to keep out dust and prevent spilling of the mercury.

Preparation of Sand. For some purposes it appears preferable to have the fat spread over a support rather than in a single layer on the bottom of the flask. For this purpose glass-makers' sand has been used, sized by sifting through silk screens and selecting the portion that passes a No. 1XX screen (opening, 0.0157 inch) and is retained on a No. 6XX screen (opening, 0.0092 inch). This is approximately equivalent to selecting the portion that passes a 40-mesh testing sieve and is retained on 60-mesh. Metal screens should not be used since they can contaminate the sand with pro-oxidant material.

Discussion

End-point of the Test. This is placed at 3 ml. oxygen absorbed per gram of fat in preference to a smaller quantity so that the rate of absorption at the end of the test will be relatively rapid. Thus errors introduced by changes in barometric pressure occurring during the test will be smaller than they would be if the end-point were taken at 1 ml. O_2 per gram. If a much greater absorption is taken as end-point, it sometimes happens that the fall in pressure in the flask, due to absorption of oxygen, ceases before the mercury reaches the contact: this probably is caused by evolution of gaseous oxidation products by the fat. It would therefore be necessary to complicate the operation by putting an absorbent in the flask to take up volatile oxidation products if the end-point selected were much greater than 3 ml. per gram.

Temperature. A temperature of 80°C. has been found to be convenient. Temperatures much above 100°C. probably are not practical for regular use because deterioration of the oil in the bath would become too rapid.

Reproducibility. It is advisable to run tests in duplicate. Results of individual tests seldom differ by more than 20% from the average result obtained by running a large number of tests on the same fat on a number of different days. There still is room for improvement here.

Representative Results. A few typical results are given in Table I.

TABLE I

Typical Oxygen Absorption Tests at 80°C.
(Hours required for 1 g. fat to absorb 3 ml. O_2 from air)

	Without sand	With oil spread over sifted glass-makers' sand
Salad Oil (winterized cottonseed oil)	70	20
Refined, deodorized cottonseed oil	63, 66	14, 16
Vegetable shortening (unhydrogenated oil base)	59	
All hydrogenated shortening	130	50
All hydrogenated shortening, bulk type	290	125
All hydrogenated shortening (long-keeping type)	Over 400	
Lard. A random sample from market (tests made on 5 different days)	53, 54 48, 50 61, 55 59, 60 58	
Lard. Another random sample from market	26, 27 28, 37	5½ 5¼, 5¼
Purified methyl esters of cottonseed oil	3.8, 3.5	1¼, 1¼
Purified methyl esters of cottonseed oil with 0.01% phosphoric acid added	3.8, 4.0	
Purified methyl esters of cottonseed oil with 0.15% alpha tocopherol and 0.01% phosphoric acid added	58, 65	
Purified methyl esters of cottonseed oil with 0.1% catechol and 0.01% phosphoric acid added	285, 291	
Purified methyl esters of olive oil	17, 17, 20 16, 18	5¼, 5¾, 5¼ 6, 6
Purified methyl esters of long-keeping type of all-hydrogenated shortening	79, 82, 86	13, 15
Same esters with 0.1% gamma tocopherol and 0.01% phosphoric acid added	477, 490	345, 352

Summary

Apparatus and procedure for testing fats by the oxygen absorption method are described. These were developed to reduce the labor required for testing numerous samples. Over a period of years they have given useful service.

Some Aspects of Recent British Studies on Antioxidants*

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WARTIME conditions of food processing, transport, and storage offer many additional problems to those encountered in normal times. One of the major difficulties is that products liable to oxidative spoilage must often be stored for long periods, and protection by gas packing imposes an additional burden on the restricted supplies of tinplate.

This paper will describe and summarize certain aspects of the research work on antioxidants carried out during the war period in Great Britain by a team of workers belonging in part to the Food Investigation staff of the Department of Scientific and Industrial Research and in part to other organizations, notably the Hannah Dairy Research Institute in Ayr, Scotland.

The immediate necessity was to discover, if possible, some effective antioxidant for each type of food requiring protection, such antioxidant also having to be non-toxic at the levels used and free from any objectionable taste or odor. Long range research, for example on the mechanism of antioxidant action, could not be included in such an emergency program

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although some interesting sidelights on this problem were obtained.

The research program set up provided for a) rapid testing of a large number of substances by accelerated tests, b) laboratory trial of the most promising materials on foodstuffs under actual storage conditions, c) commercial trials of antioxidants which appeared to satisfy all the above requirements plus the additional one of being readily available in quantity.

