TABLE II
Spectral Absorption after Conjugation of Fractions Described in Figure 1

Fraction	Specific Extinction Coefficient ($E^{0.1\%}_{1\,cm.}$)				
	3750A	3460A	3150A	2680A	2330A
Original Mixture	7.5	13.8	35.4	35.1	36.6
Fraction II[a]	14.8	23.9	45.3	44.6	45.3
Fraction III[a]	3.6	8.9	38.4	39.8	45.6
Fraction IV	18.7	30.0	47.1	44.3	46.8

[a] Recrystallized from Ethanol at —75°C.

are concentrated in Fractions II and IV with only a small quantity in the intermediate Fraction III. This difference in solubility of the urea complexes suggests that two hexaenoic acids were present in the original mixture.

Hammond and Lundberg (4) have reported a specific extinction coefficient of 28.1 at 3,750 Å for a sample of methyl docosahexaenoate of high purity. Conjugation was accomplished under approximately the same conditions as those used in our laboratory. Comparison of this figure with the 14.2 obtained for Fraction II leads to the conclusion that approximately 50% of the acids crystallizing as urea complexes at —20°C. was docosahexaenoic acid.

The specific extinction coefficients at 3,750, 3,460, and 3,150 Å show a low content of hexaenoic acid and a high percentage of tetraenoic acid in Fraction III. This indicates that the major portion of the docosahexaenoic acid had been removed as a urea complex at the higher temperature and that arachidonic with a shorter chain length constituted the main constituent precipitating at —75°C.

Fraction IV yielded a higher specific extinction coefficient at 3,750 Å than Fraction II, indicating greater percentage of hexaenoic acid. However the high iodine value, low neutral equivalent, and high refractive index all indicate that Fraction IV contains a high percentage of an acid with six double bonds but a shorter chain length than the docosahexaenoic of Fraction II. It is probably an eicosahexaenoic acid.

The eicosahexaenoic acid was present in the mixture of free acids liberated by enzymic action taking place during autolysis of the saline suspensions. It could not be detected in the acids obtained by alkaline saponification of either the neutral esters or phosphatides extracted from fresh tissue.

Summary

A mixture of fatty acids obtained from autolyzed saline extracts of beef testicular tissue was fractionated by crystallization of the urea complexes at 5°, —20°, and —75°C. Fractions rich in docosahexaenoic and in arachidonic acids were obtained as solid complexes. The filtrate remaining after precipitation of the solid urea complexes contained a high percentage of hexaenoic acid of shorter chain length than docosahexaenoic, probably eicosahexaenoic acid.

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The Effect of Various Antioxidants on the Keeping Quality of Yellow Grease[1]

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RECENTLY there has been considerable interest in the addition of fats to animal feeds. This interest was probably brought about mainly by the price situation in the lower grades of fats which gave a favorable comparison from a nutritive standpoint with wheat, corn, and other carbohydrates normally used in animal feeds. Work at various experiment stations and research centers has shown that most animals as well as poultry will show a definite gain in weight as well as over-all physical appearance when fat is used in proportions of 5-10% of the total feed formula. Matsushima and Dowe (3) of the Nebraska Experiment Station have shown that cattle when placed on a diet containing approximately 5% fat gained weight more economically than when fed grain alone.

Rice *et al.* (6) have shown that there is definitely a need for fats in animal feeds. They have shown that, in addition to the nutritive value of the fat, it controls dustiness and the physical appearance of the feed and makes it much easier to handle.

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Armstrong (1) of the American Meat Institute Foundation reports that at the beginning of 1954 approximately 200 million pounds of tallows and greases were used in mixed feeds annually. During the past year there has been a continued increase in the use of fats in animal feeds, and apparently many customers are now asking for mixed feeds which contain from 3-6% added fats.

One of the reasons that many feed users have held back on the use of fats in feed is the fact that most animals as well as poultry will not eat feed containing fats when they have become rancid except to prevent starvation. In addition to this, Quackenbush (5) of Purdue University has reported that fats which are oxidized will bring about a more rapid breakdown of the fat-soluble dietary essentials such as vitamin A, vitamin D, and vitamin K. He also reports that cases of dermatitis and failure of the reproductive organs have been reportedly caused by rancid dietary fat.

