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The Determination of Residual Alcohol in Defatted Alcohol Washed Soybean Flakes

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A method is described for determination of residual alcohol in soybean flakes washed with aqueous alcohol and desolventized to prepare a high-protein food supplement. The amount of alcohol removed from the flakes by extraction with acetone is determined quantitatively by esterification with phthalic anhydride. An excess of reagent is required since water present in the sample causes a secondary reaction which consumes phthalic anhydride. This analysis has been applied to flakes containing from 0.2 to 10% of methanol, ethanol, or isopropanol. Accuracy of the method was established by adding known amounts of the three alcohols to soybean flakes. A relative error of less than 1% was obtained with a standard derivation of 0.05%.

DESOLVENTIZING of hexane-extracted soybean flakes washed with aqueous alcohols is under study for the production of a high-protein food supplement (1).

An analytical method was developed to determine the residual alcohol content of these flakes after desolventization.

A method of determining the alcohol content indirectly has been used at this laboratory with alcohol-washed soybean flakes containing large amounts of both alcohol and water. In this procedure total volatiles are determined by drying the sample to constant weight, then obtaining the water content by the Karl Fischer method (2), and substracting the two values to find the alcoholic content of the flakes. The difference of the results indicates the alcohol content of the flakes. The method proved to be inaccurate for flakes containing 2% alcohol or less, but can be used for estimating the alcohol content.

The alcohol determination as used by the distilling industry was also explored. By this method the alcohol is distilled from the fermented mashes as the water azeotrope. From the specific gravity of the distillate the alcohol content is calculated. This method also

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gave unsatisfactory results because other volatile compounds distilled from the flakes.

Since both methods gave us unsatisfactory results, one developed by G. Verbeck was used (3). This method is based on the esterification of alcohols with phthalic anhydride. Any alcohol present in flakes is separated by refluxing with acetone. This extraction isolates the alcohol and avoids possible interfering reactions of the solids with the reagents in the subsequent analysis. In the presence of hot phthalic anhydride-pyridine solution and acetone, the alcohol reacts to form the half ester.

After the reaction is completed, the remaining phthalic anhydride is hydrolyzed with water, and the total acid content is determined by titration with sodium hydroxide.

Alcohol-washed flakes contain some moisture, which is extracted with acetone. Since the water in turn immediately hydrolyzes the phthalic anhydride-pyridine solution, the amount of reagent used in the determination must be sufficient to satisfy the esterification of the alcohol and the hydrolyzation reaction of the extracted moisture.

A blank is run in which the total amount of anhydride used is hydrolyzed. By difference, the amount of alcohol in the sample is calculated. This method has been successfully applied to the determination of methanol, ethanol, and isopropanol content in defatted soybean meals in amounts varying from 0.2 to to 10.0%.

Apparatus and Procedure

The determination is carried out with an apparatus consisting of a 500-ml. round-bottom flask, a Butt extraction tube, and a water-cooled condenser. After approximating the alcohol and water content, 1-molar phthalic anhydride-pyridine reagent was pipetted into the flask to provide a 5 to 1 ratio of phthalic anhydride to alcohol and an excess to react with the water present (1 mole of phthalic anhydride per mole of water). Fifty milliliters of acetone were then added to the 500-ml. flask. The acetone, an analyzed grade, contained a very small amount of water and methanol.

A 2½ x ⅝-in. glass vial with a bakelite cap was weighed to the nearest 1.0 mg. Approximately 4.0 g. of flakes were added to the vial, and the cap was replaced tightly. After weighing, the vial was wrapped with No. 2 Whatman filter paper, and the ends of the paper were folded over. The round edge of a second filter paper was folded toward the center about ½ in. to give a straight edge. The wrapped vial was rolled into the second sheet with the plastic cap placed away from the folded end. A piece of fat-free cotton was placed in the pocket formed at one end while the other end was folded over.

A heating unit was placed under the flask and the Butt extraction tube assembled. As soon as vapor started to rise into the Butt tube, the vial and cap were broken by a hammer blow. The filter paper containing the crushed vial was inserted into the Butt tube and the condenser placed over the extraction tube. The heat source was adjusted to provide a reflux rate of approximately 20 ml./min. After a 2-hr. extraction period the heat was removed. As soon as refluxing stopped the vial wrappings were removed and 10 ml. of water was added to the reaction flask. The heat was applied again and refluxing continued for exactly 10 min. to hydrolyze the unreacted phthalic anhydride. The flask was removed and cooled to approximately 20°C. before titration to prevent hydrolysis of the half ester by the titrant sodium hydroxide. Five drops of 1% phenolphthalein in pyridine were added to the sample, and it was titrated to a pink end-point with 1.000 N sodium hydroxide. A blank was run simultaneously with the sample. The calculation is shown below:

$$\% \text{ alcohol} = \frac{(B - S) \times N \times M \times 100}{1000\ W}$$

where

B = Blank titration
S = Sample titration
N = Normality of NaOH
M = Molecular weight of alcohol
W = Sample weight, grams.

A chart was prepared (Figure 1) for estimating the quantity of 1 M phthalic anhydride reagent needed in the determination.