In this paper I should like to discuss the methods used in the first of these projects. The results obtained in actual storage tests with such products as spray dried whole milk powder have already been summarized in a paper given at the Q.M.C. conference on dehydration last February. The substances we desired to stabilize by means of antioxidants were a) fats as such, b) the fat in various foodstuffs, such as dried milk, and c) other readily oxidizable food constituents, of which the carotene in dehydrated carrots may be taken as an outstanding example. Although carotene cannot by any stretch of imagination be included as an oil or fat, the problem of stabilizing it to oxidation is closely related to that of stabilizing a fat, and most substances which protect fat will also protect carotene.

For the rapid testing of a large number of possible antioxidants some form of accelerated test is absolutely necessary. Such tests, however, are open to the criticism that they may give fictitious results, not applicable to storage conditions where the particular accelerating influence is absent. Acceleration is commonly achieved by the use of, for example, high temperatures, irradiation by light, or the catalytic effect of heavy metals. The protective effect is usually measured either by following the rate of oxygen absorption or the rate of formation of some product of oxidation, e.g., fat peroxide. Clearly the onset of rapid absorption of oxygen is a more fundamental index of the end of the induction period than the sudden accumulation of some particular component of a complex series of oxidation reactions. Normally, however, absorption of oxygen at ordinary temperatures, even after the induction period is over, is very slow and difficult to measure accurately compared with the sensitive procedure for measuring, for instance, the amount of fat peroxide present. We believe we have a method, however, for greatly increasing the rate of oxygen uptake at the end of the induction period and also shortening this period which may not be so open to criticism as most. Before proceeding to describe the methods used, I should like to quote a few results to illustrate how deceptive may be the results obtained under certain conditions which accelerate oxidation.

In one experiment a sample of alkali-refined, peroxide-free fish liver oil was blown with air at room temperature and at 100° C. until the same level of peroxide oxygen content had been reached. Two levels of peroxide oxygen were chosen, one twice that of the other. These four samples were then tested for their susceptibility to further oxidation by measuring the rate of oxygen absorption and were found to behave quite differently (Table 1). In each case the product oxidized at 100° C. subsequently oxidized less rapidly than that originally oxidized to the same peroxide value but at room temperature. Admittedly, this was not a check on the adequacy of the peroxide test, in determining the end of the induction period, but it does suggest that there is a difference in the balance of oxidation reactions at 100° C. and at ordinary temperature.

TABLE 1

The Oxygen Uptake at 30° C. of Fish Liver Oil After Oxidation at 100° C. and at Room Temperature.

Pretreatment of oil	Peroxide value after blowing with air	Progress of subsequent oxidation at 30° C.
Blown at 100° (15 min.)	12.7	3.0 ml. absorb 89 cu. mm. in 270 min.
Blown at R.T. (4 days)	13.8	3.0 ml. absorb 156 cu. mm. in 270 min.
Blown at 100° (8 min.)	6.7	3.0 ml. absorb 83 cu. mm. in 300 min.
Blown at R.T. (3 days)	6.2	3.0 ml. absorb 112 cu. mm. in 300 min.

If carotene be dissolved in different solvents and exposed to strong light, the rate of destruction of carotene varies enormously from one solvent to another. In acetone, for instance, the carotene is far more stable than in ethyl acetate. If kept in the dark, however, carotene is much *less* stable in acetone than in ethyl acetate. In considering effects such as this it may be noted that light apparently destroys carotene by accelerating oxidation since the action is inhibited by all typical antioxidants. Further, these substances do not exert their protective action by absorbing certain wavelengths of light, e.g. ultraviolet, since they are quite ineffective if merely interposed between the light source and the carotene solution. Thus, although light-accelerated tests may be used for the rapid testing of antioxidants for carotene, interpretation of the results requires caution, and the findings cannot be assumed to be applicable to conditions in which light is excluded.

ONE method used for the rapid testing of antioxidants for fats was developed by a worker who had previously been studying the role of the muscle respiratory pigments in catalyzing the oxidation of fat in fish held in cold storage. At the time of commencement of the antioxidant work he was conducting experiments in which the effect of haematin on the oxidation of aqueous suspensions of linoleic acid was being investigated. This earlier work led him to believe that the system he was using could very well be adapted to the routine, rapid testing of possible antioxidants. I should like to discuss some of the evidence concerning the nature of this haematin-linoleic acid oxidation process.

