gums to meal in Figure 5. Moisture in meal rises rapidly with the addition of gums because of their high moisture content. Oil rises in direct proportion to the amount of gums added as gums are 100% soluble in both petroleum ether and anhydrous diethyl ether even though they contain only 12 to 13% free oil in themselves. A pound of gums added to 100 pounds of meal will increase the oil content of the meal 1%. Protein content of the meal does not decrease as rapidly as with soapstock because gums contain 12% crude protein themselves. There is a definite increase in protein solubility. Gums themselves are 100% soluble in .02N·NaOH. Of course, the gossypol picture is an adverse one for gums contain about half the gossypol normally present in crude oil. Hence the gossypol rise is identical with the soapstock picture, except that it is slightly higher. This results from the fact that much of the gossypol in soapstock has been either converted or decomposed in the harsh refining process.

FROM THE FOREGOING it appears that if an oil miller were faced with a choice of degumming his own oil or buying soapstock to add to meal, he would do well to consider degumming. Economically it is sound. Addition of gums to meal results in a) reducing of dustiness formerly inherent in solvent-process meals, b) raising of the fat content of meal pound for pound, c) weight gain in meal, d) increased protein solubility, e) no sacrifice of crude protein when added in normal amounts, *i.e.*, 1 to 3%, and f) increased nutritive value. By way of explaining this last statement, cottonseed oil gums contain about 65% crude lecithin, which is a phosphoprotein and of proven benefit in animal as well as human nutrition (9). The high gossypol content of meals with gums added should present no problem to cattle feeders, who are the prime outlet of our meal. Space does not permit a thorough study of these premises and their ramifications. but suffice it to mention that the addition of gums to meal is to be preferred over the addition of soapstock from whatever source, as viewed soley from results of chemical tests.

No mention has been made of acidulated soapstock. No thorough investigation was made, but skeletal analyses pointed in the direction we might well expect they would. Since acidulated stock is more concentrated and freer of oil, we get all the same effects we would from raw stock only more exaggerated with the exception of the rise in moisture content of the meal. Acidulated stock is drier. Its gossypol content is higher percentagewise because of the dryness. Protein solubility is inversely affected but in a more orderly fashion than is the case with raw stock.

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[Received October 16, 1957]

Problems Arising in Connection with the Use of Antioxidants in the Food Industry[1]

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RESEARCH on antioxidants started in our institute in November 1945 as a result of the growing interest in greater stability of oils, fats, and fatty products. First the antioxidative effects of oats and oat extracts were studied, but the difficulties encountered soon directed our attention to synthetic antioxidants. We were particularly interested in the work done by Boehm and Sabalitschka (3) on the lower gallic acid esters, especially propyl gallate as an effective compound of low toxicity.

Various considerations prompted us to concentrate our attention on the higher gallic acid esters, which had better fat-solubility and perhaps better carry-over properties. One of the problems however was to obtain these higher esters, not described in the literature prior to 1946, in a reasonably pure state. The direct esterification, which yielded good results in the lower esters, was attended by certain difficulties (2). Later on several syntheses were published (1, 10). The Institute for Organic Chemistry T.N.O. succeeded in developing two syntheses for the higher esters (9), the result of which was the manufacturing of octyl and dodecyl gallate on a technical scale in The Netherlands.

Coinciding with the development of the synthesis, a study was also made of the toxicity and potential applications of octyl and dodecyl gallate. The acute oral toxicity (LD_{50}) as determined with albino rats, was 4.5 g./kg. for octyl gallate and 6½ g./kg. for dodecyl gallate. Dodecyl gallate, in a concentration of 0.2% calculated on the fat fraction of the diet, did not show harmful effects for three generations (5). The harmlessness of the higher gallates has been confirmed by Van Esch (6).

As to the antioxidant properties of the gallates, it was found that they were highly effective in animal fats in concentrations of 0.005–0.01% but that in vegetable oils and margarine scarcely any effect was achieved. Good results were obtained with whole-milk powder, employing tetradecyl and dodecyl gallate in a concentration of 0.01%, calculated on the powder (12). In bakery products the results varied considerably although in some cases the incorporation of 0.05% dodecyl gallate, calculated on the fat, was

[1] Lecture delivered at the symposium on antioxidants, organized by the Low Temperature Research Station, Cambridge, April, 1957.

found to exercise a favorable influence on the flavor.

Mention must also be made of our investigations in counteracting cold-storage defects in butter made from ripened cream with the aid of antioxidants. Very good results were obtained with tetra-alkyl thiuram disulphides (18).

In recent years the action of butylated hydroxyanisole (BHA) and of butylated hydroxytoluene (BHT) as well as of mixtures of various antioxidants was also investigated. Our activities were focussed on the practical application of antioxidants in pork fat (lard), vegetable oils, margarine, and bakery products.

The investigations were based to a large extent on storage tests at normal temperature; the quality criteria were peroxide values and organoleptic evaluations. In some cases the Active Oxygen Method was also employed.

The results of this research, partly done in cooperation with industry, are discussed below. Practical applications of antioxidants and analytical problems related to the deterioration of fats were studied. Peroxide values, expressed in milliequivalents (meq) per kg. of fat, were determined by a modified Wheeler method (13). A method for the quantitative determination of the higher gallic acid esters was found, based on the reaction of the gallates with ferrous tartrate (19).

Effectiveness of Antioxidants in Pork Fat

The pork fat consumed in Europe consists essentially of a mixture of suet, dorsal fat, mesenteric fat, and loin fat with an iodine value of about 55. The greater part of this fat is consumed in unrefined, unprocessed form; in countries like Germany, for instance, the import of pork fat destined for human consumption is prohibited if such fat is refined. The poor keeping qualities of pork fat however render the addition of an effective antioxidant desirable, and the majority of European countries now permit the use of one or more antioxidants for this purpose (17).

