TABLE II

Melting Points

Wax Sample	Melting Point, °C.	
	Determined with Refractive Index	Determined from Cooling Curve [a]
Refined beeswax	65.0	65.5
Candelilla wax	69.7	69.5
Paraffin wax	51.0	51.5
10% Beeswax—90% Paraffin Wax	51.2	51.0
30% Beeswax—70% Paraffin Wax	53.0	52.8
50% Beeswax—50% Paraffin Wax	59.0	58.5
70% Beeswax—30% Paraffin Wax	61.0	61.0
90% Beeswax—10% Paraffin Wax	63.0	62.5
10% Beeswax—90% Candelilla Wax	68.0	67.5
30% Beeswax—70% Candelilla Wax	67.5	66.0
50% Beeswax—50% Candelilla Wax	67.0	66.0
70% Beeswax—30% Candelilla Wax	66.0	65.5
90% Beeswax—10% Candelilla Wax	66.0	65.0
10% Paraffin—90% Candelilla Wax	69.5	68.0
30% Paraffin—70%Candelilla Wax	67.0	66.0
50% Paraffin—50% Candelilla Wax	60.5	60.0
70% Paraffin—30% Candelilla Wax	56.0	55.0
90% Paraffin—10% Candelilla Wax	52.8	52.2

[a] Method D 87-37 for A.S.T.M. melting point.

literature are: beeswax, 61.0–65.0°C.; paraffin, 50.0–51.3°C.; candelilla, 65–69.0°C.

The melting points of the wax mixtures showing ideal behavior with respect to refractive index values lie between the melting points of the individual components. The non-ideal solutions studied do not show this linear relationship.

Studies of possible chemical interaction of these binary wax mixtures in terms of changes in physical properties are being extended to include the effect of mixing on surface tension, viscosity, freezing point lowering, light absorption, and fluorescence.

Summary

The greatest deviations in refractive index from ideal behavior of the binary wax mixtures are obtained with mixtures of vegetable waxes. These are waxes with higher unsaturation, waxes containing a higher percentage of hydroxy esters, and waxes containing glycerides or combinations of these.

Melting points and refractive indices at the melting points can be determined simultaneously with the Abbe-56 refractometer.

Acknowledgment

These studies are being made possible by a grant from the Research Corporation, New York. We wish to acknowledge our gratitude to the corporation. The authors wish to thank Miss Lois Power for technical assistance.

REFERENCES

1. Bennett, H., "Commercial Waxes," Chemical Publishing Company Inc., Brooklyn, N. Y. (1944).
2. Dobran, J., Acker, M. M., and Frediani, H. A., J. Am. Pharm. Assoc., *38*, 495 (1947).
3. Findley, T. W., and Brown, J. B., J. Am. Oil Chemists' Soc., *30*, 291 (1953).
4. Jasper, J. J., "Laboratory Methods of Physical Chemistry," Houghton-Mifflin Company, N. Y., p. 84 (1938).

[Received May 31, 1955]

The Estimation of Peanut Meal in a Premix for Bread Enrichment

D. F. LOUW and W. E. L. VAN BERGEN, National Nutrition Research Institute, Council for Scientific and Industrial Research, Pretoria, South Africa

ONE OF SOUTH AFRICA'S FOOD PROBLEMS is a deficiency in the diet of good quality protein, with its associated nutrients. In a national food enrichment program it was therefore decided to enrich bread made of 90% extraction wheat flour with skim milk powder (70 mesh), defatted peanut meal (34 mesh), and calcium carbonate (100 mesh). These supplements are previously mixed, and the "premix" is supplied to bakers for addition to 90% extraction wheat flour.

The addition of this premix to flour is controlled by the South African Wheat Industry Control Board through analysis of the enriched bread, but control of the composition of the premix is a function of this Institute.

Total protein content is determined by the improved Kjeldahl method (1, 2) whereas the contribution of skim milk powder is satisfactorily deduced from lactose content (3). Similarly the estimation of peanut meal is done indirectly. Stansbury, Field, and Guthrie (4) observed that the tannin and leucoanthocyanin of peanut skins can be converted into red products by heating in alcoholic hydrochloric acid solution. This fact was applied to estimate the skin content of peanut meals according to an elaboration by Stansbury and Hoffpauir (5). A modification of the latter method was introduced at this laboratory to estimate peanut flour content of the above-mentioned premix (3).

It was found however that the de Lange method gave inconsistent results. Very little was known about the reliability of the method and the reproducibility of results. The investigations described in this paper led to a few alterations in the method of de Lange, and it was established that the modified procedure described below gives excellent results, which are satisfactorily reproducible.