If an aqueous emulsion of linoleic acid, or other unsaturated fatty acid, is prepared and shaken with air or oxygen at ordinary temperatures, oxidation is very slow. For instance, at 30° C. 2.9 ml. of a 1.4% suspension of linoleic acid require about 180 minutes to absorb 10 cu. mm. of oxygen. If a mere trace (seven thousandths of a milligram) of haematin is added to this system, 10 cu. mm. of oxygen are absorbed in about five minutes. If the whole course of the oxidation is plotted, it will be found to follow a typical sinusoid curve, with an induction period, a period of rapid oxidation, and finally a period during which the rate of oxidation progressively diminishes. For routine work, the time taken to absorb the first 10 cu. mm. of oxygen has been taken as a measure of the induction period for at this point rapid oxidation has commenced.

If increasing proportions of haematin are added to this system, it is found that the length of the induction period is not affected, but the subsequent rapid

TABLE 2

	Conc. of Haematin 0.21 mg. in 100 ml.		Conc. of Haematin 0.42 mg. in 100 ml.		Conc. of Haematin 0.64 mg. in 100 ml.	
	Time for 0-10 cu. mm.	Time for 10-100 cu. mm.	Time for 0-10 cu. mm.	Time for 10-100 cu. mm.	Time for 0-10 cu. mm.	Time for 10-100 cu. mm.
Antioxidant	(Mins.)	(Mins.)	(Mins.)	(Mins.)	(Mins.)	(Mins.)
None	5.0	9.0	3.9	6.2	3.7	4.8
Quinol 0.11 mg. %	10.3	9.7	9.5	6.2	8.6	5.4
Quinol 0.21 mg. %	13.9	12.5	12.4	6.4	12.3	5.0
Quinol 0.32 mg. %	22.9	12.1	21.5	7.5	21.6	5.7
None	4.9	9.1	3.8	5.6	3.6	5.0
Catechol 0.11 mg. %	25.8	17.0	24.2	10.0	27.3	6.7

oxidation is accelerated and the second stage more prolonged. On these grounds alone one could conclude that, whatever else it does, haematin does not, of itself, catalyze the oxidation of linoleic acid. If antioxidants such as quinol are added to the system, the induction period is prolonged, and increasing amounts of antioxidant afford increasing protection exactly as in other systems. The antioxidants, however, usually have no effect on the subsequent rapid oxidation (e.g. with quinol) although in some cases they may alter the slope of the curve (e.g. with catechol). Typical results are shown in Table 2, in which the time taken to absorb the next 90 cu. mm. of oxygen after the induction period indicates the rate of the second phase of the oxidation.

Concerning the mode of action of haematin, the following expermental findings should be noted:

1. Haematin and antioxidants do not react with each other.

2. Haematin, which is quite stable in the absence of the emulsified linoleic acid, rapidly disappears (as shown spectroscopically) during the early stages of the oxidation. No free haematin can be detected during the stage of rapid oxidation.

3. During the third stage of oxidation, inorganic iron can be detected in the mixture. Such iron, if added, has no effect on the course of the oxidation.

4. If a trace of pyridine is added to the mixture, immediate rapid oxidation sets in. Thus the pyridine-haemochromogen, in contrast to haematin, acts as an oxygen carrier for linoleic acid.

5. No rapid oxidation sets in if the linoleic acid is present in solution, e.g., in alcohol, instead of as a suspension. If water is added to such an alcoholic solution, there is a progressive increase in the rate of oxidation, slow at first and reaching a maximum when the linoleic acid is thrown out of solution.

6. Reproducible results are obtained when linoleic acid is free from pre-formed peroxide material—i.e., when the active oxygen content is less than 8×10^{-5} g. of active oxygen per gm. If larger traces of peroxide are present, erratic results are obtained.

The theory which best seems to fit all these facts is that although haematin itself is not a catalyst for the oxidation of linoleic acid, it combines with linoleic acid peroxide as soon as this is formed spontaneously, and the resulting compound is a very powerful oxidative catalyst. Haematin would thus act as a very sensitive indicator of the first formation of peroxide. During the third phase of the oxidation the catalyst itself decomposes, resulting in the slowing down of the oxidation. It should be remembered that the concentration of haematin used (down to 3×10^{-9} gm. mol. per 100 ml. in Table 2) is minute in comparison with the concentration of linoleic acid (5×10^{-3} gm. mol. per 100 ml.), and a mere trace of peroxide would be sufficient to react with all the haematin present. The necessity of employing peroxide-free linoleic acid is thus evident.