The keeping qualities of pork fat can be rapidly determined by the Active Oxygen Method. Objections have been raised against this test (7), but it yields serviceable results from the point of view of practical evaluations in the case of animal fats.

An examination of the A.O.M. oxidation curves for pork fat (iodine value 63.8), with and without antioxidants, showed that the initial oxidation period follows a fairly linear course. The actual oxidation rate of the treated lard differs for each antioxidant

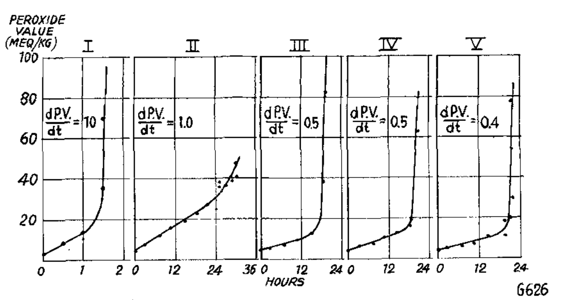

FIG. 1. Effect of various antioxidants on the peroxide formation of lard at 100°C.

I. control
II. 0.02% BHA
III. 0.01% dodecyl gallate
IV. 0.02% BHT
V. 0.01% BHT + 0.005% dodecyl gallate

(Figure 1). This can be expressed by the formula d P.V./dt (P.V. = peroxide value and t = the time in hours).

The curves for dodecyl gallate, BHT, and the mixture dodecyl gallate + BHT are practically identical. The best result is given by the mixture. The curve for the BHA-treated lard is quite different from the others.

If the end-point of the induction period (20 meq) is taken as a standard, then BHA shows the best results. If however the time to reach a peroxide value of 10 is taken as such, the conclusion is quite the reverse. Therefore it is very difficult to compare the effect of BHA with that of BHT and of dodecyl gallate. Nevertheless the progress of the peroxide value within the induction period is not without value and should be considered when investigating a correlation between A.O.M. value and storage tests.

Pork fat was also studied by means of storage tests at normal temperature. Antioxidants were incorporated in freshly rendered suet (iodine value 54.3, peroxide value 0.60), which was stored in sealed jars, each containing 40 g. of fat, at room temperature (approximately 20°C.). The fat was examined periodically, both chemically and organoleptically. The entire contents of a jar were used on each occasion. The course of the peroxide values is given in Figure 2.

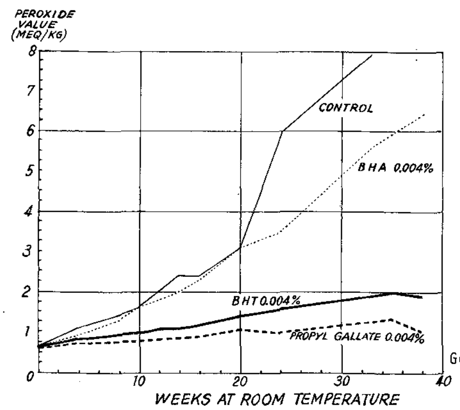

FIG. 2. Effect of various antioxidants on the peroxide formation of lard (I.V. 54.3), stored at room temperature.

During the experiment the peroxide value of the control samples increased from 0.6 to 7.9 in 33 weeks. During the first 20 weeks no influence was found for BHA at a concentration of 0.004%, but later a slightly retarded peroxide formation was observed (P.V. = 6.4 after 38 weeks). An amount of 0.004% propyl gallate however retarded peroxide formation almost completely (P.V. = 1.0 after 38 weeks); BHT at the same concentration exercised a slightly less retarding effect than propyl gallate (P.V. = 1.9 after 38 weeks).

In addition, the ultraviolet absorption of the various fats after 16 weeks of storage was measured in the 253–286 mμ region (Figure 3). Kaufmann *et al.* (8) have noted the effect of refining and autoxidation on the U.V. absorption spectra of pork fat. In the case of unrefined pork fat the curve in the region of 250–300 mμ follows a fairly even course whereas there are definite peaks at 259, 269, and 279 mμ in refined fat because of triene conjugation. As unrefined fat becomes older, the absorption level increases without any clearly defined maxima being found.

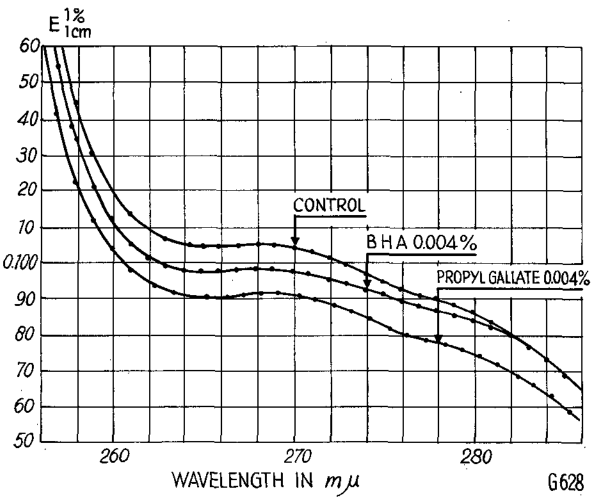

FIG. 3. Ultraviolet absorption of untreated and treated lard after 16 weeks of storage at room temperature.

In the fats examined a clear distinction—completely analogous with the deviations in the peroxide values—was found between the control samples and those with 0.004% propyl gallate; fat containing 0.004% BHA lies between these two. At the end of the 16-week period 95% of the original gallate was recovered.

