nearly 70% of the tri-glycerides, but less than 5% contains three in the same triglyceride molecule whilst linolenic groups occur (singly) in not much more than 25% of the soya bean glycerides.

Of the soya bean oil glycerides (37%) which remain in solution in acetone at —40°, about 90% contain two polyethenoid groups and about 10% contain all three acid radicals in this form whilst linolenic acid is present (singly) in about 40% of the glycerides; but over 30% of this fraction still contains one saturated acyl group.

Such a solvent-separated soya bean oil fraction would thus appear to be considerably less efficient as a drying oil than certain whole oils such as those of rubber seed or candlenut (lumbang), the latter in turn not possessing so great a linolenic acid content and content of linoleo-linoleno-(tri-unsaturated) glycerides as linseed oil itself.

REFERENCES

1. Suzuki and Yokoyama, Proc. Imp. Acad. Tokyo, *3*, 529 (1927); Hashi, J. Soc. Chem. Ind., Japan, *30*, 849, 856 (1927).
2. Hilditch and Jones, J.S.C.I., *53*, 13T, (1934).
3. Bull and Wheeler, Oil and Soap, *20*, 137, (1943).
4. Kleinsmith and Kraybill, Ind. Eng. Chem., *35*, 674, (1943).
5. Hilditch and Jasperson, J.S.C.I., *58*, 187, (1939).
6. Gunstone and Hilditch, *ibid.*, *65*, 8 (1946).
7. Walker and Mills, *ibid.*, *61*, 125 (1942); *62*, 106 (1943).
8. Gunstone and Hilditch, *ibid.*, *66*, 205 (1947).
9. Dunn and Hilditch (results not yet published).
10. Claesson, Arkiv. Kemi, Min., Geol., *23*, *A*, No. 1 (1946).
11. Hilditch and Shrivastava, Analy. ed., *72*, 1947 (in the press).

A Laboratory Method for Determining the Ability of Antioxidants to Stabilize Fat In Baked Goods[1]

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A LARGE volume of work has been published on the use of a wide variety of compounds to stabilize fats against oxidative rancidity. To a large extent the only property investigated has been the ability of the individual compounds to retard the rate of deterioration of the fat itself, as measured by various organoleptic and chemical tests. Rather recently more attention has been directed toward the development of practical antioxidants which possess such essential characteristics as nontoxicity, stability, lack of odor or flavor, fat-solubility and the ability to stabilize the fat in baked goods (1).

The literature contains relatively few references to the ability of antioxidants to carry their stabilizing effect over into the fat in bakery products. Higgins and Black (1) reported that essentially all of the stabilizing effectiveness of gum guaiac and a large portion of that of nordihydroguairetic acid (N.D.G.A.) were carried through to crackers. Lundberg, *et al.* (2), explained this partial failure of N.D.G.A. on the basis of its instability to the alkalinity of crackers. These authors found the carry-over of N.D.G.A. to be better in pie crusts, which are not alkaline, than in crackers. Tocopherols and propyl gallate have shown very little tendency to stabilize crackers (1).

The reasons for such variations were described in 1936 in a patent by Richardson, Grettie, and Newton (3), who discovered that substituted polyphenols and polyphenol derivatives which were soluble in oil or fat and relatively insoluble in water were effective not only in stabilizing the oil or fat as such but also in retarding the oxidation of the fat after it had been used as shortening in bakery products. Phenols which were relatively soluble in water were said to be extracted from the fat when the shortening is mixed with other ingredients containing moisture; this was especially true if the other ingredients were alkaline in reaction. To our knowledge, no further reference has been made in the literature to this broad concept.

One of the reasons for the scarcity of such publications has undoubtedly been the fact that many laboratories do not have the facilities for testing fats in baked goods. For that reason it was felt that it would be of value to develop a laboratory method for assaying the ability of an antioxidant to stabilize such products. The above solubility concept appeared to offer the most promising basis for such a test, and an investigation was undertaken in that direction. It has been found that the relative partition of an antioxidant between a fat and hot water can be correlated with its ability to stabilize baked goods; those antioxidants which are found almost entirely in the water phase are practically ineffective while those which are not extracted by water but remain in the fat are effective in retarding rancidity in various bakery products.