Variation in the pigment content of peanut skins with variety and geographical origin (6) may present an obstacle. Preliminary investigations on available samples suggest that the pigment content of peanut varieties used in this country for the manufacture of premix does not vary appreciably and will not seriously affect the estimation of peanut meal by a method based on the incidence of this component. This matter however is being further investigated.

Reagents

1. Ethanol 95%.
2. Sulphuric acid, 5% solution. Add 5.6 ml. of conc. sulphuric acid (C.P., S.G. 1.84) to 190 ml. of water.
3. Neutral lead acetate, 10% solution.
4. Hydrochloric acid, A.R., S.G. 1.18.
5. Tungstate-phosphate solution. Dissolve 50 g. of sodium tungstate (C.P.) and 6 g. of sodium mono-acid phosphate (C.P.) in 200 ml. of water,

add 220 ml. of 2N hydrochloric acid, and dilute to 500 ml. with water.

Procedure

Weigh 0.200 g. of premix into a 50-ml. short-necked, round-bottom flask, add 7 ml. of ethanol and 12 ml. of distilled water, and boil under reflux on a hotplate for 1 hr. with occasional swirling to prevent sedimentation. Cool the mixture to room temperature and transfer the contents of the flask quantitatively to a 50-ml. graduated centrifuge tube by rinsing with several small portions of alcohol so as to end up with a total volume of 40 ml. Place a tight-fitting rubber stopper on the tube to prevent evaporation and centrifuge at 2,500 r.p.m.

Pipette 10 ml. of the slightly turbid supernatant liquid into another 50-ml. centrifuge tube and add 2 ml. of lead acetate solution and 0.5 ml. of tungstatephosphate solution. Stopper the tube and invert it 10 times to achieve mixing. Heat the open tube for 10 min. in a waterbath at 80°C. Cool to room temperature and centrifuge for 10 min. at 2,500 r.p.m. Decant very carefully and discard the clear supernatant liquid. Place the inverted tube on a piece of filter paper and allow the precipitate to drain for 10 min. Wipe off any remaining drops with a cleansing tissue.

Add 2 ml. of 5% sulphuric acid and 5 ml. of ethanol to the precipitate in the tube, stir with a glass rod, and heat the mixture under continuous stirring for 10 min. in a waterbath at 80°C. Wash the glass rod with alcohol, cool the suspension, and centrifuge at 2,500 r.p.m. for 10 min. in order to remove the lead sulphate. Decant the supernatant liquid as quantitatively as possible into a 30-ml. test tube. Mix the residue with 10 ml. of alcohol, centrifuge, and add the liquid to that in the test tube.

Pipette 2 ml. of concentrated hydrochloric acid into the test tube, stopper and shake vigorously to mix with the alcoholic solution. Immerse the open test tube in a waterbath at 75° for 1 hr. and swirl occasionally. The pink solution is then cooled to room temperature, diluted to 20 ml. by addition of ethanol, either in the test tube having a previously determined graduation or by transferring the contents to a suitable measuring cylinder with stopper or to a volumetric flask.

Determine the extinction (E) for the diluted solution in a spectrophotometer at 545 mμ 1 hr. after cooling the solution to room temperature; use cuvettes with an optical path of 20 mm. A solution of 2 ml. of concentrated hydrochloric acid diluted to 20 ml. with ethanol serves as a blank.

The percentage of peanut meal corresponding to the above E-value is obtained from a standard graph plotted from extinctions of premixes of known peanut meal content which were similarly treated. (This standard graph is further discussed below.)

Discussion

1. *Extraction of Pigments.* The following methods of extraction have been compared: a) Soxhlet extraction (Stansbury-Hoffpauir) for 3 hrs. with 95% ethanol; b) extraction with a mixture of 7 ml. of ethanol and 12 ml. of water in direct contact with the material by stirring the mixture in a centrifuge tube heated for 15 min. in a waterbath at 80°C. (de Lange); and c) extraction as described above.

As shown in Table I, methods b) and c) give comparable results for a specific sample of premix containing 61.5% of peanut meal. These are considerably higher than for a). The values obtained by method c) vary less than those obtained by method b), presumably as a result of more complete extraction of coarser particles.

The influence of the composition of the extractant is very marked (Figure 1). Masquelier (7) observed that 60% alcohol extracts more efficiently than 95% alcohol, but in the above experiments a dilution to ± 35% was used.