The organoleptic quality of stored pork fat during the induction period constitutes a separate problem. It would be wrong to regard the organoleptic quality as invariable during the induction period although true rancidity undoubtedly occurs only during the sharp rise in the peroxide value.

After all, the following factors play a vital role in the modification of the organoleptic quality:

a) the natural flavor constituents of the fat. Some of their intensity is lost during the induction period or else their properties undergo a certain modification, resulting in a flat, old flavor;
b) the influence of added antioxidants or other additives on the flavor. Some judges, for example, were able to detect the presence of 0.01% dodecyl gallate in freshly refined coconut oil; and
c) the formation of new flavor substances, generally considered unpleasant, consequent upon oxidation of the lipides.

In our storage tests with lard stored in glass jars, there was a general tendency towards a decrease in flavor quality during the first 16 weeks. Fat treated with propyl gallate evidenced the highest quality and untreated fat the lowest. The antioxidants BHA and BHT were somewhat less satisfactory. After this period the flavor quality of the untreated lard rapidly deteriorated. Even after 38 weeks the propyl gallate-treated samples were qualified as sufficient, followed by the BHT-treated lard. The flavor of the BHA-treated lard was much better than that of the control, in spite of their similarities in peroxide development, but insufficient as compared with that of propyl gallate-treated lard.

Under other conditions however BHT was found to possess considerable advantages over dodecyl gallate. Tests were carried out with lard packed in paper containers and stored at 28–29°C. (17). These tests were initiated, *inter alia,* to compare the efficacy of 0.01% BHT and 0.01% dodecyl gallate.

The induction periods of the fat at 100°C. (Active Oxygen Method) were found to be as follows: control sample, 2,6 hrs.; 0.01% BHT, 21 hrs.; and 0.01% dodecyl gallate, 35 hrs.

The organoleptic quality of samples treated with dodecyl gallate proved to have deteriorated considerably more than one might be justified in assuming from the figures mentioned above. The fat coming into contact with the paper soon became rancid and adversely affected the bulk of the fat; BHT offers good protection against this type of deterioration.

The above-mentioned differences, whereby—depending on the conditions—BHT or gallate gave better results, are arguments in favor of the use of mixtures of antioxidants in addition to known synergistic effects of some antioxidant mixtures. In all cases investigated by us, mixtures of antioxidants yielded identical results or results better than corresponding quantities of the individual constituents.

Finally, special mention must be made of the stability of the peroxides. It is generally assumed that from the moment the peroxides begin to form, dissociation also occurs to a limited extent, leading to the formation of volatile compounds characterized by an unpleasant flavor.

There is the possibility however that these undesirable volatile components are created simultaneously with the peroxides in numerous fats and oils during oxidation and are not disintegration products of peroxides. An argument lending support to this theory is that the correlation between the peroxide value and flavor evaluation figure is often poor, as in the case of vegetable oils. Another argument is the great stability of the peroxides, which was observed in butter fat and pork fat.

The butter fat was obtained by melting butter which had become "fishy" in cold storage, after which 0.01% octyl gallate was added to the fat. On heating this fat to 100°C., while CO_2 was passed through, the following figures were yielded:

Duration of heating	Peroxide values	
(hours)	Untreated fat	Fat incorporating 0.01% octyl gallate
0	2.5	2.5
½	2.6	2.2
1½	2.4	2.5

No indication was obtained regarding dissociation while octyl gallate produced no catalytic reaction. The trials with pork fat were carried out in consultation and cooperation with S. H. Bertram (The Hague).

American pork fat (iodine value 63.1) was oxidized at 100°C. by the Active Oxygen Method to peroxide values varying between 25 and 280 meq/kg. The air supply was then stopped; part of the tubes were heated further while nitrogen was passed through another part (Figure 4).

The results show that dissociation of the peroxides is governed primarily by their concentration and that fats with peroxide values of 25 up to 50 meq are very stable at 100°C. under both conditions. The slight increase in peroxide value for both the 25 meq fats may be explained by the reaction of the oxygen, still present in the headspace of the tubes without aeration, and the traces of oxygen in the nitrogen, respectively. We hope to report on this aspect in the near future.

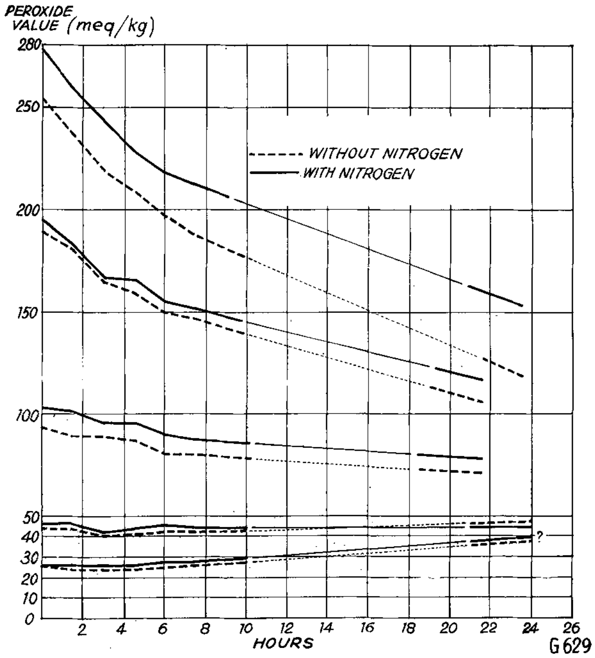

FIG. 4. Effect of heating at 100°C. on the stability of peroxides of American lard (iodine value 63.1) with and without nitrogen passage.

