phorus pentoxide, and recrystallized from the appropriate solvent (see Table I) until the nitrogen content was constant. When high boiling solvents were used, the crystals were given a thorough washing with 95% ethyl alcohol, followed by a few minutes' refluxing in absolute ethyl alcohol.

The crystals were freed of traces of solvent by heating in vacuum for 16 hrs. at 70°C. (4 hrs. at 50°C. for the *o*-nitrophenyliminogossypol). Because the arylimino compounds tend to decompose markedly on exposure to the combined action of light and air, analytical samples were preserved by storing in evacuated ampules at —15°C.

Bis(o-nitrophenylimino)gossypol. A solution of 2.07 g. (four millimoles) of gossypol and 2.76 g. (two centimoles, a 250% excess) of *o*-nitroaniline in 100 ml. of absolute ethyl alcohol was boiled under gentle reflux for 24.5 hrs. The deep red-brown solution was allowed to stand at 4–5°C. for 16 hrs., and the precipitate which formed was collected, washed twice with 15–20 ml. of cold 95% ethyl alcohol, and dried in vacuum, giving 2.2 g. of deep red micro-prisms, m. p. 229.5–230°C. (dec.). On concentrating the filtrate and washings to 30–40 ml., an additional 0.72 g. of product of the same m. p. was obtained. Further concentration of the mother liquors to 10–15 ml. gave 0.032 g. of impure material, m. p. 225–228°C. (dec.). The total yield of crude product was 2.95 g., 97% of theory. The analytical sample, m. p. 234.5–235.5°C. (dec.), was prepared by recrystallizing a portion of the first fraction four times from boiling 1-chloronaphthalene.

It was also possible to obtain a 96% yield of this compound by treating gossypol with a 1000% excess of *o*-nitroaniline (no solvent) for one hour at 80–90° C. and extracting the unchanged amine with boiling absolute ethyl alcohol.

Summary

Seventeen substituted arylimino derivatives of gossypol have been prepared and characterized. Fifteen of these are new compounds. *o*-Nitroaniline gave both a mono- and a disubstituted derivative with gossypol while *p,p'*-methylenedianiline gave only a monosubstituted derivative.

During experiments designed to prevent color reversion in stored, crude cottonseed oils by treatment with various aromatic amines, only *p*-aminobenzoic acid formed an oil-insoluble derivative with the gossypol in the oils.

Acknowledgment

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A Modified Dilatometer for Fast Evaluation of "Solids Content" in Fats

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THE DILATOMETRIC method, noted for its usefulness in phase studies (1), seems to have become increasingly popular in the edible fat industry in recent years. The estimation of "solids content" by this method is made use of in formulation and in hydrogenation control.

The fact that dilatometric methods are time-consuming has been a major drawback in their application in routine analysis of plastic fats. When dilatometry is applied in hydrogenation control, it is of particular importance to cut to a minimum the time required to carry out an analysis. Successful work to this end has been reported in this journal (3), of which one feature was a commendable tempering step introduced in the procedure.

We have made a change in the dilatometer design, which we have found very effective in cutting the time requirements of test operations. Moreover the change was a very convenient and inexpensive one to make since it involves the dilatometer stopper only, and not the bulb itself.

The change resulted in a considerable reduction in the time required to obtain temperature equilibrium throughout the fat sample. This effect results from:

a) Increased dilatometer surface area exposed to the water in the constant temperature water bath.
b) Decreased thickness of the fat body.

Apparatus

We have been using a dilatometer of the volumetric type, with a bulb volume of *ca.* 11 ml., and with a capillary graduation in cubic millimeters.[1] The original 14/20 ground glass stopper has now been replaced by a hollow stopper, made by our glassblower. Figure 1 shows the two stoppers separately, and the open-stopper dilatometer assembled.

The hollow stopper was made from Pyrex tubing with standard ground glass joint. The outside diameter of the stopper is well over one-half of that of the dilatometer bulb. Comparing the original (D_1) and the modified dilatometer (D_2), the ratio of surface area effecting heat transfer is *ca.* 1:1.5. The sample weights are close to 9 and 6 g., respectively. The 9-g. sample (in D_1) has a radius of *ca.* 9 mm. while the 6-g. sample (in D_2) is, roughly, a cylindrical ring of fat, the thickness of which is 3-4 mm.

[1] Manufactured by H. S. Martin, Evanston, Ill.

