to peanut and olive oils both in its general characteristics and composition of fatty acids; hence it can serve to supplement the supplies of the latter.

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Summary

The composition and characteristics of calabash, *Crescentia cujete L.*, seed and oil have been determined. The oil was found to have the following composition calculated from the iodine and thiocyanogen values, and saturated acid content of mixed fatty acids: saturated acids 19.7%, oleic acid 59.4%, linoleic acid 19.3%, and linolenic acid 1.6%. Comparison of the composition and characteristics of calabash seed oil with those for peanut and olive oil indicate that, except for the presence of a small amount of linolenic acid in the former, the oils are similar.

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A Qualitative Method for Detecting Surface Active Agents*

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ABSTRACT

A NEW METHOD is presented for the qualitative detection of small amounts of surface active agents which is generally applicable to all types, i.e. anionic, cationic, and non-ionic. This method is based on the solubilization in aqueous solution of certain oil soluble dyes, particularly Brilliant Oil Blue BMA.

Surface active agents, anionic, cationic, and non-ionic, are finding increasing uses in many products and processes. A large number of surface active agents are commercially available (3, 5, 11, 12, 13). It is often desirable to determine whether or not a product contains a surface active agent, which may be present in a very low concentration.

Very small concentrations of surface active agents, in pure dilute solutions, cause a marked lowering of surface tension, frequently accompanied by persistent foaming, but the presence of soluble extractives from a product under examination for the presence of a surface active compound may also lower the surface tension appreciably and render this criterion valueless.

Methods for the determination of the active component of certain types of concentrated commercial surface active agents are available (2, 6, 7, 9), but these methods do not lend themselves to the detection of small amounts. Methods for the determination of small quantities of sulfonated or sulfated surface active compounds have been offered by Scales (10), Harris (4), and Jones (5), and for the estimation of quaternary surface active agents by Auerbach (1).

These methods give satisfactory results with the types of surface active compounds for which they were developed but are not applicable to all types. A quick and easily applied qualitative test is desirable for the detection of a surface active compound, whether anionic, cationic or non-ionic.

Specification No. 100-A (Jan. 18, 1944) of the Office of the Quartermaster General, U. S. Army, entitled "Soap, Toilet, Soft, Hard or Sea Water," specifies, p. 7, section F-13, the use of a 2% solution of di-ortho-tolyl guanidine in 3% acetic acid as a precipitant to remove the synthetic detergent present in such soaps before determining chlorides. This reagent was tried for the qualitative detection of various surface active agents.

With small amounts of some surface active compounds di-ortho-tolyl guanidine reagent gives an opalescence or turbidity. It has been found in our laboratory that this reagent will detect one milligram of a sulfonate type of surface active compound in 100 ml. of solution. Semiquantitative determinations may be made with a turbidimeter if a reference sample of the known surface active compound is available.

We have found, however, that many surface active compounds do not react with this reagent. Among the compounds which give no reaction with di-ortho-tolyl guanidine are Igepon T, and the related Medialan A, Arctic Syntex M, non-ionic products such as Neutronyx 228, Triton NE, Tween 20, Igepal C, Igepal CTA Extra, Leonil FFO and cationic surface active compounds such as Roccal (lauryl benzyl dimethylammonium chloride), Fixanol, Triton K-60, Sapamine MS conc., Sapamine KW, and Retravon.

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In 1940 during the course of some unpublished work the writer found that small amounts (i.e. 0.5 milligram or less) of alkyl aryl sulfonates could be detected by their solubilizing effects on an oil soluble dye, National Brilliant Oil Blue BMA, di (alkyl amino) anthraquinone.

McBain and Merrill (8) in 1942 reported the solubilizing effects of 1% aqueous solutions of a wide variety of surface active agents on the oil-soluble dyestuffs, Oil Orange OT, and Oil Yellow AB. The degree of solubilization varied considerably with different surface active agents, ranging from 0.5 to 20 milligrams of dye per 100 ml. of 1% aqueous solution. It should be noted, however, that these authors worked with only one concentration of surface active agents, viz. 1%, and did not use this method for the detection of small quantities of surface active agents.

The writer's earlier work with National Brilliant Oil Blue BMA has been extended by applying the solubilizing test to 50 surface active agents including many of commercial importance in the anionic, nonionic, and cationic groups. Several other oil soluble dyes were tried in comparison with Oil Blue BMA, Oil Red O (Colour Index 73), and F. D. & C. Yellow No. 3, i.e., Yellow AB (Colour Index 22) were also found suitable.