Effectiveness of Antioxidants in Vegetable Oils

Our experiences with antioxidants in refined, nonhydrogenated vegetable oils have been almost invariably disappointing. It is true, that at 100°C. and at lower temperatures, considerable retardation in the formation of peroxides may be found (Figure 5). The tests at 34°C. were carried out as follows. The oils were stored in small glass cups holding 1 ml. The relative humidity of the air above the cups was as a rule regulated by means of a saturated KCl solution (R. H. 85%).

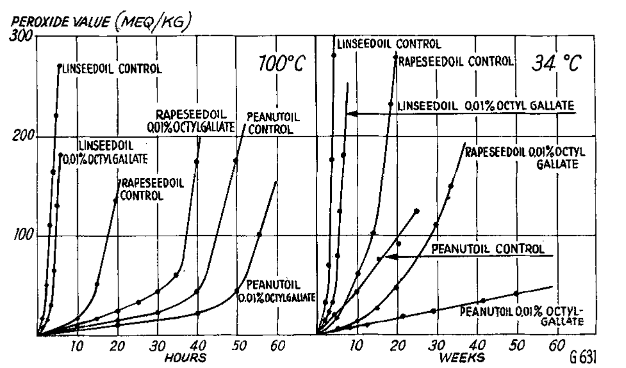

FIG. 5. Effect of octyl gallate on the stability of various vegetable oils at 100°C. and at 34°C.

In this type of experiment the determination of the peroxide value is the only practicable examination method; organoleptic evaluation is automatically ruled out. This naturally detracts from the practical value of the experiment. In addition, the quantity of oxygen, available to the oil for autoxidation, is unlimited. This does not correspond with the conditions under which the oil is marketed. The results might justify the conclusion that gallates exercise an effect in vegetable oils, but storage tests with the products packed in their customary trade packings did not agree with the above findings.

TABLE I

Peroxide Values of BHT-Treated Maize-Germ Oil in Completely Filled Bottles

Oil	Storage time in months				
	2	5½	12	17	24½
Control	0.8	1.3	1.5	24.5	7.7
0.02% BHT	0.9	1.4	9.4	5.3	12.4
0.05% BHT	0.9	1.3	3.6	4.8	5.6
0.01% DG	0.9	1.2	3.5	4.8	4.7
0.01% BHT + 0.005% DG	0.9	1.3	3.0	4.4	8.1
0.02% BHT + 0.01% DG	0.9	1.3	2.8	4.8	8.0

We have already surveyed (14) the results of storage of soybean and rapeseed oils in completely filled glass bottles. In these trials with both oils stored in diffuse daylight and in darkness the practical significance of the antioxidants examined (NDGA, octyl gallate, dodecyl gallate, TETD, and mono-octyl citrate) was negligible.

Further trials with maize-germ oil (iodine value 111), stored in filled and half-filled bottles at room temperature and at 30–32°C., were conducted to determine the efficacy of dodecyl gallate, BHT, and mixtures of these two antioxidants. Considerable attention was paid to the organoleptic evaluations by a taste panel from the factory cooperating in the experiment. In a detailed report already published (16), the antioxidants proved to have low efficacy in half-filled bottles stored at room temperature and none at all in completely filled bottles. A combination of 0.005% dodecyl gallate and 0.01% BHT proved the most effective (Figure 6).

Table I summarizes a few peroxide values of oil packed in completely filled bottles and stored at room temperature.

Antioxidants under these conditions showed no effect. It seems probable that the quantity of available oxygen appears to be of greater importance.

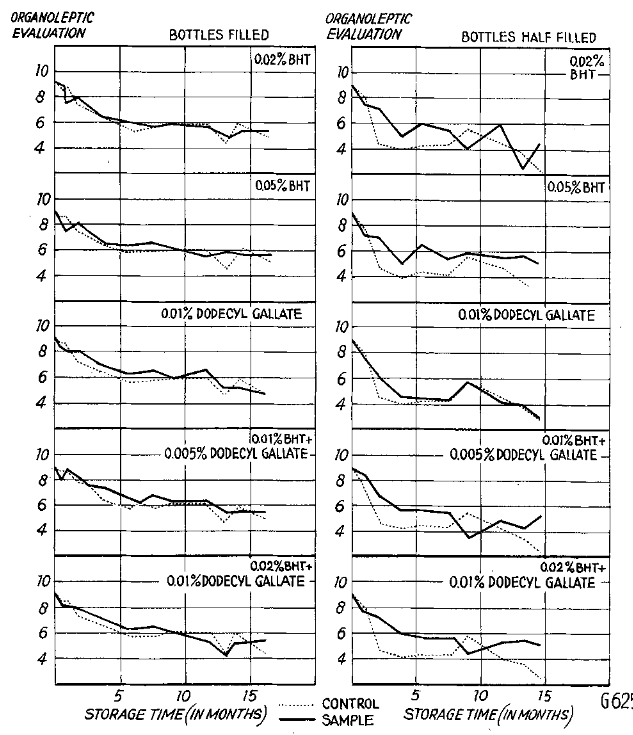

FIG. 6. Effect of various antioxidants on the organoleptic quality of maize-germ oil, stored in completely filled and half-filled bottles at room temperature.