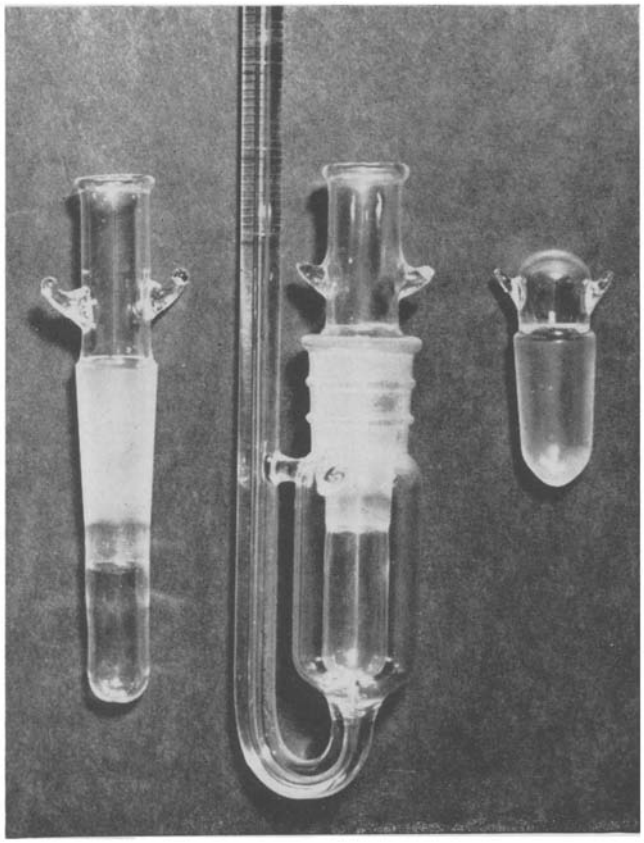

FIG. 1

It is essential that the inside of the stopper be flushed with water from the surrounding water bath. This can be done intermittently, using a hand-operated rubber bulb, or continuously with a mechanical pump. The latter method is preferable; not only is it more convenient, but it is also faster since the maximum temperature gradient is maintained.

Since the water being discharged from the pump is likely to be of a different temperature than that in the bath, we draw the water from the stoppers through glass tubes which extend to the bottom of the stoppers and which are connected through a manifold to the suction of the pump. The discharge water re-enters the bath as far as possible from the dilatometers.

Experimental Comparison Between the Two Dilatometers

Such a comparison was undertaken to establish the merits and demerits of the open-stopper dilatometer in the analysis of our edible fats. Our primary purpose was to cut the time required for the dilatometric method, having its application in formulation and hydrogenation control in mind in particular.

When details of procedures for D_2 and D_1 were worked out for use in our laboratories, the same requirements regarding completeness of freezing, tempering, and over-all reproducibility were applied to both dilatometers. The influence of deaeration conditions, freezing time, tempering time, and tempering temperatures, etc., was investigated, with the aim of determining the necessary and sufficient period of time required for each step in the methods to proceed to an acceptable degree of completeness and to obtain the reproducibility required.

Table I shows our procedures for D_2 and D_1, applicable to margarines and shortenings. Some of the figures are considerably higher than is required for most margarines. Using D_2 it is, for instance, usually possible to obtain constant readings at 15°C. and up within 3–4 minutes of holding time. Naturally the more general a dilatometric procedure is made, the less it conforms to the ideal requirements of each kind of fat. Therefore the figures in Table I give an approximate indication only of the relative time requirements. As illustrated, D_2 is 2 to 2.5 times faster than D_1.

TABLE I

Dilatometric Procedures [a]

	D_2	D_1 [b]
	min.	*min.*
Deaeration, weighing, reference reading	15	20
Freezing time	5	10
Tempering time	15	30
Refreezing time	5	15
Holding time	10	25

[a] Tentatively used for margarines and shortenings in our laboratory.
[b] D_1: The commonly used dilatometer type. D_2: Our modified dilatometer.

This factor does not however apply to every single step in the analysis. In particular, it should be noted that very soft fats may need nearly as long a freezing time in D_2 as in D_1, owing to the relatively greater influence of supercooling upon the solidification process. Such cases are however exceptions in our work, and of little importance.

While the time required for preparation of the sample is the same for the two dilatometers, D_2 will effect temperature equilibrium throughout the sample 2.5 to 3 times faster than D_1.

A special, fast procedure has been worked out for oils being hydrogenated for margarine. A one-point determination of "Solids Content," for instance at 25°C., is completed in about half an hour. Each additional observation takes 5 to 6 minutes, calculations included.

The chief disadvantage with D_2 is, in our opinion, the necessity of flushing the inside of the stopper with water. If this is done by means of a mechanical pump, the extra work is negligible.

Regarding "Solids Content" results that are obtained with D_2 and D_1, these were the same on identical samples, within experimental error, in the few comparable runs we have made. Even if this should not be so with all fats, we do not consider that to detract from the usefulness of the open-stopper dilatometer since none of the currently used dilatometers is claimed to supply anything more than an approximation of the true content of solids in natural fats (2). It is sufficient for practical use that the relative values of "Solids Content" of comparable samples are reproducible. In this respect we have found D_2 to be equal to D_1.