Since our previous work on the stabilization of the higher grades of fat, such as lard, tallow, etc., has shown that various synergistic mixtures of butylated hydroxyanisole, propyl gallate, and BHT were quite successful, it was decided to compare the effect of these antioxidants on yellow grease [2] on a laboratory scale as well as a factory scale.[3] All of the antioxidants compared in these experiments were taken from the regular production of Tennessee Eastman Company at Kingsport, Tenn. The butylated hydroxyanisole used in all experiments contained 98% of the 3-isomer and 2% of the 2-isomer.

Experimental

In several preliminary experiments on stabilizing yellow grease and similar fats it was found that there was a rather wide variation in the different greases which were used as test materials. For this reason it was felt advisable to do the basic work on one grease which had a fairly high initial AOM stability in order that our results would be more comparable. Since the Active Oxygen Method for determining stability is fairly standard throughout the industry, the stability of the various greases was compared by this method as described by King *et al.* (2).

Experiment 1. Portions of yellow grease having an initial AOM stability of 12 hrs. were stabilized with propyl gallate, butylated hydroxyanisole, and butylated hydroxytoluene so the finished sample would contain .005, .01, .02, .03, .04, and .05% antioxidant. The AOM stability of these greases was determined, using a peroxide value of 20 meq. as the rancidity point. AOM stabilities were plotted against antioxidant concentrations, and the resulting curves are shown in Figure 1.

In Figure 1 it may be seen that propyl gallate produces the highest AOM stability, reaching a peak at approximately .03% and decreasing slightly thereafter. Butylated hydroxyanisole, or BHA, is practically equal to propyl gallate at concentrations up to approximately .01% but does not show the sharp rise thereafter. It does not reach a levelling point however and again equals propyl gallate at approximately .05% concentration. Butylated hydroxytoluene, or BHT, shows a continual rise with increased concentration but shows no levelling effect at practically any concentration. This graph is quite interesting from the point of view that BHA does not level off in effectiveness at approximately .02% as it does in the higher quality fats. Higher quantities of propyl gallate must be added to yellow grease before maximum efficiency is reachd. BHT in yellow grease as in lard shows a continual rise with increased concentration.

Experiment 2. Since mixtures of antioxidants show a marked synergism in stabilizing lards and other high quality fats, it was believed desirable to have a concentration study of BHA and propyl gallate, BHA and BHT, and BHA and citric acid mixtures in yellow grease. Since propyl gallate will discolor in the presence of iron, it was decided to add a small quantity of citric acid to the BHA-propyl gallate mixture to prevent the formation of iron gallate in the presence of small quantities of iron normally found in yellow grease which might interfere with the antioxidant activity of the mixture.

The following mixtures were used in this concentration study:

No. 1	BHA — 20% Propyl gallate — 6% Citric acid — 4% Propylene glycol — 70%
No. 2	BHA — 20% Citric acid — 20% Propylene glycol — 60%
No. 3	BHA — 20% BHT — 20% Cottonseed oil — 60%

The yellow grease used in Experiment 1 was stabilized with quantities of the above mixture so that portions of it would contain .005, .01, .015, .02, .03, and .04% total antioxidant, including citric acid. Previous experience had led us to believe that the instability of some lots of yellow grease was due to the catalytic effects of some substance in the fat which greatly shortened the induction period normally found in most yellow greases. It was believed that the quantity of citric acid in an antioxidant formula had a definite bearing on its effectiveness and that the citric acid should be included as an active ingredient in any data reported. Figure 2 shows the AOM stability of the various samples of grease described above. This graph shows that mixtures of BHA and citric acid produce slightly better AOM stabilities in yellow grease although the BHA plus propyl gallate is practically equal. The mixtures of BHA and BHT, while producing much lower initial stability than the other mixtures, show a steady rise with increase in concentration and at concentrations of .04% antioxidant improve and increase the stability of the yellow grease well beyond either of the other mixtures.