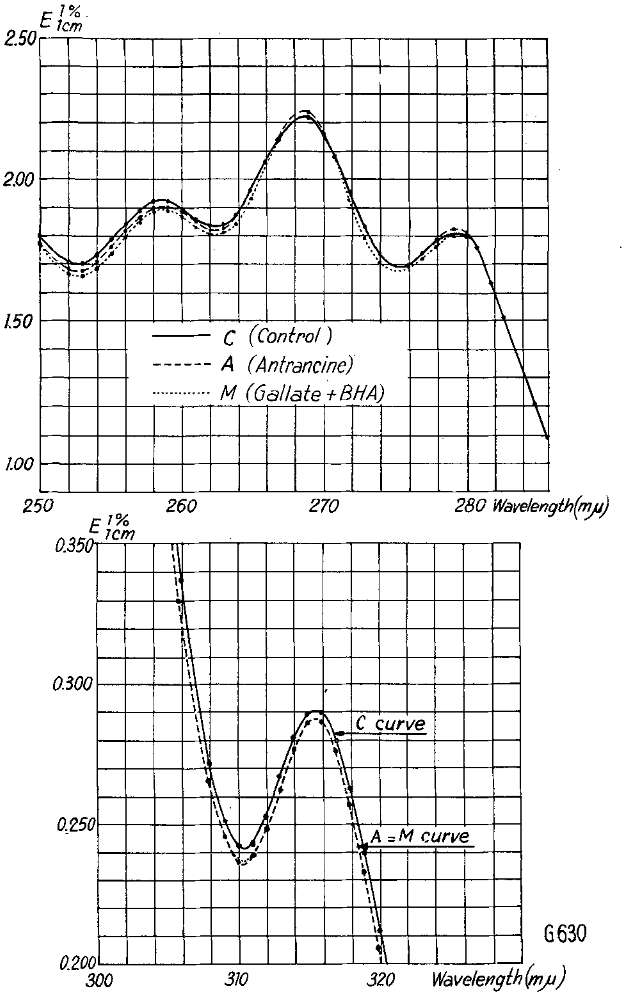

FIG. 7. Ultraviolet absorption of untreated (C-series) and treated (A- and M-series) soybean oil after three and one-half months of storage at 30°C.

Results in connection with the oils stored at 30–32° C. were very disappointing; the use of antioxidants for this purpose has practically no significance.

A new series of investigations was recently initiated with maize-germ oil, peanut oil, soybean oil, and palm oil. All these oils were refined and treated with citric acid during the refining process. The antioxidants incorporated in these oils were a mixture of 0.01% BHA and 0.005% dodecyl gallate (M-series) and 0.04% Antrancine [1] (A-series). In addition, control samples were also examined (C-series).

The oils were partially packed in 1-kg. tins and stored at 30°C.; these tins were opened and analyzed at the end of three and one-half months. The remainder was packed in 180-ml. bottles and stored at room temperature; five bottles of each category were employed. Each bottle was examined three times for chemical and organoleptic qualities. They naturally became emptier during the course of the storage test, which duplicated domestic use in Europe.

The analyses of the tins after three and one-half months of storage at 30° yielded the peroxide values shown in a table.

Oil	Peroxide-values		
	C-series	A-series	M-series
Maize-germ oil	1.6	2.0	2.1
Soybean oil	2.3	2.7	2.5
Peanut oil	2.7	2.6	3.0
Palm oil	3.3	2.1	2.5

Here too the antioxidants had no influence. As far as the flavor evaluation figures are concerned, no correlation was found with the peroxide values. The only oil to react favorably to the addition of antioxidants was peanut oil (flavor scores: control 5, Antrancine 7, BHA + dodecyl gallate 8). For the maize-germ oil and the palm oil the flavor evaluations were inferior to those of the control. With soybean oil the influence of the antioxidants on the flavor was evident although difficult to evaluate. Interestingly, the measurement of the U. V. spectrum (Figure 7) of the soybean oil, stored at 30°C., in contrast to unrefined lard, revealed no difference between the control oil and the M- and A-oils. At the end of the storage period (three and one-half months at 30°C.) 85% of the dodecyl gallate was found back in the M-oil. The result of the experiment at 30°C. is not very encouraging. The same can be said for the oils stored in glass at room temperature. During a storage period of 20 weeks the peroxide value increased on the average from 0.4 to 1.3 with the incorporated antioxidants exercising little effect. This is equally the case with the flavor scores.

On the basis of practical research on vegetable oils for several years the use of antioxidants has proved of such little value to date that there is no justification for their incorporation. Strictly speaking however, this conclusion must be limited to high-grade, refined vegetable oils.

Although the reasons why antioxidants in animal fats yield considerably better results than in vegetable oils falls outside the scope of this paper, we should like to mention these points.

a) Vegetable oils generally have a higher iodine value than animal fats. Antioxidants however exercise a stronger influence on the oxidation of oleic acid esters than on linoleic and linolenic acid esters. Although oxidative rancidity does not constitute a major problem for coconut oil, the positive action of phenolic antioxidants on this lowly unsaturated vegetable oil has been demonstrated in storage tests.
b) Vegetable oils contain many more natural antioxidants than animal fats. The supplementary addition of antioxidants has less point for the vegetable group.
c) Fats as a rule are packed entirely differently from oils; packed fat generally has unlimited quantities of oxygen available.
d) The natural flavor constituents in vegetable oils and fats are entirely different from those present in animal fats and react differently to the addition of antioxidants.

Effectiveness of Antioxidants in Margarine

Margarine in The Netherlands is prepared from a mixture of liquid and hydrogenated vegetable oils and, in many cases, hydrogenated train oil, which are emulsified with an aqueous phase, consisting either of water or else of chemically ripened, skimmed milk or whey. The pH of the aqueous phase is kept low (4.5–5.0) in order to ensure the efficacy of the benzoic acid permitted as a preservative. The aqueous phase contains a minute quantity of copper, corresponding with 50–100 μg/kg. of margarine. The margarine is lightly salted (about 1%) and flavored, *e.g.*, with diacetyl, colored with annatto and vitaminized to 2.000 I. U. per 100 g. with vitamin A concentrate. Depending upon the time of the year, a harder or softer consistency is selected for the mar-

[1] Antrancine: Chemische Fabriek "Naarden," The Netherlands, is a solution of dodecyl gallate, BHA, BHT, and citric acid in hexylene glycol.

garine. It is interesting to note that margarine is solely available in The Netherlands in packages of 250 g.

