of the seven oils one of the two currently prescribed lyes gave a good refining.

Conclusions that seem to be established by the summarized data, attached, are:

1. Regular Referee Board collaborators obtained about the same, or slightly poorer, order of agreement with the modified cup method on degummed crude oils as they obtained by the regular cup method on other types of crude soybean oil. The degree of agreement varies considerably on oils, and somewhat by seasons, so that the performance of the modified method among collaborators is not yet well established. The *close* agreement (.12% actual) between duplicates of the individual collaborators suggests the need for a more uniform technique among all collaborators.
2. There seems to be no advantage in changing present lye prescriptions for the modified method.

It is the Committee's recommendation that before the method is made official, it be tested as tentative for another season on one or more check samples by the Referee Board, and by experience of others.

The Refining Committee is again appreciative of the excellent work done by the subcommittees made up as follows:

Kettle Refining Method—Sorensen, chairman; James. Milner, Kruse, Mitchell.
Centrifugal Method—James, chairman; Ayres, Moore, Sanders, Tuttle.
Modified Cup Refining Method—Sanders, chairman; Barrow, Freyer.

H. S. MITCHELL, chairman
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L. A. SPIELMAN
S. O. SORENSEN
W. T. MAXWELL

Effect of Feeding and Injecting Hogs With Tocopherols on the Susceptibility of Pork Fat to Rancidity*

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ABILITY of the rat to store excess tocopherols from the diet in the abdominal fat has been clearly demonstrated by Lundberg *et al.* (1). These workers found maximum storage of single large doses of alpha tocopherol 7 to 10 days after feeding, with concurrent large increases in the induction period of the fat.

The work with rats suggests a possible control of rancidification in the fat of meat animals. Pork fat is notoriously low in tocopherols, and more subject to spoilage by rancidification than are the common vegetable oils. Estimates of the tocopherol content of pork fat range from .0005 to .003% (2, 3) as compared to values ranging from .05 to .11% in the common vegetable oils. Rancidity may be prevented in rendered lard by the incorporation of antioxidants after rendering (4, 5), but such incorporation in pork that is to be preserved by curing, freezing, etc., is impractical. Tocopherol is apparently the only antioxidant of a large number which has been tried which is capable of being stored by the animal itself (6).

The present investigation is an attempt to obtain storage of tocopherol in hog fat, as evidenced by increased resistance to rancidification, through (A) increasing the tocopherol content of the ration, (B) injecting tocopherols subcutaneously.

Experimental

Experiment I. As a preliminary trial, advantage was taken of a hog-feeding project already under way in the Department of Animal Husbandry, described in more detail elsewhere (7). The hogs used were divided into four groups. Groups II and III were maintained on purified rations composed of casein, sucrose, lard, mineral mix, and the following synthetic vitamins: thiamine, riboflavin, niacin, pyridoxine, pantothenic acid, choline, and vitamins A, D, and K. (The amount of thiamine was varied but these variations are not significant in the present study.) The pigs in Group III received, in addition, 50 mg. of vitamin E supplied as a distillation mixture containing 34% mixed tocopherols.[1] Group IV pigs were fed a natural ration consisting of wheat 46%, barley 35%, tankage (a dry rendered meat meal, 55% protein) 13.5%, alfalfa 5%, and iodized salt 0.5%. The pigs in Group V received a different natural ration composed of wheat 36%, barley 35%, tankage 7.0%, alfalfa 5%, wheat germ 15.5%, oyster shell flour 1.0%, and iodized salt 0.5%. The length of the feeding period was 56 days and the hogs averaged 80 pounds when slaughtered. The hogs from all four feeding groups used in this study had been slaughtered and the carcasses had been hanging approximately four months in a refrigerator at 0° C., at the time they were used for this investigation.

Leaf fat was stripped from the kidney region of each of these hog carcasses. Several samples from each group were ground individually in a meat grinder and rendered in beakers set in a boiling water bath for approximately 1 hour. The rendered fat was filtered while holding in an oven at 65° C. A weighed amount of the filtered fat was made to volume with chloroform and aliquots withdrawn for determination of rancidity by a quantitative Kreis test described elsewhere (8).

Since the degree of rancidity was very high in several of these samples, the rendering process was omit-

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[1] Mixed tocopherols obtained through courtesy of the Lederle Laboratories, Pearl River, New York.

ted with the samples of fat from the remaining hogs in each group. Instead, 25 gm. samples of the ground fat were whirled for 2 minutes in a Waring blendor with 30 gm. sodium sulfate as a drying agent and 100 ml. of chloroform. The blended mixture was filtered into glass stoppered graduates and an aliquot of the clear chloroform was drawn off and dried to constant weight to determine the concentration of fat. Suitable aliquots were then used for the determination of the iodine number and of rancidity by the Kreis test and the peroxide value (8).

