A Colorimetric Procedure for the Estimation of Fat Acidity in Peanuts and Peanut Meals

G. S. BAINS, S. V. RAO and D. S. BHATIA, Central Food Technological Research Institute, Mysore, India

Abstract

Modification of a colorimetric method for the estimation of free fatty acids in peanuts and its products is described. The material is extracted with benzene and a measured aliquot shaken with aqueous cupric acetate. The absorbance of the filtered coloured benzene layer is measured spectrophotometrically at 670 mμ.

The concomitant presence of peanut oil in the benzene solution of copper oleate has a negligible effect on the intensity of colour. The procedure can also be used for determining the fat acidity in sesame meals.

Introduction

FAT ACIDITY IN GRAIN is usually determined by extraction of oil with either petroleum ether or benzene followed by titration of the free fatty acids (FFA) in the oil. In the method of the Association of Official Agricultural Chemists (1) extraction is carried out with petroleum ether in a Soxhlet apparatus or with benzene by shaking the contents in bottles, and the FFA titrated in a solution of benzene-alcohol with standard potassium hydroxide. Baker et al. (3) described a rapid method for the determination of fat acidity utilizing a grinder-extracter. More recently Baker (4) reported a colorimetric method. The method is based on the reaction of the fatty acids to form metal soaps. The intensity of the blue colour (in benzene solutions) measured as percentage transmission at 640 mμ gave a linear relationship with the fat acidity. In our laboratory we applied this reaction to determine the fat acidity in peanut kernels and peanut meals. Absorption, instead of percentage transmittance was measured on a Beckman DU Spectrophotometer. Compared to grains, peanuts contain a large amount of oil. It was, therefore, necessary to study the influence of excessive amount of oil in the determination of FFA. Effect of fatty acids other than oleic acid was also studied.

Experimental

Absorption Spectra of Copper Soaps of Fatty Acids. Peanut oil fatty acids are predominantly oleic (56%) and linoleic (26%) with lesser amt of palmitic (8.3%) and stearic (3.1%) (2). Ten ml aliquots of benzene containing 1–8 mg/ml of individual fatty acids were shaken by hand in glass stoppered tubes with 2-ml portions of 5% aqueous cupric acetate. The two layers were allowed to separate and the upper benzene layer decanted through a Whatman No. 1 filter paper. Optical density was measured at different wave lengths (640–690 mμ) on a Beckman DU Spectrophotometer. Stearic acid forms copper soap which is difficultly soluble in benzene, as such absorption spectra of the acid could not be studied. A linear relationship between concn and absorbance of the copper soaps of oleic, linoleic and palmitic acid was observed. Copper soaps of these acids showed maximum absorption at 670–690 mμ.

Results obtained with oleic acid are presented in Figures 1 and 2.

Analysis of Peanut Kernels or Meal. 5 and 20 g of ground peanut kernel and meal were mixed, respec-

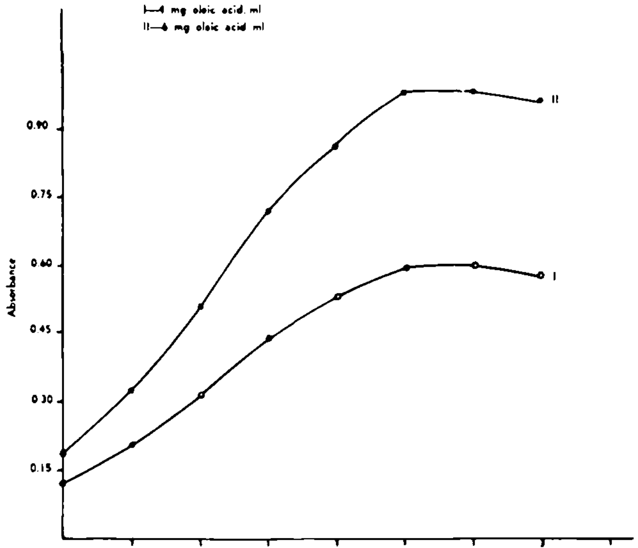

FIG. 1. Effect of oleic acid concn on the absorption spectra of copper oleate in benzene.

