basic salts such as sodium bicarbonate, sodium acetate, disodium hydrogen phosphate, and sodium tetraborate. These drying oils may prove useful in paints and varnishes.

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Estimation of Skin Content of Peanut Meals and Relative Skin Pigment Content of Isolated Proteins

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ANY residue of red skins in peanut meals imparts color to them and to the protein products derived from them (1, 2). This color is objectionable to the use of these products for industrial purposes and may be an important consideration in the future use of extracted peanut meal in foods. The pigments in the red skins have been shown to consist predominantly of a catechol tannin and to include small amounts of a leuco-anthocyanin, a phlobaphene, and a flavanone (4, 5, 8, 9). A method has been developed for estimating the skin content of peanut meals and relative skin pigment content of isolated proteins based on a characteristic color reaction of the catechol tannin with hydrochloric acid on heating in solution with ethanol (8). It involves Soxhlet extraction of the pigments with ethanol, their precipitation with neutral lead acetate, and treatment with alcoholic hydrochloric acid to give a red product having a characteristic absorption which can be measured colorimetrically. Details of the method are as follows:

Reagents

1. Ethanol, 95% U.S.P.
2. Neutral lead acetate, 10% solution. Dissolve 50 g. of lead acetate, $Pb(C_2H_3O_2)_2 \cdot 3H_2O$, in 450 ml. of water.
3. Sulfuric acid, 5% solution. Add 5.6 ml. of concentrated sulfuric acid to 190 ml. of water.
4. Hydrochloric acid. Concentrated reagent grade.
5. Petroleum ether, commercial pentane.

Analytical Procedure

Weigh 20 g. of meal or protein, ground to pass a 1-mm. sieve, into a paper extraction thimble (preferably 33×118-mm. size for meals and 33×80-mm. size for proteins). Place a cotton plug in the top of the thimble. Extract the sample in a Soxhlet extractor with 125-150 ml. of 95% ethanol for 3 hours at a rate to provide syphoning approximately every 6 minutes. Concentrate the extract to approximately 30-35 ml., taking care not to concentrate to less than this volume. Transfer the concentrate to a 50-ml. conical-tipped centrifuge tube, rinsing the extraction flask with several small portions of ethanol to give approximately a 40-ml. volume in the centrifuge tube. Centrifuge for 10 minutes at 2,000 r.p.m. and decant the supernatant into a clean 50-ml. centrifuge tube. This centrifugation step may be omitted in the case of proteins.

Add by pipette 5 ml. of 10% neutral lead acetate solution to the ethanol extract. Mix by swirling. Heat the tube in a water bath at 75°C. for 10 minutes with occasional swirling. Remove from the bath and allow to cool for approximately 30 minutes. Separate the coagulated lead salts by centrifuging for 10 minutes at 2,000 r.p.m., decanting and discarding the supernatant liquid. Add by pipette 1.5 ml. of 5% sulfuric acid, stir for several minutes with a slender glass rod to allow decomposition of the lead salts, and then add sufficient ethanol to make a total volume of 15 ml. Wash the walls of the tube with ethanol, rubbing with the rod. Replace the tube in the water bath and heat for 10 minutes, stirring occasionally with the rod. Remove from the bath, rinse off the rod with ethanol, then centrifuge for 10 minutes to remove the lead sulfate. Decant the supernatant through a small filter of medium retentivity paper into a 50-ml. volumetric flask. Wash the filter with several portions of ethanol so that the volume of liquid in the flask is about 25 ml. Pipette 5 ml. of concentrated hydrochloric acid into the flask, mix by swirling, stopper, immerse in the water bath, and heat for 1 hour at 75°C. with occasional swirling. Remove, cool to room temperature, make to volume with ethanol, and mix. At the same time run a reagent blank consisting of ethanol and hydrochloric acid.

Determine the transmittance at approximately 540 mμ. An Evelyn photoelectric colorimeter equipped with a No. 540 filter was used in this investigation.[2] If the final solution is slightly turbid due to the presence of lipids, shake gently in the colorimeter tube with about ⅓ volume of petroleum ether, allow to layer, and determine the color intensity of the lower layer.

Compare the intensity of the color obtained with that of suitable standards in order to estimate the amounts of skins or the relative amount of pigments. In the case of meals these standards can be prepared

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

[2] Mention of trade products does not imply that they are endorsed or recommended by the U. S. Department of Agriculture over similar products not mentioned.

by applying the procedure described above to synthetic meals, containing known amounts of skins from the same or similar peanuts. Standards for proteins can be prepared similarly from proteins of known processing history and characteristics.