Experimental

General Method of Partition Analysis. A standard method was used in all the partition analyses. It will be evident in the subsequent discussion that many variations of the method may well be employed; for reliable comparison, however, it is felt that the same partition method should be used throughout any given series of tests. In the present work sufficient antioxidant was added to lard to represent 0.05% concentration, assuming complete solubility. Although the lard was then held at 170° F. for about 2 hours, some of the compounds were not completely dissolved. In all cases the lard was filtered to remove undissolved material.

To a 100-gm. sample of the solution of antioxidant in lard was added 100 gm. of distilled water. The fat-water mixture was heated to the boiling point of water and then held on a steam bath for 15 minutes with intermittent stirring. After being transferred

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to a separatory funnel, the mixture was shaken vigorously to assure solubility partition equilibrium. After settling, the water layer was removed and filtered through a water-wetted filter paper. The fat layer was then extracted with three successive 100-ml. portions of hot 80% methanol or 80% ethanol. The alcoholic extracts were combined, chilled in an ice water bath, and finally filtered, the filtrate then being analyzed for antioxidant content by the methods described below.

From the quantity of antioxidant in the above alcoholic extract and the quantity found in a similar extract of the original lard solution of antioxidant, the proportion of antioxidant remaining in the lard after the hot water partition-extraction could be calculated. Further details and the methods used for the analysis of the various antioxidants are presented in subsequent sections. Table I contains a summary of all partition data as well as the stability data for all samples.

Stability Measurements. 1. The stabilities of the various samples of fat were measured by two methods:

(a) Active oxygen method (AOM) as commonly employed and reported as the number of hours before the fat becomes rancid at 98° C. under agitation with a constant stream of air (4, 5).

(b) An accelerated oven test in which the fats were stored at 140° F. in loosely covered jars and the stability was measured as days of storage prior to the appearance of rancidity.

2. The efficiency of carry-over of the antioxidant effect of the various compounds into baked goods was tested by baking the fats into pie crusts and crackers. The former were found to give more uniform and reproducible results so only a few tests were made with crackers. The stabilities of the baked goods were determined by storing the pie crusts and crackers in jars at two different temperatures, 140° F. and 95° F. The data are based on the number of days prior to the first detection of a rancid odor in the jars and are reported as protection factors.

Antioxidants. 1. Gallic acid (GA) was chosen as representative of the water soluble phenolic antioxidants. Because of the water solubility of GA the fat layer from the original water partition was extracted with three 100 ml. portions of hot water rather than with 80% alcohol as was used for the other antioxidants. The original 100 ml. of aqueous solution from the partition and the 300 ml. combined extract of the fat after partition were analyzed for GA by ultraviolet absorption as measured by a Beckman spectrophotometer according to the method of Mattil and Filer (6). It was found that 93% of the original GA was extracted in the original partition with water, leaving only 7% in the fat. From this it would be presumed that GA would be practically ineffective in stabilizing baked goods, and the experimental data support this presumption (Table I). The minor amount of carry-over which occurred may have resulted from the partial binding and deactivation of pro-oxidants by the GA as well as the slight retention of antioxidant in the fat.

2. For pyrogallol (PG), the second representative of water-soluble antioxidants, it was necessary to acidify the original water phase in order to prevent destruction of PG. For this purpose 1 ml. of glacial acetic acid was added to the 100 ml. of hot water. The method of analysis for PG was based on its ultra violet absorption curve in 80% methanol which showed a characteristic peak at 268 mμ ($E^{1\%}_{1cm.}$ = 76 for the sample used, which was a technical grade. This value obviously will vary with the purity of the material and should not be used as an absolute reference standard).

The fat containing PG was run through the partition analysis and extracted as described with 80% methanol. No tract of PG could be found in the methanol extract; as would be expected, essentially all of the PG could be accounted for in the original water extract. The correlation of the water solubility of PG with its highly inefficient carry-over to pie crusts is evident from the data in Table I.