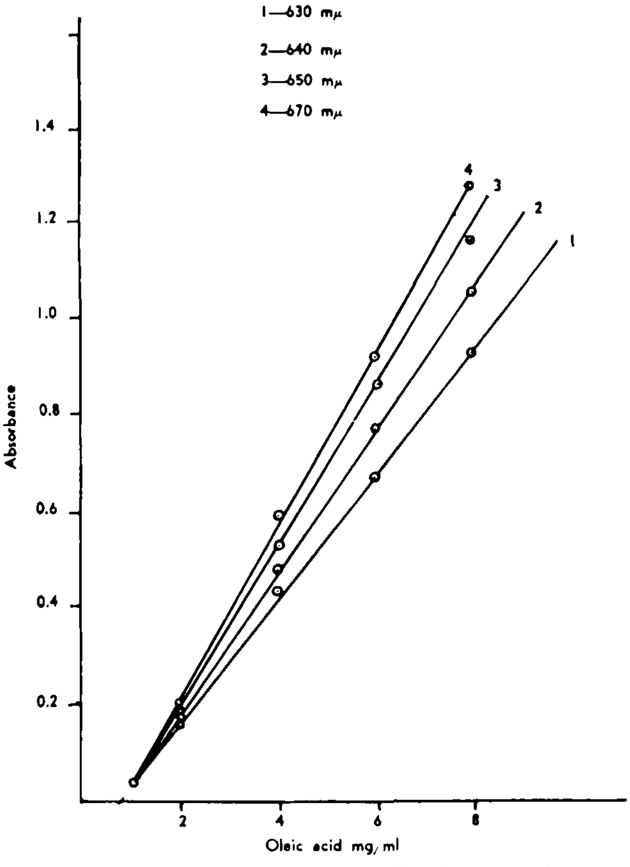

FIG. 2. Relation between the concn of oleic acid and absorbance.

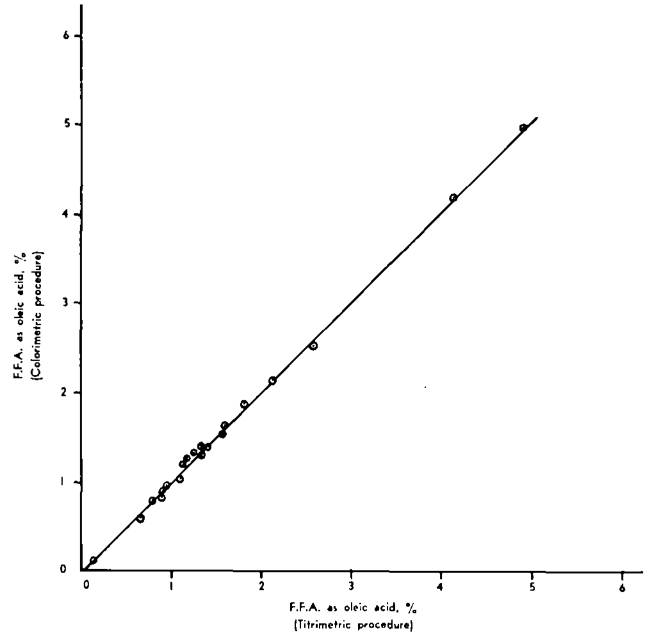

FIG. 3.

tively, with 25 and 50 ml of benzene in stoppered bottles. The mixture was shaken periodically for 45 min by hand. The contents were then centrifuged at 3500 rpm. A 5–10 ml aliquot was taken from the upper benzene layer for colour development with aqueous cupric acetate. Assay was carried out on a Beckman DU Spectrophotometer at 670 mμ using oleic acid as standard.