Development of the Method

In preliminary experiments the effect of a number of reagents on the alcoholic extracts of peanut meals containing known amounts of skins was investigated. Of these, only alkalis caused appreciable intensification of the color of the extracts. This color intensification was almost as great for skin-free meals as for those containing 5% of the original skin content, indicating that the intensification of yellow color was probably due primarily to some constituents extracted from the peanut endosperm rather than from the skins. The arseno-tungstate method (6) for tannins gave colors which were neither stable nor reproducible.

Since it has been shown that the tannin and leucoanthocyanin of peanut skins can be converted to red products with characteristic absorption (maximum at 548-550 mμ) by heating in alcoholic hydrochloric acid solution (8), the development of such a red color directly in the ethanol extracts of peanut meal was investigated. In contrast to results obtained by other investigators (3) who extracted meals directly with alcoholic hydrochloric acid, brown colors were obtained which were unsuitable for colorimetric evaluation, presumably due to the presence of sugars, proteins, and other constituents. However if the pigments were precipitated from the ethanol extracts as their lead salts, sufficient purification was achieved to give a satisfactory red color on subsequent heating with alcoholic hydrochloric acid. The color produced in this way was fairly reproducible and quite stable, showing no change in intensity after 1 hour and only a slight change after 24 hours. Spectrophotometric curves for the colored solutions produced from several extracts showed characteristic absorption in the region of 525-540 mμ.

In order to establish the optimum period of extraction, several peanut meals and protein preparations were analyzed by the proposed method using extraction times of 1 to 3 hours. The transmittance values obtained are shown in Table I. These data indicate that an extraction time of 2.5 to 3 hours is optimum for both meals and proteins.

TABLE I

Variation of Transmittance Values Obtained by Specified Method With Time of Extraction

No.	Sample	Hours extracted			
		1	2	2.5	3
1	Peanut meal containing 5% of normal skin content	64.5	57.4	.....	52.1
2	Skin-free peanut meal	76.9	78.9	.....	78.2
3	Protein prepared from peanuts with normal red skin content	50.9	24.1	22.2	19.3
4	Same as protein No. 3 except alcohol washed	78.0	71.7	63.9	66.5
5	Protein prepared from lye-dipped red skin peanuts	96.8	92.8	94.3	95.0
6	Protein prepared from white skin peanuts	98.0	90.9	89.3	91.5

The method, using an extraction time of 3 hours, was applied to a sample of skin-free meal and to a series of synthetic peanut meals prepared from skins and skin-free meal so as to contain 1, 2, 3, 4, and 5% of the normal skin content, assuming the normal skin content to be 3%. The transmittance values obtained are shown in Table II. Although the values for the six meals are not entirely in the anticipated order because of the value obtained for the skin-free meal, numbers 3, 4, and 5 are decidedly lower than numbers 1, 2, and 6, indicating that differentiation may be made between 95-97% and 98-99% of skin removal in the preparation of peanut meals. It is recognized, as pointed out by Dangoumau *et al.* (3), that the pigment content of peanut skins varies with type and environment under which the peanuts are grown. Therefore in order to estimate the skin content of peanut meals the intensity of the color developed with alcoholic hydrochloric acid should be compared with that obtained by the analysis of synthetic meals containing skins from the same or similar peanuts.

Transmittance values obtained for a series of proteins of known processing histories are also shown in Table II. Four of these samples were prepared from various grades of red-skinned Spanish peanut kernels which had been lye-dipped to remove a considerable portion of the skin pigments (1). One of these, the protein prepared from lye-dipped oil mill stock which contained damaged and shriveled kernels, was quite dark in color; yet it contained approximately the same amount of skin pigments as each of the other proteins prepared from lye-dipped peanuts. Thus the method might be useful in differentiating between the color of proteins due to skin pigments and that due to other factors such as deterioration. The remaining proteins were prepared from peanuts blanched to remove most of the skins, and from hand-picked, skin-free peanuts, respectively (7). The transmittance obtained for the protein from blanched peanuts approximates those for the first four proteins. As anticipated, the protein from skin-free peanuts had the lowest content of skin pigments, giving a transmittance value of the same magnitude as that obtained for the protein from white-skinned peanuts (Table I). Proteins Nos. 7 through 10, Table II, prepared from lye-dipped peanuts, gave transmittance values approximating 75%