Results, summarized in Table 1, show a marked difference between the pigs fed the natural as compared to the purified rations in the extent of rancidity during the storage period. Accepting a peroxide number of 20 and a Kreis value of 10 as approximate points of organoleptic rancidity (8), it will be seen that 8 out of 10 hogs on purified rations were below the rancid point, whereas 5 of the 6 hogs fed the natural rations were definitely rancid and the sixth was on the border line.

The difference between pigs on the purified ration plus tocopherol as compared to those on the same ration without tocopherol are less marked but show some protective action from the tocopherol.

The differences in susceptibility of the fats to oxidation were not due to variations in the degree of unsaturation. No correlation was obtained between the iodine number and the two tests for rancidity. Fat from animals on the tankage ration was more saturated but less stable than that from those on synthetic rations. Kreis values and peroxide numbers correlated well.

Rancidity tests were not run on the rations or their components. Fresh wheat germ is known to be an excellent source of tocopherol, but rancidification of the germ would bring about destruction of this vitamin.

It is also quite possible that while tocopherol may be the only antioxidant stored in the fat, the composition of the muscle tissue may cause great differences in the relative rate of rancidity development of rendered fat from hogs on various rations as compared to meat from the same hogs. Preliminary investigations indicate that such differences may exist. Rancidity development in frozen sausages from these and other hogs on a variety of rations will be reported at a later date.

Experiment II. A second feeding trial was set up to compare keeping qualities of the rendered fat from hogs on a number of natural rations, one of which was supplemented with extra amounts of tocopherol. Six lots of pigs were placed on controlled natural grain rations plus various protein supplements as follows:

Lot I—
Wheat, barley, tankage, 5% alfalfa, and minerals
Lot II—
Wheat, barley, tankage, 15% alfalfa, and minerals
Lot III—
Wheat, barley, cull peas, 5% alfalfa, and minerals
Lot IV—
Wheat, barley, cull peas, 15% alfalfa, and minerals
Lot V—
Wheat, barley, soybean oil meal, 5% alfalfa, and minerals
Lot VI—
Wheat, barley, soybean oil meal, 15% alfalfa, and minerals.

TABLE 1

Rancidity Tests on Internal Fat From Hog Carcasses Refrigerated for Four Months

Diet group	Heat rendered fat		Fat extracted without heat rendering			
	Code No.	Kreis value	Code No.	Kreis value	Peroxide number	Iodine number
II. Purified ration without added tocopherol	148 240 266	8.6 30.4 14.0	152 186	4.2 3.2	8.3 5.0	57.3 56.0
III. Purified ration plus 50 mg. tocopherol daily	268 162 151	4.0 7.0 2.3	162 269	2.7 3.2	3.5 4.8	54.7 63.0
IV. Natural grain ration with tankage as a protein supplement	183	20.8	153 265 208	9.4 13.9 17.8	18.5 28.6 36.1	50.5 50.0 51.8
V. Natural grain ration with wheat germ plus tankage supplement	184	65.5	221	17.8	28.0	59.3

The pigs in all lots were fed ad libitum. The amount of protein fed in each lot was equalized so that regardless of the protein supplement fed, the pigs were obtaining the same amount of total protein in the feed. The following levels of protein were used:

Pigs 50 to 75 lbs. in wt. 20% total protein in the ration.
Pigs 75 to 125 lbs. in wt. 16% total protein in the ration.
Pigs over 125 lbs. in wt. 14% total protein in the ration.

The six pigs in Lot I were divided into three groups of 2 pigs each. Group 1 was given no added tocopherol, Group 2 was given .6 gm., and Group 3, 2.4 gm. pure alpha tocopherol per animal[2] by capsule every other day for 14 days. The pigs were slaughtered 3 days after the last dosage. The pigs in Lots II to VI received no added tocopherol.

Peroxide numbers were run on the chloroform extract from the mixed rations as well as on several of the individual constituents of the rations. The tankage showed a high degree of rancidity (peroxide number 86.3). The alfalfa meal extract contained so little fat that only slightly more than .1 gm. could be taken for the peroxide determinations in place of the .5 gm. required for comparable tests. Also the green color interfered to some extent with determinations of the end point. The peroxide value of 95 obtained for the alfalfa meal is probably not highly accurate; nevertheless, it undoubtedly indicates considerable peroxide formation. The peroxide values of the remaining ration constituents were all much lower, ranging from 1.6 in the soybean oil meal to 10.8 in the peas. Peroxide numbers for the mixed rations are shown in Table 2.

The six hogs in Lot I and the two hogs from each of the other lots were slaughtered at approximately 6 months of age. Their weights ranged from 190-248 pounds, although most of the pigs weighed around 200 pounds. The carcasses were chilled overnight. A strip of external fat was removed from the shoulder of each animal. All samples of fat were frozen at —18° C. overnight.