Microbiological deterioration of margarine is a phenomenon that only occurs sporadically at present and generally manifests itself in the form of soapiness. The flavor of margarine deteriorates slowly during storage. The aroma becomes "flatter" while flavor defects make their appearance. The margarine becomes characterized by an "old" flavor, sometimes by a "paper" or "cardboard" flavor. The peroxide value gradually increases; a value of 5 is to be regarded as particularly high. True oxidative rancidity, like that observed in highly oxidized lard with a high peroxide value, is seldom met with in margarine. After storage for approximately four weeks at room temperature there usually is a distinct deterioration in the organoleptic qualities, therefore the circulation time is limited to this period.

Experiments with margarine, incorporating antioxidants, have generally belied our anticipations.

a) The determination of the induction period at 100°C. of the melted margarine fat had no significance for the evaluation of the keeping qualities of the margarine.
b) A number of antioxidants, like the gallates and NDGA, did actually retard peroxide formation but only exercised a very slight influence on flavor deterioration. A marked influence on organoleptic quality was seldom found (15).
c) BHA scarcely retards peroxide formation in margarine and has little influence on flavor deterioration.
d) The best results have been obtained with a mixture of antioxidants, for example, a mixture of 0.01% BHA and 0.005% dodecyl gallate. The influence of this mixture on the flavor is described as "organoleptic synergy" (17).

Not in all cases however have the same results been obtained with the mixtures. The strongly varying composition of margarine, both with the aqueous as well as the fat phase, has played a role in this respect. The way in which the organoleptic evaluation is carried out also influences the results. We prefer to take a cylindrical sample, including both the top and bottom of the packet. If the external layer is removed and only the internal contents of the packet is used, any eventual influence of the antioxidants is noticed to a considerably lesser degree. This is readily understandable as oxidation and the attendant flavor deterioration first become apparent and are most marked on the external layer of the packet; it is there, too, that the possible efficacy of the antioxidants is most clearly defined.

The results of a recent experiment may be summarized as follows. A mixture of 0.01% BHA and 0.005% dodecyl gallate, or 0.05% Antrancine (which contains almost an identical quantity of primary antioxidants) was added to a quantity of "Votator" margarine on a milk basis. A storage test, including both treated and untreated samples, all packed in packets of 100–150 g., was initiated at room temperature (12–18°C.). The peroxide value and organoleptic qualities were evaluated periodically as previously described. The samples were evaluated blind by two tasters; two of each were tested each time. The results of this experiment are summarized in Figure 8. The curves for the BHA/dodecyl gallate mixture have been omitted as they run parallel with those for Antrancine. The results were found to correspond with "organoleptic synergy" (17). It should be noted that the margarine proved to have excellent keeping qualities at 15°C. and that the effect of the antioxidants on the flavor, although unmistakable, was not great. The influence on peroxide formation

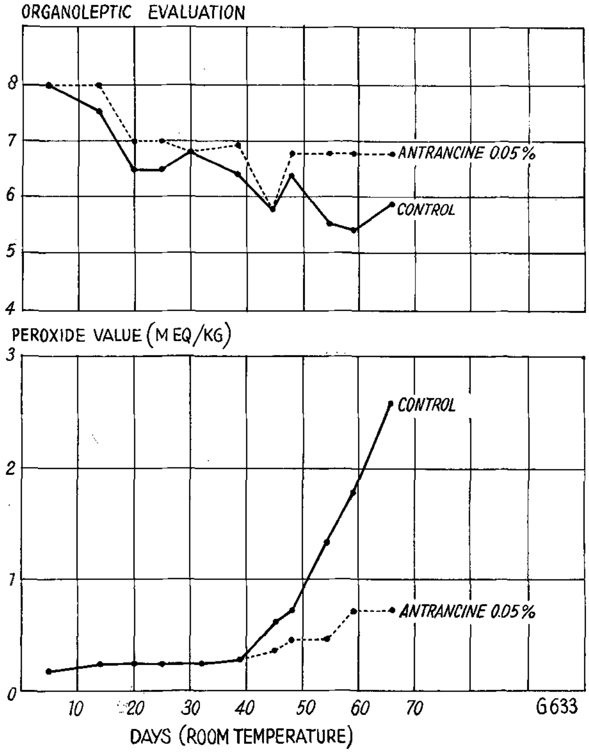

FIG. 8. Effect of Antrancine on the peroxide formation and organoleptic quality of margarine, stored at room temperature.

however was remarkable. At the end of 66 days it was found that the peroxide value of the untreated margarine had risen to approximately 3 as compared with 0.6 in the margarine containing antioxidants.

To ascertain the influence of the composition of the fat and aqueous phases on the activity of the antioxidant mixtures, we shall carry out further testing in cooperation with as large a number of industries as possible.