The proposed new qualitative method is carried out as follows:

Reagent

Prepare a finely ground mixture of 2 parts National Brilliant Oil Blue BMA and 98 parts of sodium chloride by micropulverizing or ball milling.

(Note: Oil Red O and Oil Yellow AB may be used in place of Brilliant Oil Blue BMA, but are not, in our experience, quite as universally applicable.)

Preparation of Sample

(1) *Dry Materials.* Grind sample to a fine powder if not already in that condition. Digest a 10-gram sample with 50 ml. of hot 50% alcohol* (Formulae No. 30 or 2B) and filter through paper with suction. Evaporate extract to dryness. Digest residue with 3 ml. distilled water by warming on steam bath or hot plate. Filter water extract through a wet filter paper in a 1-inch funnel receiving filtrate in small test tube.

(2) *Liquids or Pastes.* Extract a 10-gram sample with 3 successive 20 ml. portions of an immiscible organic solvent such as benzol, chloroform, or trichlorethylene. Combine the extracts, filter if not clear and evaporate to dryness. Digest residue in 3 ml. of distilled water and proceed as in (1).

Method

Add 0.02 gram of the dry 2% dilution of Brilliant Oil Blue BMA in salt to the water extract from (1) or (2) above. Shake and let stand an hour or more. Compare with a blank of 0.02 gram of the dye reagent in 2 ml. distilled water.

A concentration of 0.5 milligram, often less, of the organic component of surface active agents in 2 ml. of water will solubilize the Brilliant Oil Blue BMA producing a blue solution. The blank test of the same amount of dye in water only will show a faint reddish purple tinge, becoming clear and colorless as the finely suspended, insoluble dye settles.

* Other solvents such as benzol and chloroform may be used if the sample is largely inorganic, or if it is an organic product low in fat content. In testing for the presence of traces of surface active agents in water soluble inorganic mixtures or in organic products containing relatively high proportions of sugars, soluble proteins and other extractives it is impractical, of course, to use water as the initial solvent.

Results

The following table shows the extent to which three oil soluble dyes gave positive tests with 50 surface active agents, in comparison with di-ortho-tolyl guanidine. The surface active agents are identified in the attached list, in most cases by reference to the trade mark under which they are offered.

	Brilliant oil blue BMA	Oil red O	Oil yellow AB	Di-o-tolyl guandidine
Positive	48	44	44	22
Negative	2	6	6	28

The two surface active compounds which gave a negative test with Brilliant Dil Blue BMA were Alframine DCA and the cationic product, Retravon.

If the test is made as above described, the presence of 0.002% Nacconol NRSF in a 10-gram sample can be readily detected.

Soap also gives a positive test by solubilizing all three dyes. If soap is suspected, it may be eliminated by acidulating the sample or an extract from it, filtering without heating through a wet filter and neutralizing. The test can then be applied to the neutralized filtrate.

The principal difficulty in detecting small amounts of surface active compounds in commercial products is the difficulty of extracting them, and the analytical chemist must at times use his ingenuity in selecting a solvent or succession of solvents to obtain the surface active compound as free as possible from interfering substances and in as concentrated form as possible.

List of Surface Active Agents Tested

Aerosol OT	Nacconol NRSF
Aerosol 22	Naccosol A
Alframine DCA	Naccotan A
Alipal D	Neutronyx S
Aquarex D	Neutronyx 30
Cetyl Dimethylamine Oxide	Neutronyx 228
Detergent NE	9-Octadecenyl Dimethylamine Oxide
Detergent NS	Oronite A
Detergent 92	Orvus Paste
Dodecyl Dimethylamine Oxide	Retravon
Dreft	Santomerse No. 1
Fixanol	Santomerse No. 3
Igepal C	Sapamine KW
Igepal CTA Extra	Sapamine MS Conc.
Igepal NA	SV-1959
Igepon AP EXTRA	Syn-O-Tol L Conc.
Igepon T	Tergitol No. 4
Lauryl Trimethyl Benzyl Ammonium Chloride	Tetrone
Leonil FFO	Triton K60
Medialan A	Triton W30
MP 189	Triton 720
Nacconol FSNO	Tween 20
Nacconol HG	Ultrawet
Nacconol LAL	Ultrawet 40B
Nacconol NR	Vel

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For description of the chemical designation of most of the products listed the reader is referred to paper 8 by McBain and Merrill and to paper 12 by Van Antwerpen in the References above; also to the "Handbook of Material and Trade Names" by O. T. Zimmerman and I. Lavine (Industrial Research Service, Dover, N. H., 1946).