The fact that almost no reaction takes place in a homogeneous solution emphasizes the importance of the physical state of the fat as affecting its stability. Presumably the water causes orientation of the fat with the carbon chains exposed to more ready attack. The frequent presence of fat as an emulsion, or at least in association with water, in foodstuffs should be kept in mind. Fat is probably also oriented to some extent in dry products, such as cereals.

The haematin-linoleic acid method is easy to use in routine testing. It is carried out in a Warburg bath with manometers of the type used in research on biological oxidations. The linoleic acid suspension, together with the antioxidant under test (in aqueous solution) are pipetted into the manometer flask, and the haematin solution into the side tube. After the necessary equilibration period the haematin is mixed with the suspension and manometer readings taken at convenient intervals. Full experimental details and discussion have been published (1).

A CONSIDERABLE number of pure compounds, together with many natural antioxidant materials such as extracts of flower petals, have been tested by this method and classified in order of potency. It should be pointed out that, while the results are fully reproducible using the same substrate, somewhat different values are obtained for certain antioxidants if other fatty acids are used, e.g., if a mixture of oleic and linoleic acids is employed. Thus, while quinol has the same protective factor when measured using linoleic acid as when using an oleic-linoleic mixture, catechol is a much more powerful antioxidant for the oleic-linoleic mixture. It seems possible that we are dealing here with differences in the reactivity of the fatty peroxides as affecting the stability of the antioxidants, but more evidence is needed on this point.

A considerable number of possible antioxidants were also tested by the more conventional method of adding them to a fat such as butter fat or lard and following the development of peroxide at 100° C. Many of the results obtained were also correlated with the absorption of oxygen, the bleaching of carotenoid pigment, and the development of tallowy odor and flavor. Some corroborative tests at 37° C. were also run.

As a measure of the length of the induction period all these methods gave comparable results. Uptake of oxygen and development of peroxide followed practically identical courses. On the average, fats with a peroxide value of 2-3 (ml. of 0.002N—thiosulphate per gm.) were beginning to show signs of tallowiness and bleaching, at 5 they were definitely tallowy and about half bleached, and at values above 10 they were intensely tallowy and nearly or quite

white. For routine purposes a peroxide value of 10 was taken to indicate the end of the induction period.

On the whole, the results obtained at 100° C. and 37° C. were in good agreement in placing various substances in order of efficiency as antioxidants. The most marked discrepancy was found with a condensation product of pyrogallol and acetone, which was relatively more effective at 37° C. than at 100° C. Stability of the antioxidant may be a factor here.

Rapid testing of antioxidants for carotene has involved using a light-accelerated method. The carotene, either pure or more generally as a crude extract of carrots, was used as a dilute solution in ethyl acetate, and exposed to uniform light intensity. The antioxidants were dissolved in the ethyl acetate and, as might be expected, showed quite different stabilities and hence different activities from those obtaining in the previously described work. The effect of light on a few of them was quite noticeable and had to be taken into account.

In contrast to the haematin-linoleic acid method, reproducibility was poor in the carotene experiments, and in order to classify compounds in order of activity it was necessary to include a quinol standard as well as a blank in each set of determinations. Values for other compounds were then corrected in accordance with an average value for quinol. In spite of these irregularities there was usually no difficulty in duplicating the relative order in which a series of antioxidants was graded for potency. It was the absolute values which could not always be reproduced.

One of the outstanding causes of variability was the effect of traces of moisture in the ethyl acetate. While moisture alone had almost no effect on the stability of the dissolved carotene, it had a pronounced effect on the activity of certain antioxidants, while having little effect on others. The most outstanding effect of water was noticed with the ethanolamines—mono-, di-, and tri-. In the presence of moisture all these compounds were powerful antioxidants, at least as strong as quinol. This, by the way, is in marked contrast to their ineffectiveness in the usual fat systems and in the haematin-linoleic acid system: In the absence of water monoethanolamine lost most of its antioxidant activity and in some experiments became a powerful pro-oxidant. The other two ethanolamines were also much reduced in activity but never became pro-oxidants.