Effectiveness of Antioxidants in Bakery Products

The following remarks may be based on investigations of antioxidants in bakery products.

a) During storage tests carried out at 20–30°C. it was observed in some cases that the peroxide value, after increasing initially, subsequently tended to decrease again. As the organoleptic quality during storage evidenced a constant tendency toward deterioration, the peroxide value is not always a reliable criterion for the evaluation of quality of the product.
b) During organoleptic evaluation it is essential to pay attention not only to any eventual rancidity (this does not rapidly occur, incidentally, in high-grade bakery products) but also especially to the fullness of the flavor. Many products are flavored with vanillin or rasped lemon, for example, and the effectiveness of antioxidants with good carry-over properties, such as dodecyl gallate, BHA, and BHT, on the keeping qualities of the flavor is unmistakable. The extent to which fat oxidation and loss of flavor are interrelated processes is an open question.
c) The quality of the packing of baked products is of the greatest importance in the preservation of the flavor properties. Baked goods rich in fat readily permit a certain amount of this fat to be absorbed by the paper in which the goods are packed; the fat in and on this paper may rapidly oxidize and become rancid so that the flavor of the entire contents of the package is naturally adversely influenced to a high degree. The quality of the paper, in turn, depends to a large extent on the copper content. Investigations have shown that the copper content may vary between 2 and 220 p.p.m., depending on the type of packing paper used.

We have conducted a number of tentative experiments to ascertain the significance of the copper content of the paper and the result of treating the paper with antioxidants in order to study the stability of the fat coming into contact with it. In all these experiments a paper disc was placed at the bottom of a Petri dish, 30 g. of lard were poured on top, and the whole was subsequently sealed off with a similar paper disc. Three wax-coated types of paper, used by the food industry, with copper contents of 1.6, 20, and 113 mg. per kg., were investigated. Each of these types was examined, both in the natural state and after the application of the antioxidants BHA, BHT, and dodecyl gallate in a concentration of 0.05% calculated on the wax. The dishes were stored at 22°C., and the peroxide value of the fat was determined periodically. The total quantity of fat in a dish was used each time. The results are shown in Figure 9.

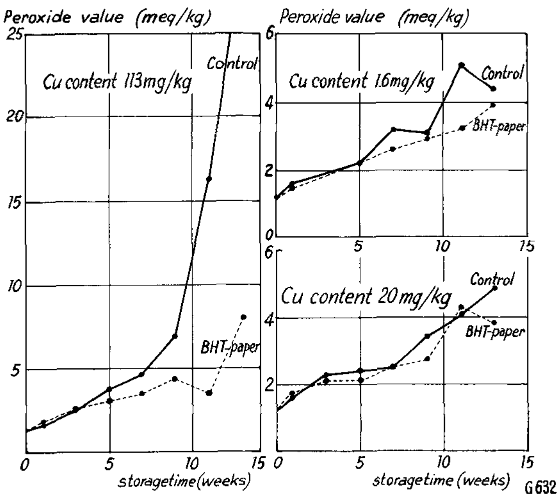

FIG. 9. Effect of copper content and BHT-treatment of packing paper on the stability of lard, stored at 22°C.

Since the antioxidants under examination behaved similarly in the three cases, only the effect of BHT is shown. The antioxidant property is obvious: the paper contained 113 mg. Cu/kg. With the other papers the effect is questionable. There is practically no difference between the papers containing 1.6 and 20 mg. Cu/kg. Our impression is that the beneficial effect of the antioxidants in packing papers strongly depends upon the copper content of the paper.

In recent years extensive research has been carried out on the effectiveness of antioxidants in "botersprits," a kind of Dutch shortbread made with butter. Parts of the results have already been published (17); dodecyl gallate, BHT, and a mixture of these two antioxidants exercised a favorable effect. A recent investigation was initiated as follows:

A dough was prepared from 3½ kg. of flour, 2½ kg. of butter, 1½ kg. of sugar, and 50 g. of salt and vanillin. The dough was divided into two parts: 200 g. of butter fat (obtained by melting 250 g. of butter) were added to one half, and a similar 200 g. of butter fat, containing 300 mg. of BHT, to the other half. Both dough mixtures were deposited on the tray with a biscuit molder and baked for 30 min. at 180–190°C. The resulting shortbread had a moisture content of 1.8% and an equilibrium relative humidity of 45% at 20°C., determined with a Philips lithium-chloride cell (11).

Part of this shortbread was packed in air-tight tins and stored at room temperature. Some of the tins were lined with packing paper (copper content 20 mg./kg.), others with the same paper treated with BHT.

Another part of the shortbread was powdered and stored in glass jars at temperatures of 22, 37, and 55°C. In the trials at 55°C. the humidity of the air in the jars was varied with the aid of saturated salt solutions.

The following humidity percentages were employed: 81% (sat. KCl), 75% (sat. NaCl), 64% (sat. KI), 50% (sat. NaBr), 27% (sat. NaI), and <5% (silica gel), according to Carr and Harris (4); the I- and Br-solutions stabilized with 0.1% Na_2CO_3.

The relation between moisture content and relative humidity of the atmosphere at 55°C. for the shortbread is given in Figure 10. "Keeping tests," at elevated temperatures with bakery products such as shortbread, should be carried out in an atmosphere with a humidity corresponding to that of the product at that temperature. This point generally has been neglected in the literature dealing with the accelerated testing of bakery products (*e.g.*, by means of the Schaal-Oven Test). Figure 10 makes it clear that, to be in accordance with the natural humidity of the shortbread, storage tests have to be carried out at low humidity levels.

The results of the storage tests with powdered shortbread at 55°, 37°, and 22°C. as well as with shortbread packed in tins, lined with untreated and BHT-treated paper, at 22°C. are summarized in Tables II, III, and IV.

From these results the influence both of moisture and of BHT is very clear. Peroxide formation is retarded by increasing moisture content both in the control samples as well as in the BHT-treated samples. The same influence of moisture has been found in whole-milk powder. In the case of oils and fats however peroxide formation was found generally somewhat more rapid at a relative humidity of 85% than in a dry atmosphere (14). BHT suppresses peroxide formation in the shortbread to a certain degree, especially at low moisture content.

It is obvious from these data that at 37°C. BHT retarded peroxide formation but that at 22°C. no effect of the antioxidant could be observed. It is possible that the storage time at 22°C. was not long enough to show a beneficial effect from BHT.