TABLE I

Influence of Extraction Method on E-Values

Extraction method	Extinction (E) at 545 mμ
a	0.062, 0.063, 0.063
b	0.139, 0.145, 0.130
c	0.141, 0.141, 0.145

From Figure 2 the influence of time on extraction method c) will be observed. The decrease in E-value after ± 90 min. can possibly be ascribed either to thermal decomposition of the extracted pigment or to some other internal reaction. A period of 60–90 min. gave maximum results.

2. *Spectrum of the Red Product.* In Figure 3 were plotted E-values between 400 and 600 mμ for a) 0.200 g. of a premix containing 61.5% peanut meal, 30.8% skim milk powder, and 7.7% calcium carbonate, and b) a sample of pure defatted peanut flour corresponding in weight to that present in a). In both cases the maximum absorption is at 545 mμ whereas E-values for a) and b) coincide at this wavelength. Previously a maximum absorption(4) at 548–550 mμ and a characteristic absorption (5) in the region 525–540 mμ were determined for the red product obtainable from peanut pigments.

From Figure 3 and from Table II it is obvious that no identical red pigment has its origin in the skim

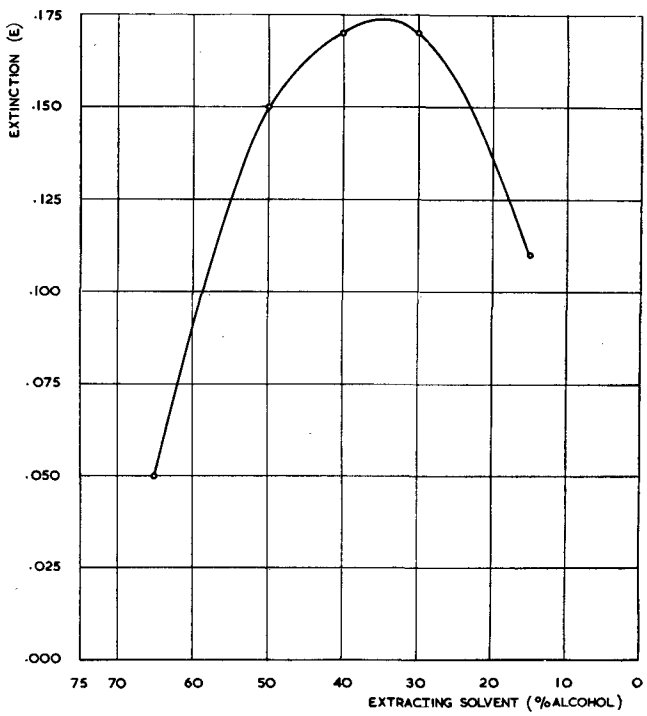

FIG. 1. Influence of alcohol concentration on extraction of pigments from peanut meal.

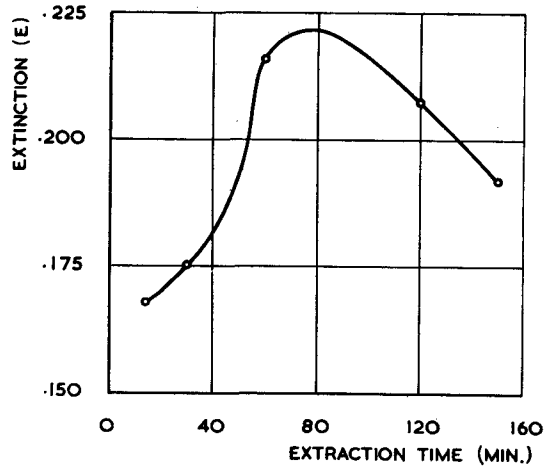

FIG. 2. Influence of time on the extraction of pigments from peanut meal.

milk powder or the $CaCO_3$, nor do these components interfere with the extraction of the pigment or with the development of the pink color.

In the following table A = weight of defatted peanut meal, B = weight of skim milk powder, and C = weight of calcium carbonate.

3. *Preparation of a Standard Graph.* Vacuum-dried samples of defatted peanut meal (34 mesh) and skim milk powder (70–80 mesh) were mixed with calcium carbonate (100 mesh) to give premixes of varying (known) peanut meal content. These mixtures were then treated as described above and their E-values determined. The results are graphically represented in Figure 4 and show a marked deviation from the linear relationship between E and % peanut meal at concentrations over 70%. This can be ascribed to either incomplete extraction or deviation from Beer's law at these high concentrations. In the range 50–75% the graph is practically a straight line, and it is this part of the graph which is important for control of the premix used at present.

4. *Calculation of Standard Deviation.* A series of 10 portions of a premix containing exactly 60% peanut meal was analyzed as above, and a standard deviation of 2.1% was calculated for the E-values observed [min.=174.5, max.=186.0, average=181.6].