Certain compounds proved to have an activity quite out of proportion to what one would expect from their structure, and from their activity in other systems. Thus, a synthetic analogue of α-tocopherol, in which the phytyl chain was replaced by a methyl group, was about three time more active than either tocopherol or the various dimethyl tocols. A startling result was obtained with one compound. Resorcinol can be condensed with quinol to give either a monoresorcinyl-quinol or a diresorcinyl-quinol. Resorcinol itself is a weak antioxidant for this system. The monoresorcinyl-quinol is about as active as quinol itself, but the diresorcinyl compound has about 50 times the activity of quinol. This enormous antioxidant activity is not shown in other systems, nor was it possible to detect this effect when the substance was added to dehydrated carrots. This last is another problem however, which, as was pointed out in the paper at the Dehydration Conference, applies to all antioxidants tested on dehydrated carrots.

One or two phenomena noted in these studies are of interest in connection with the chain theory of the mode of action of antioxidants. It is assumed in this theory, and it can frequently be shown to be the case, that the end of the induction period coincides with the destruction of the antioxidant. No one seems to have tested the truth of this with such reported antioxidants as phosphoric acid, and, as I mentioned at the Dehydration Conference in describing our work on the deterioration of dried milk, ethyl gallate, functioning quite well as an antioxidant, did not disappear in this system when the induction period was over. In the carotene experiments it was found that the ethanolamines were completely stable and could be recovered unchanged long after the induction period was over by merely distilling off the ethyl acetate. At the concentrations used, these compounds are volatile in the vapor of boiling ethyl acetate. Such recovered ethyl acetate would exert the full protective effect of the ethanolamine on a fresh lot of carotene.

A RELATED phenomenon is the commonly observed one that certain antioxidants, but not all, can affect the slope of the oxidation curve after rapid oxidation has set in. I have already instanced the difference between quinol and catechol in this respect in the haematin-linoleic acid system. Some marked examples of this were found in the carotene studies, one of the most interesting being a stepwise destruction of carotene invariably observed when the methyl analogue of α-tocopherol was employed. Following a long induction period of complete carotene stability, there was a sudden loss of some color, followed by another period of complete stability, and so on for many successive steps before continuous destruction finally set in.

The phenomenon which is, in my opinion, irreconcilable with the chain theory is the variable effect of concentration on activity. Under the chain theory one would expect a direct proportionality between the concentration of any particular antioxidant and the protective effect obtained. In a great many cases, this is actually found. There are, however, sufficient examples of erratic results to upset any such generalization. In the dried milk experiments quoted at the Dehydration Conference, for instance, it was found that high concentrations of ethyl gallate were no more effective than lower ones. In the haematin-linoleic acid system it was found that the superiority of catechol over quinol was more marked at higher concentrations, since catechol is relatively more active at higher concentrations, while quinol increases in activity with concentration in a regular manner. In tests on butterfat at 100° C., while many substances, including ethyl gallate in this case, exhibited a direct proportionality between protective effect and concentration over the admittedly small range of concentrations tested, other antioxidants showed little increase in effect with increasing concentration. Thus, with the tocopherols, there was a progressive decrease in extra protection afforded with each successive increase in concentration, and 0.04% was less than three times as effective as 0.004%.

The carotene studies afforded some striking instances of variable effects of concentration. For many substances, e.g., quinol, a strict proportionality between concentration and activity could be observed

over a wide range. For other substances, e.g., hexylresorcinol, increasing concentration was almost without effect on the protection afforded, at least over a certain range. A frequently encountered phenomenon was a disproportionately large activity at very low concentrations even when the rest of the activity-concentration curve was normal. One or two substances, however, exhibited a zone of minimal activity, at concentration levels of the order of 0.01%. Either higher or lower concentrations than this exhibited increased antioxygenic effect. The final result of dilution of antioxidant was, of course, a lessening of protective effect so that the activity-concentration curves for such substances exhibited both a minimum and a maximum near the lower end of the activity curve. An example of such a substance is haematoxylin, while the related compound brazilin exhibited a normal activity-concentration curve over the corresponding range of concentrations. Ethyl alcohol, a very weak antioxidant in this system, also showed this effect. Table 4 shows the relation between concentration and activity for a number of compounds.

If the results obtained on the same list of compounds by the three methods are compared, numerous discrepancies are found. Some of these have been noted already, e.g. the disproportionate effectiveness of diresorcinyl-quinol in the carotene-light system, as also the powerful antioxidant properties of the ethanolamines in this system, contrasted with their inactivity in the haematin-linoleic acid system.