Experiment 3. After the above data had been completed and it became apparent that yellow grease could be stabilized quite efficiently with food grade antioxidants, it was decided to test the synergistic mixtures under actual factory conditions. After some discussion with various members of the rendering industry, it was found that particularly the smaller plants, as well as some of the larger ones, could not add antioxidants to the finished fat without the purchase of extra mixing equipment. The idea was brought forth that perhaps the more stable antioxidants, such as BHA and BHT, could be added to the rendering kettle along with the charge and stabilize the meat scraps as well as the rendered fat itself. Various antioxidant mixtures were added to the rendering tanks along with the charge by adding an antioxidant solution when approximately half of the charge had been filled into each tank. The percentage antioxidant was based on the estimated grease yield of each tank. The raw material for all batches was well mixed, and portions were put in each tank in an endeavor to obtain as uniform raw material as possible for this experiment. One group of three rendering tanks was run without any antioxidant, and this grease was collected for control grease. Samples of this control grease were stabilized in the laboratory with the same antioxidant mixtures used above so that each sample, stabilized before rendering, would have a corresponding sample stabilized after rendering. The treatments, the stability of each sample, as well as its actual antioxidant content (determined spectrophotometrically), may be seen in Table I.

The data in Table I indicate that, when using an

[2] Supplied by the Carolina By-Products Company, Greensboro, N. C., and Darling and Company, Chicago, Ill.

[3] Factory experiments carried out at Greensboro Plant of Carolina By-Products Company (a dry rendering operation).

TABLE I

Stability of Yellow Grease as Affected by Method of Antioxidant Application

Treatment	AOM stability in hrs.			
	Treatment before rendering		Treatment after rendering	
	Antioxidant content	AOM value	Antioxidant content	AOM value
Control	0	12	0	12
.01 BHA[a] + .01 CA[b]	.003	41	.011	116
.02 BHA + .02 CA	.009	78	.019	165
.01 BHT[c] + .01 CA	.007	55	.006	42
.02 BHT + .02 CA	.017	82	.017	123
.005 BHA + .005 BHT	.013	75	.01	67
.01 BHA + .01 BHT	.025	83	.021	128
.02 BHA + .02 BHT	.034	136	.038	185
.0075 propyl gallate + .1 CA	0	12	.0066	124
.010 propyl gallate + .1 CA	0	20	.0095	198

[a] Butylated hydroxyanisole.
[b] Citric acid.
[c] Butylated hydroxytoluene.

average grease, either BHA or BHT in combination with each other or with citric acid produces a fairly stable product. Much better stability is obtained, with the same amount of antioxidant, when the antioxidant is added after the rendering and settling are complete. The antioxidant analysis indicates that the stability of the grease varies directly with its antioxidant content. It also indicates that a part of the antioxidant is not retained by the finished grease after the rendering operation. An analysis of the meat scraps and tank bottoms indicate however that a large part of the antioxidant remains in the grease portion of the meat scraps as would be expected. Propyl gallate does not carry through the rendering process and does not improve the stability of the finished grease to the same extent as either BHA or BHT. When added after rendering however, the results are similar to Experiment 1.

It was noted, while making various stabilizations, that citric acid was dissolved in hot grease with difficulty. It seemed that a simple method was needed whereby the citric acid could be well mixed without violent agitation and still maintain the economy of adding the antioxidant after rendering. It was decided to stabilize several rendering tanks by adding solid citric acid to the tank (basing the concentration on the estimated yield) and adding the antioxidant to the settling tank after the rendering was complete.

Samples of the completed grease were taken and compared with a control sample taken immediately preceding the above batches.

Table II shows the effect of adding the citric acid along with the rendering charge and adding the antioxidant after the rendering is complete. In this particular case a grease with an initial stability of 20 was used, and the results cannot be accurately compared with the previous data. They do indicate however that citric acid can be added along with the charge and the antioxidant added after rendering is complete.