Comments

The open-stopper dilatometer is time-saving but not work-saving. The former is sometimes of primary importance. We would like to carry further the principle of increasing the effective surface of the dilatometer and reducing the thickness of the fat sample. One could think of several ways of doing this, one being simply to make the open stopper slightly larger, and, if one wishes to maintain the size of the sample,

to compensate by making the dilatometer bulb slightly longer.

If the dimensions were chosen so as to make the fat sample smaller, one could reduce the capillary bore in order to maintain the present sensitivity of the dilatometer, which is now about 2.8 and 1.8 mm./1% "Solids Content" for a 9- and 6-g. sample, respectively. The diameter of the capillary bore is well over 2 mm. so there seems to be room for such a reduction.

Summary

A change was made in the dilatometer design, allowing a substantial reduction in the time required to complete a "Solids Content" determination in edible fats.

The modified dilatometer has a hollow stopper that extends nearly to the bottom of the dilatometer bulb, thereby giving the fat sample the shape of a hollow cylinder. The surface area effecting heat transfer is increased, and the thickness of the fat sample is reduced, resulting in a considerable reduction in the time required to obtain temperature equilibrium throughout the fat sample.

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The Antioxidants of the Osage Orange Fruit[1]

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AMONG the natural materials which have been shown to contain potent antioxidants for fats is the mature fruit of the osage orange (*Maclura pomifera* [Raf.] Schneider). Initial work in this laboratory (3) showed that carbon tetrachloride extracts of the powdered dried fruit increased the keeping time of lard by more than one hundredfold; non-crystalline allophonate and acetyl derivatives prepared from the extract were inactive but became active antioxidants after hydrolysis.

In subsequent work (2) osajin and pomiferin were isolated from the extracts. Tests with the pure compounds showed osajin only slightly active while pomiferin accounted for a large part of the total activity. However a non-crystalline fraction contained a synergistic substance. Clopton (1) reached similar conclusions apparently from studies with concentrates since no tests with pure pomiferin were presented.

This paper describes the chromatographic separation of antioxidants from the osage orange extracts. Besides osajin and pomiferin, each of which constitutes 3 to 4% of the dried fruit, a second group of highly active pigments representing 0.5 to 0.75% of the dried fruit has been separated. Chromatography has revealed this group to contain at least eight different components, three of which preponderate in quantity.

Experimental

Methods. Mature fruits from the osage orange tree were collected, sliced, and set in a forced-air oven at 80°C. until thoroughly dry, then stored at room temperature. Before extraction, the material was ground to pass a 1-mm. sieve.

Antioxidant potencies were determined in oven tests at 100°C. with bleached and deodorized lard,[2] low in antioxidants. This lard was received in 25-lb. lots and was stored in 2-qt. glass containers at −18°C. The antioxidant material to be tested was dissolved in chloroform or ethanol, and an appropriate aliquot was added to 10 g. of the lard in a 9-cm. Petri dish. The same amount of solvent was added to a second 10-g. of lard (control). The solvent was evaporated on a steam table, the dishes were covered and placed in an oven equipped with a circulating fan (100 ± 1°C.). A 0.2-g. sample was withdrawn periodically for a peroxide determination, method of Wheeler (4) modified for small samples. At a peroxide number of *ca.* 20 organoleptic evidence of rancidity was generally observed.

Comparison of Solvents for Extracting Antioxidants from the Osage Orange Fruit. To compare the relative effectiveness of different solvents in concentrating the active substances from the plant material, a series of 50-g. samples of the dried meal was extracted in a Soxhlet type of extractor (Table I). Although ethyl acetate, trichloroethylene, and chloroform produced extracts with the highest activity per gram of extract, methanol was the most effective of the solvents tried in extracting the total activity of the osage orange powder.

TABLE I

Comparison of Solvents for Extracting the Antioxidants

Solvent	Yield[a]	Induction period of lard (10 g.) containing	
		100 mg. of solvent-free extract	Total extract from 1 g. of powder
	%	*hrs.*	*hrs.*
Petroleum ether	12.8	50	50
Acetone	22.8	110	175
Carbon tetrachloride	20.4	110	210
Ethyl acetate	23.9	140	210
Ethyl ether	25.5	115	200
Trichloroethylene	18.2	155	230
Chloroform	21.8	155	230
Methanol	51.8	110	350

[a] Percentage, by weight, of total osage orange powder extracted.

Antioxidant Properties of Pomiferin and Osajin. Each of the two compounds was prepared in quantity and purified by recrystallizing twice from xylene and finally from alcohol. The melting points, pomiferin 200° and osajin 189° (uncorrected), agreed well with literature values reported by Wolfrom and coworkers (6).

[1] Journal Paper No. 913 of the Purdue University Agricultural Experiment Station. The subject-matter of this paper was undertaken initially in cooperation with the Committee on Food Research of the Quartermaster Food and Container Institute for the Armed Forces. The opinions or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or endorsements of the War Department.

[2] Generously prepared and supplied by Swift and Company, Chicago, Ill.