A comparison of a number of more conventional substances which were effective antioxidants in all systems tested is interesting. Five such compounds are compared in Table 3. The protective factors for the different systems, at the concentrations shown, indicate how wide in some cases are the differences in effectiveness in these three systems. At a different concentration the order might well be different in any of them. It may be noted that these protective factors are expressed on a strictly comparable basis by calculating them as the conventional type of factor minus one. Thus an inactive material would have a factor of zero instead of the more customary value of one.

It is quite clear that the results obtained in testing for antioxygenic activity in any particular system cannot be assumed to apply to any other system—they may, or they may not. As an amplification of the differences shown in Table 3, it may be mentioned that when tested on a "margarine" (water-in-oil emulsion) made from beef tallow and exposed in thin layers to air, quinol, catechol, and pyrogallol were effective to a decreasing extent in the order given, i.e. still another order of relative potencies. In addition to differences due to the nature and degree of orientation of the fat in the system, the stability of the antioxidants will vary in the different systems, and will presumably be less in the presence of water. An association of fat and water is common in many foodstuffs as already noted.

TABLE 3

Antioxidants in Order of Decreasing Activity in Different Systems.

Haematin-linoleic acid (0.0001%)	Butter Fat at 100°C. (0.01%)	Carotene-light (0.01%)
Catechol (3.7)	Pyrogallol (9.0)	1.5-Dihydroxynaphthalene (8.6)
1.5-Dihydroxynaphthalene (1.6)	Quinol (6.9)	Pyrogallol (7.0)
Quinol (1.2)	1.5-Dihydroxynaphthalene (6.0)	Quinol (2.6)
Pyrogallol (0.8)	Ethyl gallate (5.3)	Catechol (2.1)
Ethyl gallate (0.7)	Catechol (4.0)	Ethyl gallate (1.4)

N. B. Concentrations shown are percentages of antioxidant in total system.

Some fairly consistent findings were observed for each particular system concerning relationships between chemical structure and antioxidant activity. Very few of these could have general applicability, in view of the differences shown in Table 3 and also mentioned elsewhere in this paper. In all systems, introduction of a carboxyl or carbonyl group into the benzene nucleus led to reduced antioxidant activity, and in some instances converted a weak antioxidant into a definite pro-oxidant. Methylation of one hydroxyl group of catechol or quinol does not eliminate antioxidant activity, although in some cases it reduces it. Methylation of both hydroxyl groups destroys the antioxygenic properties. Meta-substituted polyphenols (resorcinol and phloroglucinol) are very poor antioxidants and in the naphthalene series hydroxyl groups in the α position are more effective than in the β.

Before any adequate consideration can be given to the relationship between chemical structure and antioxidant properties, a far bigger range of types of compound must be studied, including hetero-cyclic compounds and aliphatic compounds, as well as homocyclic compounds having other than a benzene or naphthalene nucleus. Most of the data so far available have been obtained on hydroxy- or amino-derivatives of benzene or naphthalene. As an indication of what may be found when other classes of compound are equally thoroughly studied, I may mention that in the carotene-light system many aliphatic compounds show weak antioxidant activity (in addition to the strong effect of the three ethanolamines). Ethylene glycol and glycerol are both very weak but trimethylene glycol is markedly more active. Factors at 0.1% concentration are: glycerol 0.02, ethylene glycol 0.2, trimethylene glycol 2.1.

In contrast to the detrimental effect of introducing a carboxyl group into the nucleus of a phenolic inhibitor, the synergistic effect between certain organic acids and polyphenols is well known. It may be of

TABLE 4

Effect of Certain Compounds at Different Concentrations on the Destruction of Carotene in Solution in Ethyl Acetate.

	Protection factor and concentration (%)					
Antioxidant	0.002	0.005	0.01	0.05	0.1	0.2
Pyrocatechol	.....	2.6	2.1	.....	.....	.....
Quinol	.....	1.6	2.6	9.0	18.3	41.3
Hexylresorcinol	.....	0.2	0.4	1.7	1.9	2.6
ψ-Cumoquinol	1.1	1.6	2.1	.....	.....	.....
Diresorcinylquinol	.....	60.9	120.4	.....	.....	.....
Protocatechuic acid	.....	0.9	1.2	.....	.....	.....
Gallic acid	2.3	2.4	1.7	.....	.....	.....
α-Naphthol	.....	1.9	2.1	.....	.....	.....
6-Hydroxy-2:2:5:7:8-pentamethylchroman	9.1	10.8	21.1	.....	.....	.....
Haematoxylin	.....	3.0	2.2	18.4	44.1	.....
Brazilin	.....	4.0	7.8	16.9	.....	.....

interest to note that this synergistic action was well marked when tested on the carotene-light system.