While still partially frozen, each sample of fat was ground in a meat grinder. A uniform weight of the ground fat (150 gm.) was rendered by placing in Erlenmeyer flasks in a boiling water bath. The fat rendered nicely in 15 minutes. The rendered fat was

[2] Supplied by Merck and Co., Rahway, New Jersey.

TABLE 2

Accelerated Rancidity Tests on External Fats From Hogs on Various Natural Rations

Diet group		Peroxide numbers of mixed diet	Time required for external fat to turn rancid at 93° C.
			hours
Lot I Tankage Alfalfa 5%	Group 1—No added tocopherol.	27.8	4.5 3
	Group 2—0.6 gram alpha tocopherol per pig every other day for 14 days.		3.5 4.5
	Group 3—2.4 gram alpha tocopherol per pig every other day for 14 days.		5 4
Lot II	Tankage Alfalfa 15%	25.5	2.5 3
Lot III	Cull Peas Alfalfa 5%	15.6	3 3
Lot IV	Cull Peas Alfalfa 15%	15.6	3.5 3
Lot V	Soybean oil meal Alfalfa 5%	12.2	3 3
Lot VI	Soybean oil meal Alfalfa 15%	15.9	3 3
Mixed fat from the 2 animals in Lot I, Group 3			4.5
Mixed fat 2 above plus .05% tocopherol added after rendering			28.5

filtered in an oven at 65° C. The filtration required 1 hour. Accelerated tests were then run on the samples of external fat as follows: Uniform 2 gm. portions of the melted fat were weighed out into 100 ml. beakers, which were set in an air oven held at a temperature of 93° C. Beakers were removed at half-hour intervals and analyzed for peroxide oxygen. Time required to reach a peroxide number of 20 is given in Table 2.

It is apparent that little variation in keeping quality was obtained in fat from hogs on the several natural grain rations studied. Also the addition of large amounts of tocopherol to one of these rations (Lot I, groups 2 and 3) had only a slight effect on retarding rancidity as compared to the very large amount of protection secured by the addition of small amounts of tocopherol to the rendered fat. It is possible that in this experiment the pigs were slaughtered too soon after the beginning of tocopherol feeding (begun 17 days before slaughter) to get the maximum amount of stored tocopherol in the fat.

Experiment III. Injection of Tocopherol. Since it was considered probable that a large proportion of the tocopherol fed the hogs in Experiment II was not absorbed from the digestive tract, a third experiment was set up to determine the effect of injecting the pigs with tocopherols on the susceptibility of their fat to oxidative rancidity.

A group of 8 hogs on a natural ration (fed a mixture of wheat, barley, tankage, and iodized salt on Sudan grass pasture) were selected for this experiments. Three pigs from this group, the controls, were given no tocopherol supplement. The remaining five pigs were given 3 injections of the distillation mixture of plant tocopherols (containing 34% mixed tocopherols), subcutaneously, just behind the ear. The first injection of 4.5 ml. of the mixture (1.5 gm. tocopherols) was made 58 days before slaughter, the second of 5 ml. (1.7 gm. tocopherols) 38 days before slaughter, and the third, also of 5 ml. was given 17 days before slaughter.

The day following slaughter, comparable strips of fat were taken from the internal (kidney) fat and from the outside of the ham. The fat samples were frozen, ground, and rendered as described in Experiment II. Internal fat samples were rendered in 10 minutes, while the external fat required 3 hours at this temperature. The rendered samples were filtered for 1 hour in an oven at 37° C. Accelerated tests at 93° C. were then run as previously described on all samples.

Results (Table 3) showed no differences between the control and the injected groups. Apparently the injected tocopherols were not absorbed, since it was noted at the time of slaughter that pockets of the injected material remained in the subcutaneous fat at the site of the injections. Further experiments on the injection of water-soluble tocopherols on smaller animals are being carried out and will be reported elsewhere. At present, sufficient quantities of the water soluble material has not been obtained for experiments with pigs.

TABLE 3

Effect of Injecting Hogs With Mixed Tocopherols on Susceptibility of Their Fat to Oxidation

Treatment of animal	Time in hours to turn rancid at 93° C. (Each figure represents a separate animal.)	
	Back fat (required 3 hrs. to render)	Internal fat (required 10 min. to render)
Controls.— No added tocopherol.	5 5 6.5	10 9.5 9.5
Injected with a total of 4.9 gm. mixed tocopherols.	5 6.5 6 6 5	8 10 8.5 9 9.5

Discussion

While the evidence would indicate that some deposition of extra dietary tocopherol occurred in both experiments in which tocopherol was added to the rations, the magnitude of the effect was too small to be of great practical significance.