Hygroscopic Equilibrium of Cottonseed

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Introduction

ATMOSPHERIC conditions in sections of the cotton-producing area during the harvest season are often such that the seed may contain more moisture than is safe for storage. Such seed may undergo spontaneous heating and rapid deterioration. It is common practice under these conditions to draw air through the seed piles to reduce the temperature and lower the moisture content of the seed. If, however, the air drawn through the seed has a high relative humidity, it is conceivable that the moisture content of the entire seed pile might be increased instead of decreased, thus aggravating rather than mitigating the deteriorative processes. Investigations on the storage and respiration of cottonseed have shown that moisture content is a dominant factor in the biological activity of this seed. Cottonseed of high moisture content respires more vigorously, develops free fatty acids more rapidly, and generally deteriorates to a greater extent upon storage than seed of low moisture content (1, 2). Cottonseed like most other seeds tends to assume a moisture content in equilibrium with that of the surrounding atmosphere. Consequently, the relative humidity, the nature of the hygroscopic equilibrium, and the rate at which it is attained are of considerable importance in determining the storage properties of the seed.

Thornton and Briggs (3) measured the effect of the relative humidity of air circulating through cottonseed on the rate of absorption of moisture. Their experiments were of relatively short duration (165-180 hours) and, except where air of low relative humidity was used, equilibrium was not attained. Thornton and Bishop (4) attempted to use static-air conditions to achieve equilibrium. Here, too, equilibrium was not attained at the end of their experiments (675 hours) and the only conclusion which could be drawn was that the rate of absorption of water in cottonseed increased with rise in temperature. Franco (5) determined the hygroscopic equilibrium of the I. A. 7387 variety of cottonseed grown in the state of Säo Paulo, Brazil. His experiments were conducted at 19°C. and lasted 20 days, the period which was required to attain equilibrium. Simpson and Miller (6) determined the equilibrium moisture content of Stoneville variety of cottonseed by storing samples at 25° C. in air of known relative humidities maintained by sulfuric acid solutions. Moisture determinations were made at the end of 8 and 12 weeks of storage. The results of the analysis after equilibration for 12 weeks were considered to be equilibrium values.

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In this investigation the hygroscopic equilibrium of cottonseed was determined at 26° C. in air adjusted to constant relative humidities within the range 31 to 93%. The distribution of moisture in cottonseed between the kernels and hulls was also determined within the above mentioned range of relative humidities.

Materials and Methods

Saturated solutions of pure salts and salt mixtures provide an excellent means of obtaining a wide range of relative atmospheric humidities in closed spaces. Saturated salt solutions are superior to sulfuric acid solutions for this type of investigation because the former are usually not corrosive and are easier to handle, and because they maintain a constant relative humidity of air in contact with materials of high or low moisture content as long as an excess of the solid salt persists. Spencer (7) lists a number of saturated salt solutions which maintain relative humidities ranging from 30% to 93%. The salts used in this investigation and the relative humidity values of their saturated solutions at 25° C. are listed in Table I.

TABLE I

Relative Humidity of Confined Air in Contact With Saturated Solutions of Various Salts and Salt Mixtures at 25° C.

Salts	Relative humidity
	%
$CaCl_2\,6H_2O$	31
K_2CO_2	43
NH_4NO_3	62
$NH_4Cl + KNO_3$ (mutually sat.)	71.2
$(NH_4)_2SO_4$	81.1
$NH_4H_2PO_4$	93

The cottonseed used in this investigation was D & PL-45 variety from the 1944 crop grown at the Delta Branch of the Mississippi Agricultural Experiment Station, Stoneville, Miss. The seed as received contained 9.55% moisture. Analysis on a moisture-free basis gave: nitrogen, 3.49%; free fatty acids, 0.80%; lipids, 24.32%; ash, 4.17%; and crude fiber, 23.35%. Germination was practically 100%.

Saturated salt solutions, together with an excess of solid salt, were placed in the bottoms of large desiccators, and 200 g. samples of cottonseed were suspended above these solutions by means of wire gauze mats. The desiccators were kept in a room maintained at 26° C. Every few days they were opened to remove duplicate samples of 5-10 g. each which were weighed immediately in closed moisture dishes. All moisture determinations were made by heating the products at 101° C. for 16 hours in a forced-draft oven.

At the end of 36 days a 10-15 g. sample of seed was removed from each humidifying chamber. This sam-