TABLE II

Effect of Other Components on Estimation of Defatted Peanut Meal

Composition (mg.)			Extinction (545 mμ)
A	B	C	
120	0	0	179,179
120	80	0	179,183
120	0	20	175,180
120	80	20	185,181

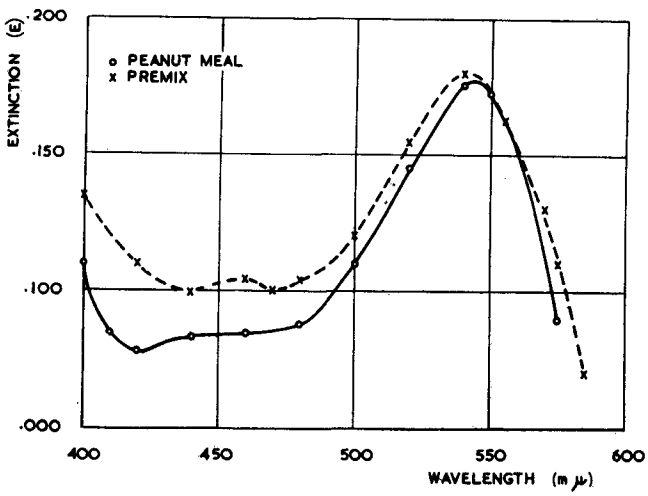

FIG. 3. Spectrum of red product obtained from pigments of premix and pure peanut meal.

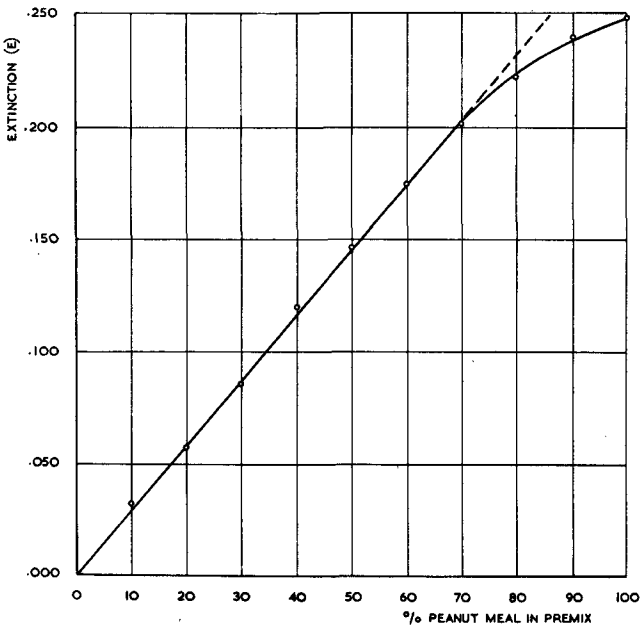

FIG. 4. Influence of concentration of peanut meal in premix on the development of a pink color.

Summary

By modifying existing methods a dependable procedure has been developed for the estimation of defatted peanut flour in a premix containing this component in addition to skim milk powder and calcium carbonate. The method is based on extraction of the catechol tannin pigments of the peanut skin by refluxing with diluted alcohol, purification of the pigments by precipitation as the lead salts, and development of a red color in alcoholic hydrochloric acid. This red product has a maximum absorption at 545 mμ, and the extinction is directly proportional to the concentration of peanut meal in a range of 0–70%. Deviations from the linear relationship occur at higher concentrations.

Using a premix containing 60% peanut meal, a standard deviation of 2.1% has been calculated for this procedure, indicating excellent reproducibility for control determinations.

Acknowledgment

This paper is published with the permission of the S. A. Council for Scientific and Industrial Research.

REFERENCES

1. Perrin, C. H., Anal. Chem., *25*, 968 (1953).
2. Louw, D. F., J. South African Chem. Inst. (New Series), *8*, 39 (1955).
3. Government Gazette (Extraordinary) Union of S. A., No. 5172, p. 17 (Oct. 1953). Details of these methods were worked out by D. J. de Lange, who has since joined the staff of the National Nutrition Research Institute.
4. Stansbury, M. F., Field, E. T., and Guthrie, J. D., J. Am. Oil Chemists' Soc., *27*, 317 (1950).
5. Stansbury, M. F., and Hoffpauir, C. L., *ibid.*, *29*, 370 (1952).
6. Dangoumau, A., Debruyne, H., and Cluzan, R., Oleagineux, *6*, 402 (1951).
7. Masquelier, J., Bull. soc. chim. France, *1948*, 1175.

[Received July 25, 1955]