Effect of Excessive Amounts of Oil in the Benzene Extract. Peanut kernel and cake, when extracted in the manner described, yield ca. 80 and 20 mg/ml of oil, respectively. The meals also contain a considerable amt of the testa of the kernel. The effect of the excessive amt of the oil on the reaction was studied by adding 0, 20 and 80 mg of oil to the benzene solutions of oleic acid (3 and 6 mg/ml), prior to reaction with aqueous cupric acetate. Testa (5 g) was extracted with 50 ml of benzene. To 10 ml extracts, were added 30 and 60 mg oleic acid for reaction with aqueous cupric acetate. Neither the excessive oil, nor testa extracts had any appreciable influence on the colour intensity of the copper-oleic acid soaps.

It was observed that the copper soap solutions in benzene were sometimes turbid even after filtration. This presumably is due to the excessive moisture in the benzene layer which was not adsorbed by the filter paper. Addition of ethyl alcohol (0.5 ml) to 5 ml filtered benzene soap solution gave clear extracts which could be read on the spectrophotometer. Addition of alcohol did not influence the development of colour of oleic acid soaps.

A mixture of oleic, linoleic, palmitic and stearic acid (28,13,4.2 and 1.6 mg/10 ml benzene) was used to determine the influence on the colour development of fatty acids as compared with an equivalent quantity of oleic acid alone. The proportion of oleic to remaining fatty acids, viz., linoleic acid etc., was chosen on the basis of the approx fatty acid composition of peanut oil. The intensity of the colour was found to be proportional to the amt of total acidity (as oleic acid) in the mixture.

Comparison of the Colorimetric and Titrimetric Procedures. A number of samples of decorticated peanut kernels and of commercial peanut meal were extracted with benzene in the manner described. Suitable aliquots of the extract were taken for simultaneous estimation of FFA as oleic acid by the present method and the titrimetric method described by Association of Official Agricultural Chemists (1). There was a close agreement in the values obtained by the two methods (Fig. 3).

Discussion

Although the grinder was not used, it is believed that the extraction technique described by Baker et al. (3) can be applied to the peanut products.

Using the Beckman DU Spectrophotometer, it has been found that the maximum absorption by the copper soap solutions in benzene was in the range of 670–690 mμ. The absorbance in this range is considerably higher (Fig. 2) than at 640 mμ suggested by Baker (4). Since there was not much difference in the absorptions at 670 and 690 mμ, respectively, the assays were carried out at 670 mμ.

As the intensity of colour of mixed copper soaps comprising the major fatty acids of peanut oil was nearly the same as that of an equivalent quantity of oleic acid, the method is suitable for determining the fat acidity of peanut products; the added advantage is that the colour is quite stable.

The concomitant presence of peanut oil in amt of 20–80 mg/ml usually encountered in benzene extracts of peanut products showed negligible effect on the intensity of colour of copper-oleic soaps dissolved in benzene. It was also observed that the benzene extracts of testa did not cause any interference in the method. The extracts of decorticated peanut products are lighter hue as compared with the yellow coloured extracts of cereals studied by Baker (4) and therefore absorbance can be measured at a higher wave length such as 670 mμ. In the case of coloured extracts, blanks are prepared by using 2 ml water in place of cupric acetate, filtered and clarified with alcohol before recording absorbance for correction.

There is a linear relationship between concn and absorbance of copper-soap solutions of oleic, linoleic, and palmitic acids, respectively, when present in amt of 1–8 mg/ml of benzene. Below 1 mg/ml of the fatty acid, there is no stoichiometric relationship between concn and absorbance. Copper-stearic acid soaps by themselves cause turbidity when dispersed in benzene, but when present along with linoleic acid, oleic and palmitic soaps, the solutions are clear. This may be ascribed to the intersolubility of different soaps in benzene.

The average fat acidity value (as oleic acid) of 20 different samples determined by the modified colorimetric method was 1.58 as compared with 1.56 obtained by the titrimetric procedure. The difference in the mean values of fat acidity was statistically insignificant.

Similar close agreement of fat acidity values determined by the proposed colorimetric method and by the A.O.A.C. method has been observed in the case of sesame meals.

ACKNOWLEDGMENTS

Keen interest in the investigation by V. Subrahmanyan; technical assistance from Shri Haridas Rao.

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[Received May 6, 1963—Accepted August 10, 1964]