The work described in this paper was carried out in the main as part of the program of the Food Investigation Board of the Department of Scientific and Industrial Research (Great Britain) and is published by permission of the latter. The work on antioxidants in milk powder was carried out in collaboration between the Food Investigation staff of the Department of Scientific and Industrial Research and the staff of the Hannah Dairy Research Institute, Ayr, Scotland. The full results have been published in more detail in the following papers:

Some Factors Affecting the Control of Oxidative Rancidity. T. P. Hilditch. Chem. & Ind., 1944, p. 67.

A Method for Studying the Effect of Antioxidants on the Oxidation of Aqueous Suspensions of Unsaturated Fatty Acids. A. Banks. J. Soc. chem. Ind., Lond., 1944, *63*, 8.

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Oil and Meal Yields in Peanut Milling

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This paper describes a continuous processing test which was made in a commercial oil mill to determine the nature and amount of the so-called invisible oil loss which has been reported to occur in milling peanuts. Under the conditions of processing of this test run no invisible oil loss was observed.

Introduction

BEFORE the establishment of national programs for crop adjustment and soil conservation most of the domestic peanut crop was used in confectionery and food products. Under these programs there has been a marked increase in the acreage planted to peanuts throughout the Cotton Belt. These programs have, except in the later war years, resulted in the production of peanuts in excess of those which the confectionery and food industries could consume and these surplus peanuts have been diverted to crushers for the production of oil and meal. Under the pressure of wartime economy and notwithstanding extensive increases in the acreage planted in peanuts there has been a strong demand both for edible grade and oil stock peanuts.

In the past the market standards of quality for peanuts have been determined by qualities which adapt them to confectionery and food uses. Under the present wartime system of marketing (1) all peanuts are handled through the Commodity Credit Corporation and those allocated to oil mills are sold to the crushers on the basis of total kernel content, with adjustments in price made on the basis of chemical analysis (2).

Methods for analysis of whole peanuts and shelled stock were devised in 1939-40 by the Peanut Committee of the American Oil Chemists' Society (3) for the evaluation of peanuts in terms of prospective yields of oil and meal. These methods were adopted as tentative by the American Oil Chemists' Society (4) and were incorporated in the rules of the National Cottonseed Products Association (5). In the formula which is used for the calculation of crushing yields from analytical data on whole peanuts, the anticipated oil yield, calculated from the chemical analysis and an assumed oil mill efficiency, is multiplied by a factor of 97% to obtain the available yield of oil. This factor of 97% is called the invisible loss factor. Stated in another way, this means that 3% of the oil which would appear to be obtainable from a given lot of peanuts, based on the analysis of the peanuts, cannot be accounted for in the yield of oil or in the amount of extractable oil left in the meal and hulls.

A difference between the oil yield obtained on milling peanuts and that predicted on the basis of analysis has also been reported by Sethne (6) who discusses in considerable detail the various factors involved. He presented data on the analysis and oil yield of peanuts covering five years of operation on shelled stock in a mill using hydraulic presses of European construction. When the determination of the oil content of the peanuts and the cake were made using ethyl ether as a solvent for extraction, the oil yield difference over a five-year period averaged 0.70 $\pm$ 0.15 in percentage of weight of shelled peanuts milled. However, the use of petroleum ether as a solvent for the oil determinations reduced the oil yield difference to about half this value. When placed on a basis comparable with the A.O.C.S. yield calculation, this would be equivalent to an invisible oil loss of about 1% or an invisible loss factor of 99%.

This so-called invisible oil loss is of considerable importance in processing peanuts for oil and meal, and several suggestions have been made by various agencies and organizations that the factors responsible for this loss should be investigated as a service to the oilseed processing industry and to the producers or growers of peanuts. Since problems of this type form part of one of the projects of the Southern Regional Research Laboratory, such an investigation was undertaken at the first opportunity. The work involved was carried out in cooperation with a very progressive oil miller operating an eight-press cotton oil mill and peanut shelling plant. This mill employed the usual cleaning equipment, such as boll reel and shakers for separating the hay and sticks,

[1] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.