TABLE II

Stability of Yellow Grease When Citric Acid Is Added Before Rendering

Treatment	AOM stability in hrs.
Control	20
0.1% CA + 0.005% BHA + 0.005% BHT	115
0.1% CA + 0.01% BHA + 0.01% BHT	260
0.1% CA + 0.01% BHT	72
0.2% CA + 0.02% BHT	127

Experiment 4. Our laboratory experiments as well as some of our sales service problems had shown that an occasional lot of yellow grease was extremely difficult to stabilize if practical quantities of antioxidants were used. Reports from the rendering industry also showed that some renderers had apparently found occasional lots of grease which were extremely difficult to stabilize under commercial conditions. These greases almost invariably have an initial AOM value of 5 hrs. or below. The foregoing data indicated that the amount of citric acid which was used during the stabilizing of yellow grease had a definite bearing on the final stability of the grease itself. It was decided to take a grease which we had been unable to stabilize, and add various antioxidants with varying quantities of citric acid to determine the effect of the citric acid on the stability of this poor quality grease. All samples of this grease were stabilized according to standard laboratory procedures, and AOM stabilities were determined. Table III lists these treatments and shows that citric acid alone, if used in sufficient quantities, greatly improves the stability of a poor grease. When BHT or BHA was added to the citric acid, a further increase was obtained. It may also be seen that when synergistic mixtures of BHA and propyl gallate, and BHA and BHT were added to the poor quality grease along with citric acid, stabilities were obtained which would be acceptable to most yellow grease purchasers.

TABLE III

The Effect of Citric Acid on the Stability of Yellow Grease

Treatment	AOM stability in hrs.
Control	1
0.05% CA	2
0.1% CA	2
0.2% CA	17½
0.4% CA	33
0.02% BHT + 0.1%CA	6
0.02% BHT + 0.2%CA	28
0.02% BHT + 0.3%CA	55
0.01% BHA + 0.01% CA	2
0.01% BHA + 0.06% CA	2
0.01% BHA + 0.26% CA	53
Control	1
0.01% BHA + 0.003% PG[a] + .002% CA	1½
0.01% BHA + 0.003% PG + 0.05% CA	2
0.01% BHA + 0.003% PG + 0.10% CA	13
0.01% BHA + 0.003% PG + 0.20% CA	50
0.01% BHA + 0.003% PG + 0.40% CA	75
0.01% BHA + 0.01% BHT + 0.05% CA	2
0.01% BHA + 0.01% BHT + 0.2% CA	53
0.2% BHA + 0.02% BHT + 0.2% CA	73
0.01% BHA + 0.01% BHT + 0.4% CA	90

[a] Propyl gallate.

Experiment 5. In view of the effect of citric acid on the stability of poor quality grease, it was believed that the iron content of the grease itself had a direct bearing on the effect of any antioxidant. Several samples of grease having an initial AOM stability varying from 1 hr. to 65 hrs. were analyzed for ferric oxide, using a gravimetric method described by Pierce and Haenisch (4). The results of these analyses may be seen in Table IV. This table shows that most of the greases with low initial stability have an iron content in the neighborhood of .03% and above. Those greases having a high initial stability have iron contents in the neighborhood of .01%. Since time was limited on these particular experiments, a more complete analysis could not be made. Further work will

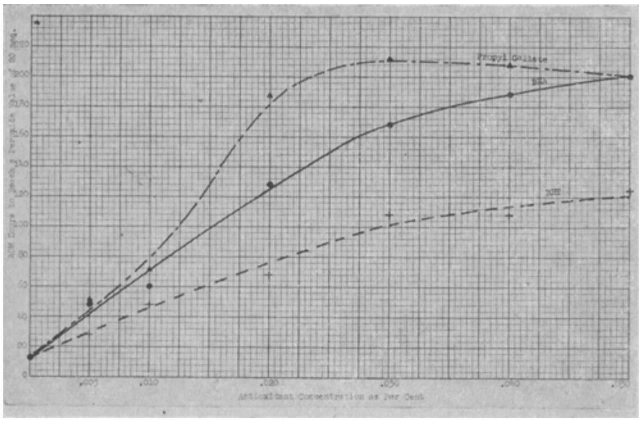

FIG. 1. Relationship of basic antioxidant concentration *vs.* AOM stability of yellow grease.