TABLE I.

Data Showing Correlation Between Relative Solubilities of Antioxidants in Lard and Their Ability to Carry Their Stabilizing Effects Into Baked Goods When Added to Lard at 0.05% Concentration.

Antioxidant	Per cent retention in lard after partition with water	Stability data as protection factors[1]				
		Lard+antioxidant		Pie crusts		Crackers
		AOM	140°F.	140°F.	95°F.	140°F.
GA	7	7.6	4	1.5	1.4	1
PG[2]	0	6	6	1.5	1.4	....
DMPG	82	3.3	2	3.5	....	1.5
GAT	84	7.3	5	2	5.4	2.5
Guaiac	91	2.6	2	2.5	3.4	....
NDGA	90	8.3	5.6	2	5.4	....

[1] Protection factor is the ratio of the stability of the experimental sample to that of the control. A factor of unity represents no protection; a factor of two indicates that the stability was doubled by the addition of the antioxidant, etc.

[2] PG was added at 0.005% concentration for stability data.

3. The dimethyl ether of pyrogallol (DMPG) is one of the compounds cited by Richardson, *et al.* (3), as being in the class of phenolic derivatives which are sufficiently insoluble in water to enable them to stabilize baked goods. The experimental data support this statement. The analysis for DMPG was based upon its absorption peak at 270 mμ in 80% methanol ($E^{1\%}_{1cm.}$ = 100 for this sample). It was found that 82% of the DMPG originally in the fat remained after partition. This would suggest that DMPG should stabilize baked goods, and the experimental findings show (Table I) that DMPG is about as effective in pie crusts as in lard itself. Its decreased effectiveness in crackers is probably due to alkalinity.

4. Gallacetonin (GAT), a condensation product of acetone and pyrogallol, is another compound cited by Richardson, *et al.* (3) as falling within the scope of their fundamental concept. As GAT did not give any characteristic peak in the ultraviolet region a method for analysis was developed based upon its color reaction with $FeSO_4$ in alcoholic solution. The color intensity increased on standing but appeared to be changing slowly enough after 30 minutes to permit reasonable estimations to be made. The GAT-$FeSO_4$ complex showed an absorption peak at 600 mμ, as measured by a Coleman spectrophotometer, but neither this value nor the intensity of absorption can be considered as reference values inasmuch as the condensation reaction by which GAT is prepared results in a mixture of somewhat variable composition.

Using the above method it was found that 84% of the GAT added to lard remained after water-partition. Correspondingly, GAT was found to effectively stabilize baked goods (Table I).

5. Gum guaiac, an antioxidant patented by Newton and Grettie (7), was found to have a characteristic

absorption peak at 281 mμ which was then used as a basis for analysis ($E^{1\%}_{1cm.}$ for the sample used was 116). After water partition 91% of the original dissolved guaiac still remained in the fat phase. Again this relative insolubility in water correlates well with the carry-over of its antioxidant effect to baked goods (Table I). It should be pointed out in order to explain the apparent discrepancies between these and previously reported stability data for gum guaiac (1) that in the present work the antioxidant was added merely by stirring it into warm lard. In the work of Higgins and Black (1) the gum was incorporated into deodorized lard by means of an acetic acid solution which greatly enhances its effectiveness (8). Failure to recognize this effect may explain why various investigators have obtained unsatisfactory results when using gum guaiac to stabilize lard.

6. N.D.G.A. was also determined on the basis of its absorption curve which peaked at 283 mμ ($E^{1\%}_{1cm.}$ = 188). Again, its 90% retention in the fat phase correlated with its carry-over into pie crusts (Table I).

Discussion

The data assembled in Table I appear to substantiate the hypothesis of Richardson, Grettie, and Newton that phenolic antioxidants which are relatively less soluble in water than in fat on a partition basis such as found in a bakery composition will carry their stabilizing properties into the fat in the baked goods; conversely, those antioxidants which were more soluble in water evidently are extracted from the fat phase and consequently are unable to exert their stabilizing effect in the final product.