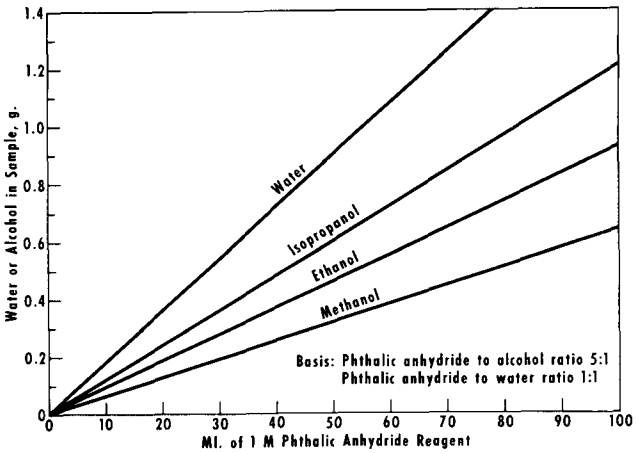

FIG. 1. Phthalic anhydride required for the determination of alcohol in the presence of water.

Results and Discussion

The accuracy of this method was determined by adding known amounts of alcohol to hexane-defatted soybean flakes. The quantity of alcohol recovered was in most cases slightly less than the amount added. This difference may be attributed to incomplete extraction. The relative error was similar for the three alcohols and was less than 1% (Table I). These alcohols are probably subject to hydrogen bonding with the flake protein. Incomplete extraction could also occur if the 2-hr. extraction time were not sufficient. Extraction

TABLE I

Data Showing Accuracy of Method for Three Alcohols

Alcohol	Alcohol added	Alcohol found	Relative error
	g.	g.	%
Methanol:			
Sample 1	0.2082	0.2060	−1.06
Sample 2	0.2500	0.2484	−0.56
Ethanol:			
Sample 1	0.3245	0.3224	−0.65
Sample 2	0.1865	0.1871	+0.32
Isopropanol:			
Sample 1	0.1182	0.1189	−0.59
Sample 2	0.2677	0.2674	−0.11

time was studied over a range of 1–3 hr.; no significant increase in yield was found after 2 hr. Another source of error occurred if the subsequent water hydrolysis was allowed to continue longer than 10 min. (4).

Two samples of flakes—one defatted with methanol, the other with ethanol—were analyzed five times to measure the precision possible with this method. Residual methanol ranged from 0.86 to 0.99% with a standard deviation of 0.05% and ethanol from 1.11 to 1.24% with a standard deviation of 0.05% (Table II).

TABLE II

Standard Deviation of the Results for Methanol and Ethanol

Number of tests	Residual alcohols	
	Methanol	Ethanol
	%	%
1	0.92	1.24
2	0.91	1.21
3	0.86	1.20
4	0.99	1.12
5	0.86	1.11
Standard deviation	0.05	0.05

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Minor Components of Olive Oils. I. Triterpenoid Acids in an Acetone-Extracted Orujo Oil[1, 2]

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Two triterpenoid acids have been isolated from an acetone extracted orujo oil (sulfur olive oil). One of these, amounting to 0.1% of this oil, has been shown to be oleanolic acid, 3β-hydroxyolean-12-en-28-oic acid. The other, amounting to 0.4% of the oil, has not been completely characterized, but is apparently a novel dihydroxy triterpenoid acid.

Introduction

OLIVE OIL is produced in Mediterranean countries by hot-pressing the pulped olives in open presses. The residual pressed cake is subsequently extracted with carbon disulfide, trichloroethylene or some other solvent and a low grade of oil is obtained. This solvent extracted oil is called *aceite de orujo* in Spain and *sulfur olive oil* in English speaking countries. In this series of papers it will be referred to as orujo oil.

The oil remaining in the pressed pulp generally undergoes some enzymatic hydrolysis and oxidation before it is extracted with solvent (1,2). The resulting orujo oil therefore often contains a relatively high proportion of free fatty acids, some oxidation products, and some non-fatty compounds. These impurities in the orujo oil considerably reduce its value as an edible oil. The main outlet for the oil is in the soap industry although a high proportion of oxygenated acids reduces its value because these complicate the technical processing and result in reduced yields of soap.

As orujo oil accounts for some 10% of total olive oil production, a study of those components of orujo oil which are not present in high grade olive oil may be of economic importance. We have undertaken a program of research in which we hope to isolate and characterize some of these minor components. This paper records studies of a crystalline acidic material which was extracted with an orujo oil, and from which we have isolated two triterpenoid acids. One of these is identified as oleanolic acid and the other, although not fully characterized, is shown to be a dihydroxy triterpenoid acid.

Experimental

The orujo oil used in this study was prepared in the laboratories of the Instituto de la Grasa, Seville, Spain, by acetone extraction of the pressed cake of olives which were free of leaves, etc. The oil was mixed with ten times its volume of petroleum ether and the insoluble residue filtered off, washed with water, and dried. This material was a brownish white solid, readily soluble in dioxane or ethanol, but insoluble in petroleum ether or water. It amounted to 0.9% by weight of the orujo oil.

Separation of Components

Thin-layer chromatography (TLC) on Silica Gel G,[4] according to Stahl (3) and Mangold and Malins (4,5), demonstrated the presence of four main components, as shown in Figure 1, sample b. The solvent system used for developing the plate was diethyl ether-hexane (1:1) and spots were made visible by heating after spraying with 50% sulfuric acid (6). Spots 1 and 3, during this heating, gave a color transition of yellow through brown to black, the color sequence given by normal aliphatic compounds such as glycerides and free fatty acids. Spots 2 and 4 had color transitions of violet to black and reddish-purple to

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