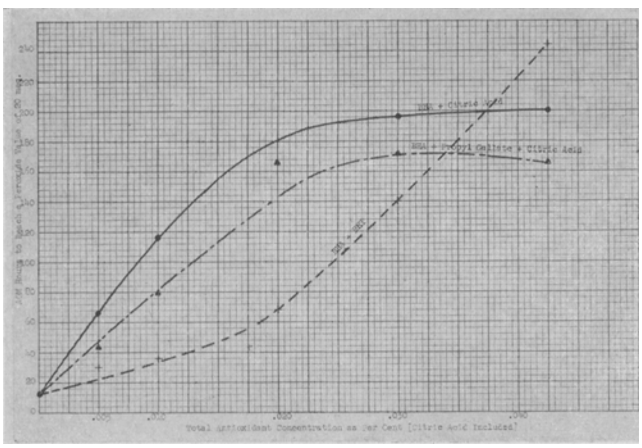

FIG. 2. Relationship of synergistic antioxidant mixtures *vs.* AOM stability of yellow grease.

be done however to determine if the metal content can be used as an indication of the ability of a yellow grease to be stabilized.

Discussion

The foregoing data show that yellow grease can be effectively stabilized by the use of phenolic type antioxidants. The data are not as clear-cut as those of previous work on lard but definitely show the trends which can be expected when stabilizing yellow grease under commercial conditions.

Propyl gallate is a slightly better antioxidant than BHA when used alone. BHA is practically as good at concentrations of .02% and lower. BHT is not as good at any concentration tried as the other antioxidants. The synergistic mixture of BHA and citric acid produces the best AOM stability with BHA plus propyl gallate and citric acid a close second in the grease used as a test medium. These data strongly bring out the point that citric acid or similar metal scavengers are definitely needed in the stabilization of a complex fat, such as yellow grease. Normally one would expect the mixture of BHA and propyl gallate to produce much better AOM stability tha nthe BHA citric acid mixture. Apparently, without an excess of citric acid, various metals and other materials catalyze oxidation to the point where the action of the antioxidant is inhibited.

BHA and BHT alone and in combination with each other with citric acid have excellent carry-through

TABLE IV

Iron Oxide Content *vs.* Stability of Yellow Grease

% Fe_2O_3	AOM stability in hrs.
0.0356	7
0.0422	4
0.0419	1
0.0350	5
0.0140	6
0.0099	18
0.0096	21
0.0142	20
0.0094	43
0.0140	38

properties and can be added to the fat during the rendering process. Propyl gallate however does not carry through and, if used, can only be effective when added after rendering is complete. More effective results from the same amount of antioxidant is obtained if the antioxidant is added after rendering. This would be expected since a large amount of the antioxidant remains with the grease in the meat scraps and is not effective for stabilizing the rendered fat. If a renderer has difficulty dissolving citric acid in the fat, it may be added to the rendering tank with the antioxidant added to the finished grease. A part of the citric acid is lost however since it remains with the meat scraps and is not as effective as when added after rendering.

Greases which are difficult to stabilize apparently have heavy metallic contamination. It is also believed that a high protein content may affect the properties of the phenolic antioxidants by tying up the active groups in the molecule. In all cases however a grease which was difficult to stabilize could be stabilized by the addition of a good antioxidant mixture plus an excess of citric acid. Work will be continued on this problem.

Summary

Yellow grease was stabilized with varying quantities of BHA, BHT, and propyl gallate under laboratory conditions. Propyl gallate was the best antioxidant with BHA a close second.

Yellow grease was stabilized with mixtures of BHA and citric acid; BHA, propyl gallate, and citric acid; and BHA and BHT under laboratory conditions. The BHA-citric acid mix proved to be the best mixture.

Grease was stabilized by adding various antioxidant mixtures before and after rendering. It was found better to add the antioxidant after rendering. Propyl gallate showed almost no carry-through properties. It was also shown possible to add the citric acid before rendering and the antioxidant after rendering.

It was found that extra citric acid (.1 to .5%) added with an antioxidant increased the effectiveness of the antioxidant when greases of low stability were encountered. The iron content of the initial grease seems to have some bearing on the ease with which it could be stabilized.

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