On this basis a laboratory test is proposed for the evaluation of the probable baking carry-over of an antioxidant, to be used as a substitute for baking tests when the latter are not feasible or practicable. The test is based upon the partition of the antioxidant between the fat in which it is dissolved and an equal volume of hot water. If, after thorough agitation of the two phases, a relatively large proportion of the antioxidant remains in the fat phase, it can then be assumed that the antioxidant will carry its effectiveness into baked goods, providing, of course, that it is not destroyed by heat or by one of the constituents of the baking composition.

For most phenolic antioxidants ultraviolet absorption spectroscopy appears to be the most convenient method for the determination of the amount of antioxidant in the two phases. When a spectrophotometer is not available, colorimetric analyses may readily be developed, as was shown above for gallacetonin.

Summary

Verification is offered for the hypothesis that the ability of an antioxidant to stabilize the fat in baked goods is a function of its solubility characteristics. Thus, to be effective in baked goods, an antioxidant may not be sufficiently more soluble in water than in fat that it will be washed out of the fat by the moisture in the other ingredients. On the basis of this observation a laboratory method involving the partition of antioxidants between equal amounts of fat and hot water has been developed. Good correlation has been found between this laboratory test and actual bakeshop results.

REFERENCES

1. Higgins, J. W., and H. C. Black, Oil and Soap *21*, 277 (1944).
2. Lundberg, W. O., H. O. Halvorson, and G. O. Burr, Oil and Soap *21*, 33 (1944).
3. Richardson, W. D., D. P. Grettie, and R. C. Newton, U. S. Patent 2,031,069 (1936).
4. King, A. E., H. L. Roschen, and W. H. Irwin, Oil and Soap *10*, 105 (1933).
5. Mehlenbacher, V. C., Oil and Soap *19*, 137 (1942).
6. Mattil, K. F., and L. J. Filer, Jr., Ind. Eng. Chem. Anal. Ed. *16*, 427 (1944).
7. Newton, R. C., and D. P. Grettie, U. S. Patent 1,903,126 (1933).
8. Doegey, J. L., U. S. Patent 2,308,912 (1943).

Report of the Smalley Foundation Committee 1946-1947

A YEAR ago the Smalley Foundation Committee was enlarged to include all types of collaborative work to improve analytical results. As a result it was divided into three sub-committees:

1. Sub-Committee on Oilseed Meal, R. W. Bates, chairman
2. Sub-Committee on Crude Vegetable Oils, A. S. Richardson, chairman
3. Sub-Committee on Oilseed, R. T. Doughtie, Jr., chairman

Each of these sub-committees has worked faithfully in getting out samples and checking results, and it was thought best to allow each sub-committee chairman to make a report of the work of his group at this time. As chairman of the Smalley Foundation Committee, I want to express my thanks and appreciation for the careful work done by each of these sub-committees.

J. J. VOLLERTSEN, general chairman.

WE are presenting herewith the 29th report of the Sub-Committee on Oilseed Meal of the Smalley Foundation Committee of the American Oil Chemists' Society. During these past twenty-nine years considerable progress has been made in the accuracy of the determination of Oil and Nitrogen on cottonseed meal. The results obtained in practically all determinations were slightly higher than last year.

As usual, 30 samples of cottonseed meal were distributed to the collaborators. Last year we recommended that, in order to obtain better results in the determination of moisture, certificates be awarded to the collaborators having the highest and next highest averages in the work for the year.

There are attached to this report five tables indicating the standing in percentage of the members taking part. Table No. I gives the standing of 48 collaborators who reported moisture determinations on all samples. Table No. II gives the standing of 49 collaborators who reported oil results on all samples. Table No. III gives the standing of 52 collaborators who reported nitrogen on all samples. Table No. IV gives the standing of 49 collaborators who reported on oil and nitrogen on all samples. In these tables we have taken into consideration the results of those reports which were received within the time specified in our original announcement of the Smalley Founda-