

The washed polymerized oil was used for the preparation of the ethyl esters by alcoholysis, using caustic soda at the rate of 0.5% by weight of the oil as catalyst (Feuge and Gros, '49). The esters were washed with large volumes of hot distilled water in a large separatory funnel. The total yield of mixed esters reckoned on the weight of oil taken was approximately 90% (varying from 88 to 92 with different batches).

Fractionation of the ethyl esters for the feeding trials

A portion of the total ethyl esters was subjected to vacuum distillation as described by Crampton et al. ('51b). The non-distillable fraction, comprising the ethyl esters of dimeric fatty acids, constituted fraction 6. The distillable portion, comprising the ethyl esters of monomeric acids, constituted fraction 3.

Immediately after distillation Tenox II * (Tennessee Eastman Corp.) was added to the portions of fractions 3 and 6 reserved for feeding at the rate of 0.1%. The protected ester fractions were at once removed to the cold room (5°C.), and held there until incorporated in the diets.

To another portion of fraction 3, Tenox II was added at the rate of 0.025%. This portion was subjected to fractionation by urea adduct formation (Schlenk, '49). The following technique was used.

Five hundred grams of the esters were dissolved in 2 l absolute commercial ethanol in a 4-l beaker. Solid urea was added slowly with stirring, while maintaining the mixture at 50°C. In all 2 kg urea were added, since approximately 3 gm urea are required for each gram of C18 ester for adduct formation. The mixture was maintained at 50°C. for about 30 minutes and then allowed to stand at room temperature overnight. Next morning the adduct and excess urea were filtered off and washed on the Buchner funnel with absolute ethanol saturated with urea. The adduct was decomposed by being thrown into warm water. Immediately the oily layer separated. Tenox II was added at the rate of approximately 0.25% of the adduct-forming material. The oily material was washed with water and dried under high vacuum with shaking. This material constituted fraction 4, and presumably consisted mainly of straight chain monomeric esters (see table 5). The non-adduct-forming material in the supernatant and ethanolic washings was separated by the addition of copious amounts of water. No Tenox

* Tenox II is a preparation containing 20% butylated hydroxyanisole, 4% citric acid, 6% propyl gallate and 70% propylene glycol.

II was added, since presumably that present before adduct formation would tend to be concentrated in this fraction, which constituted fraction 5. The addition of small quantities of sodium chloride facilitated the separation of both fractions 4 and 5 from the aqueous phase. Fraction 5 was dried in the same fashion as was fraction 4. Both fractions were now further protected against autoxidation by the further addition of 0.1% Tenox II and stored under the same conditions as fractions 3 and 6.

Another portion of the total esters was separated into adduct-forming (fraction 1) and non-adduct-forming fractions (fraction 2) by the technique described above, except that the total weights of the esters and ethanol were correspondingly reduced, since the total esters of heated oil contained a much smaller proportion of straight chain monomeric material than did the distillable esters (fraction 3). The same precautions were taken against peroxidation as when preparing fractions 4 and 5.

The analytical characteristics of the fractions are presented in table 5.

TABLE 5

Yields and characteristics of fractions of ethyl esters used in the feeding trials

FRACTION NUMBER	YIELD AS % TOTAL ESTERS OF HEATED LINSEED OIL	IODINE NO.[1]	REFRACTIVE INDEX n_D^{25}	MEAN MOLECULAR WEIGHT	HYDROXYL[2] VALUE AS PER CENT (—OH)
1 Adduct-forming fraction of total esters	46	118.2	1.45345	293	0.23
2 Non-adduct-forming fraction of total esters	54	162.7	1.47561	472	0.46
3 Distillable esters	60	130.1	1.45684	294	0.22
4 Adduct-forming fraction of distillable esters	49	124.8	1.45494	293	0.11
5 Non-adduct-forming fraction of distillable esters	11	170.7	1.46986	300	0.39
6 Non-distillable esters	40	159.9	1.48017	550	0.64

[1] Iodine numbers by the method of Benham and Klee ('50), using one hour reaction time (Klee and Benham, '50).

[2] Hydroxyl values by the method of Ogg, Porter and Willits ('45).

EFFECTS OF PROLONGED INGESTION OF XYLOSE ON RATS

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Xylose is a pentose sugar which might conceivably be incorporated into foods for human consumption. In terms of sweetness it has a value of 40 when compared to sucrose at 100. Monogastric animals are unable to utilize this 5-carbon sugar (Pflüger, '05; Miller and Lewis, '32; Anderson, '50), which suggests that it might be used as a dietary sweetening agent by individuals who desire to reduce body weight. Xylose might also serve as a sweetening agent for diabetics, either in addition to or in lieu of saccharin. The tendency of this sugar to cause diarrhea in laboratory animals suggests its possible use in laxative preparations. In the event a use is found for xylose, it could be produced in quantity from cottonseed hulls and corn cobs, thanks to the methods of production developed by Dunning and Lathrop ('45).

The possible inclusion of xylose in foods immediately raises the question as to whether any health hazard would be involved. The literature concerning the utilization of this substance by man is neither very extensive nor conclusive, but some experimental work has been done using laboratory animals. Darby and Day ('39) reported on the cataractogenic action of xylose in rats. These same workers (Darby and Day, '40) and others (Anderson, '50; Blatherwick et al., '36) observed diarrhea and abdominal distension when rats received this sugar orally. Blatherwick and co-workers ('36) fed

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NUTRITIVE VALUE OR TOXICITY OF HIGHLY UNSATURATED FATTY ACIDS. I

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According to the result from the experiments on the nutritive value of fish oils conducted by Ozaki (*1*) and Yoshida (*2*), highly unsaturated fatty acids in fish oils give toxic effects on the growth of rats. So far, this has been generally conceded, and the existence of highly unsaturated fatty acids in fish oils has been thought to account for the inferiority of fish oils to other edible oils from nutritive point of view. If this is actually the case, it might be surmised that eating of sardines, for example, in a large amount at a time or successively at short intervals would bring about harmful effects on our health.

It is a well known fact that highly unsaturated acids are rapidly oxidized when exposed to air. For this reason, a special attention must be paid when we carry out a feeding experiment by administrating highly unsaturated acids to test animals, and even if the experiment is started with genuine highly unsaturated acids, there will be a danger of supplying more or less autoxidized acids to the animals.

As has been reported in the previous paper (*3*), we examined the nutritive values of ethyl ester of highly unsaturated acids with iodine value of 365 and their partially reduced products. During the assay period the rats receiving ethyl ester of these acids showed a symptom of losing hairs around their mouths and legs. The ethyl ester used for the previous test was preserved in a brown bottle, and every time the bottle was opened, CO_2 gas was introduced, so that the acid might be kept from oxidation. In the later course of the experiment, however, we found that it was impossible to prevent the acid from deterioration by such a treatment as described above. Therefore, there was a possibility that we were measuring the nutritive value of the acid which was not so genuine as we thought to be, but which had become slightly oxidized. On the other hand, it was revealed by a successive experiment carried out by us that the partially reduced products of highly unsaturated acids, though they had fairly high iodine values, were almost as nutritious as oleic acid.