During the organoleptic evaluation of the powders stored at 37°C. and 55°C. (silica gel) the effectiveness of BHT was marked by the more powerful flavor of the samples incorporating the antioxidant.

TABLE II

Peroxide Value of Powdered Dutch Shortbread, Stored at 55°C. at Different Levels of Relative Humidity for Two Months

Storage time	Relative humidity					
	Shortbread (control)					
	81%	75%	64%	50%	27%	<5%
(days)						
5	0.5	0.7	0.7	0.7	0.7	0.5
9	0.6	0.9	0.5	0.5	0.7	0.9
15	0.5	0.3	0.7	0.9	1.2	1.2
22	0.6	0.9	0.5	1.0	1.4	1.3
30	1.1	0.4	0.5	1.0	1.2	1.9
40	1.5	1.1	1.3	1.7	2.2	3.1
58	1.3	1.0	1.2	1.5	4.5	4.0
Storage time	Shortbread with BHT					
	81%	75%	64%	50%	27%	<5%
(days)						
5	0.2	0.5	0.6	0.6	0.6	0.6
9	0.5	0.4	0.6	0.5	0.5	0.4
15	0.2	0.2	0.6	0.5	1.0	0.5
22	0.6	0.4	0.4	0.4	0.6	0.8
30	0.4	0.6	0.2	0.5	0.7	0.7
40	0.8	0.7	1.5	1.0	1.0	2.0
58	0.7	1.2	1.0	0.6	1.1	1.8

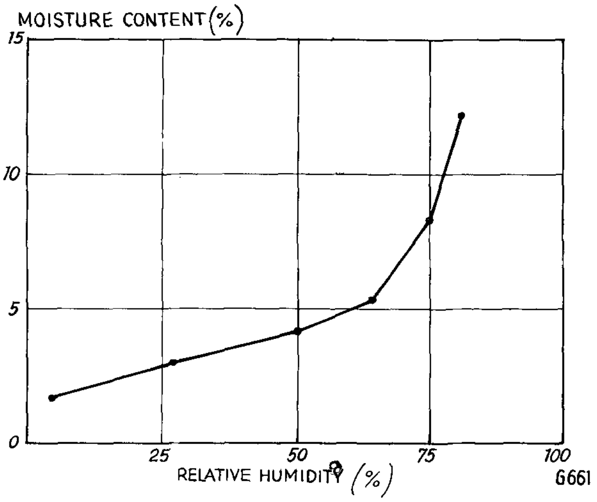

FIG. 10. Relation between moisture content and relative humidity at 55°C. of Dutch shortbread.

TABLE III

Peroxide Value of Powdered Dutch Shortbread, Stored at 37°C. and 22°C., with and Without BHT for Eight Months

Storage time	At 37°C.		At 22°C.	
	Control	With BHT	Control	With BHT
(days)				
30	0.5	0.7	0.7	0.6
58	0.9	0.7	1.5	—
73	1.1	0.6	—	—
159	1.7	1.0	0.8	0.5
252	3.1	1.6	1.0	0.6

TABLE IV

Peroxide Value of Dutch Shortbread, Stored in Tins Lined with Untreated and BHT-Treated Packing Paper at 22°C. for Eight Months

Storage time	Control		BHT-treated shortbread	
	Untreated paper	Treated paper	Untreated paper	Treated paper
(days)				
30	0.7	0.7	0.6	0.6
73	0.9	0.9	1.2	0.6
252	1.3	1.3	0.7	0.7

The vanillin component especially was characteristic. As to the powders stored at 22°C. hardly any difference could be tasted.

Even after eight months of storage all the samples qualified as very good. One may say that BHT, incorporated in the shortbread, had some slightly depressing effect on the formation of peroxides. The similarity between the peroxide values of the samples packed in BHT-treated and in untreated paper indicated that during the storage period BHT had no effect.

From results obtained with shortbread stored under different conditions, the conclusion may be drawn that BHT can afford good protection against peroxide formation as well as against flavor deterioration.

Summary

Investigations on the use of the gallic acid esters, butylated hydroxyanisole, and butylated hydroxytoluene as antioxidants in animal fats, vegetable oils, margarine, and bakery products are described. It was found that the gallates and BHT were highly effective in lard; BHA was of lesser importance.

In vegetable oils scarcely any effect was observed although some antioxidants might be able to suppress peroxide formation in these products; the flavor evaluations however are not always in accordance with these findings.

In margarine it was found that some antioxidants had a promising effect on the peroxide formation but only a slight effect on the flavor. Mixtures of antioxidants however could exercise a synergistic effect on the organoleptic qualities.

In bakery products, *e.g.*, Dutch shortbread, BHT can afford good protection against flavor deterioration.

Mention is made of the fact that the dissociation of peroxides is governed primarily by their concentration and that fats with a peroxide value of 25 to 50 meq/kg. are very stable on heating at 100°C. without air-passage.

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[Received June 26, 1957]

Preparation of 3-Stearoyl-D-Glucose—A Bread-Softening Agent

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BREAD STALING has long been a problem to the baking industry. One important aspect of bread staling is the increase in firming of the crumb, and it is desirable to develop products which will prolong crumb softness and thus shelf life of bread (4). To find more effective softening agents acceptable for use in bread a study was made of the relationship of bread-softening action to the chemical structure of compounds derived from glucose (6). Of the products synthesized and evaluated, the most promising was 3-stearoyl-D-glucose. This substance was found to have a high crumb-softening action comparable to that of polyoxyethylene monostearate (4), one of the most effective bread softeners available. Improved machinability of doughs and increased water absorption of doughs, both desirable properties