This does not necessarily mean that the hog is less able than the rat to store dietary tocopherol in the fat. Although Lundberg *et al.* (1) show much greater differences between control- and tocopherol-fed rats than any here reported for pigs, it should be pointed out that their control rats were completely lacking in vitamin E, whereas in our one experiment with pigs on purified rations, no attempt was made to exclude vitamin E in the control group by rancidification of the lard in the ration. In the second and third experiments reported here, the pigs were on natural rations which probably contained considerable amounts of vitamin E.

Also the amounts of tocopherol fed in relation to the weight of the animals did not approach the larger dosages fed by Lundberg *et al.* These workers fed amounts ranging from 50 to 500 mg. to rats weighing 175 grams (approximately .03 to 0.3% by weight). In our experiments with pigs on purified rations, a total of 2.8 grams of tocopherols were fed to pigs weighing 80 pounds (.007% by weight). The largest dosage fed pigs on natural rations amounted to approximately .02% by weight (16.8 gm. tocopherol per 200-pound hog).

However, the latter figure represents a much greater amount of tocopherol than would be obtained

by manipulation of natural rations. From a practical standpoint it does not seem feasible to attempt to improve the keeping qualities of pork fat by increasing the tocopherol content of the diet, unless means can be found to bring about more economical storage of diet tocopherol in adipose tissues.

Furthermore, while tocopherol and the degree of saturation may be the only important factors in determining the keeping quality of rendered hog fat, other factors probably influence to a large extent the keeping qualities of the fat present in refrigerated, frozen or cured pork. Among such contributing factors may be mentioned the lipoxidases present in muscle and adipose tissue, the effect on such oxidases of salt, pH, etc. (9), and possible variations in synergistic antioxidants present in muscle tissue. Further work along these lines will be reported at a later date.

Summary

1. Fat from hogs on several natural rations became rancid more rapidly than fat from similar hogs on purified rations with or without added tocopherol.

2. Feeding of tocopherols to hogs on purified and natural rations resulted in small decreases in the susceptibility of their fat to oxidative rancidity. The magnitude of the protective action of dietary tocopherol was too small to be of practical significance.

3. Subcutaneous injection of a distillation mixture of tocopherols resulted in no added protection to the fat, probably because of lack of absorption.

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The Stability and Constitution of Monoglycerides

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IT has long been reported in the literature (1) that glycerol and fatty acid triglycerides will react at temperatures of 180-290° C. to form mono- and di-esters of glycerol. It has been commonly accepted and more recently reported (2) that conversely, glycerol and triglycerides are formed when distillation is attempted of mono- and diglycerides in vacuo.

$$\begin{array}{l} CH_2OOCR \\ | \\ CHOOCR \\ | \\ CH_2OOCR \end{array} + 2 \begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OH \end{array} \rightleftharpoons 3 \begin{array}{l} CH_2OH \\ | \\ CHOH \\ | \\ CH_2OOCR \end{array}$$

As stated in a recent review (3) it is generally acknowledged that these reactions "can be considerably speeded up if catalysts are used." However, we feel that it would be more useful to adhere to the restricted view that such reactions between polyhydric alcohols and polyhydric alcohol esters proceed at an appreciable rate only in the presence of catalysts. The earlier chemists realized the value of catalysts in promoting reaction rates but later chemists have neglected to recognize the possible catalytic effect of impurities on the reversibility of these reactions and upon the stability of their products.

We have found that pure anhydrous glycerol and fatty acid triglycerides do not react appreciably below decomposition temperatures but will do so readily in the presence of water or other catalysts. We have also found (4) that pure mono- and di-esters of fatty acids and glycerol can be distilled without disproportionation.

In this discussion the words "pure," "catalyst," and "appreciable" are used in a limited sense and not with their absolute meanings. Thus a preparation is described as pure when it contains no decomposition or reversion catalyst, or when such catalyst is inhibited.

Reaction Between Glycerol and Triglycerides

In Table I can be seen the results of heating glycerol (0.2 moles) and a triglyceride (0.1 mole coconut oil) with and without water in an agitated autoclave.

The refined coconut oil used had been refined by bleaching and so-called "caustic refining" and thus contained 0.013% of soap. The "pure" coconut oil was prepared by treating an ether solution of this refined coconut oil with acetic acid, washing with water and finally drying, removing the solvent and

TABLE I.

	Weight Oil	Weight Glycerine	Weight Water	Temperature	Time	% Free Fatty Acid	Acetyl Value	Calculated Approx. % Monoglyceride
	g.	*g.*	*g.*	*°C.*	*hrs.*		*%OH*	*%*
Refined coconut oil	66.0	18.4	2.0	270	2	5.6	.....	50
Refined coconut oil	66.0	18.4	None	270	2	0.5	2.16	24
Pure coconut oil	66.0	18.4	2.0	240	2	6.3	.....	50
Pure coconut oil	66.0	18.4	None	240	2	0.6	0.45	3