TABLE II

Transmittance Values Obtained by Specified Method for Peanut Meals and Proteins

No.	Sample	Transmittance[1]
	Synthetic meal[2]	
1	1% of normal skin content	83.5
2	2% of normal skin content	77.4
3	3% of normal skin content	67.1
4	4% of normal skin content	53.3
5	5% of normal skin content	52.1
6	Skin-free meal	78.2
	Precipitated proteins from:	
7	Lye-dipped selected sound kernels	75.5
8	Lye-dipped U. S. No. 1 peanuts	76.0
9	Lye-dipped U. S. No. 2 peanuts	75.2
10	Lye-dipped oil mill stock peanuts	74.4
11	Peanuts blanched to remove skins	71.0
12	Skin-free peanuts	91.3

[1] Average of duplicate determinations for meals; single determinations for proteins.
[2] Prepared from oil-free skins and skin-free meal, assuming a normal skin content of 3% in peanut kernels.

as compared with a value of 95% obtained for another protein (No. 5, Table I), previously prepared from a different lot of lye-dipped peanuts. This variation may be due to differences in skin pigment content of peanuts from different sources and treatments.

Summary

A method for estimating the skin content of peanut meals and relative skin pigment content of peanut proteins is described. The method is based on the fact that the pigments consist predominantly of a catechol tannin and related compounds which give a red product with characteristic absorption when heated with alcoholic hydrochloric acid.

Any residue of red skins imparts color to peanut meals and protein products derived from them. Since this color is objectionable to the use of these products for industrial purposes and may be an important consideration in the future use of extracted peanut meal in foods, the method was applied to meals containing known amounts of skins and to proteins of known processing history. The results obtained indicate that the method may be used to estimate the degree of skin removal in the preparation of peanut meals and also to evaluate proteins for skin pigment content.

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Volatile Cleavage Products of Autoxidized Soybean Oil[1]

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THE isolation and identification of volatile substances which, in trace amounts, contribute to the odors and flavors of food products constitute a most difficult field of research. There is no physical or chemical test which can rival the human sense of smell or taste. Research in this field must necessarily involve a many-fold concentration of volatile flavor principles and use of highly developed micro-chemical techniques.

A pioneering step in the study of the flavor problem of soybean oil and of soybean shortening was taken by Daubert and co-workers (9, 12, 14), who collected the volatile materials from as many as 275 successive "reversions" and deodorizations. In this condensate they were able to identify acetaldehyde, α-heptenal, and 2,4-decadienal from soybean oil and maleic dialdehyde, di-n-propyl ketone, and α-heptenal from soybean oil shortenings as their respective 2,4-dinitrophenylhydrazones (DNPH's). Since Daubert's "reversion" procedure for the soybean salad oil consisted of heating the oil to 200°C., it appeared that isolation and identification of odor constituents formed at low temperature would be of considerable practical interest with regard to the storage flavor of soybean salad oil. A further innovation in the work to be described consisted of chromatographic fractionation of the odor constituents upon silicic acid into what has been characterized by experienced taste panel members as "reverted" and "rancid" components prior to formation of the 2,4-dinitrophenylhydrazone derivatives. After another chromatographic separation step the colored derivatives, and consequently the original aldehydes, were identified by melting points, ultimate analyses, and R_f values.

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[2]One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture. Report of a study made under the Research and Marketing Act of 1946.

Materials and Methods

The pentane-hexane used for ultraviolet spectroscopy was prepared by distillation and by passage through silicic acid according to the method of Graff (7). For chromatographic separation of DNPH's purification of the pentane-hexane was by distillation.

Chromatographic grade silicic acid (100-mesh size), procured from the Mallinckrodt Company[3] (Lot 2847, Control Van-1), was mixed with Johns-Manville Super-cel[3] (code A66050) and served as adsorbent.

The absolute ethanol was purified by refluxing over zinc and potassium hydroxide and was distilled through a fractionating column.

Diethyl ether (Baker's (A.C.S.) was used without further treatment.

The stock solution of *m*-phenylene diamine hydrochloride used for the detection of α-β unsaturated carbonyls was prepared by dissolving 1.0 g. of the reagent in 250 ml. of water (16).

Five-milliliter fractions from the chromatographic column were caught in a collector manufactured by the Technicon Chromatography Corporation.[3]

2,4-Dinitrophenylhydrazones were prepared by dissolving the samples in 100 to 150 ml. of aldehyde-free ethanol in the presence of 1 ml. of concentrated hydrochloric acid and 1 to 2 g. of 2,4-dinitrophenyl-

[3]The mention of this product does not imply that it is endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.