## LITERATURE CITED

- American Association of Cereal Chemists, AACC Approved Methods, Revised, St. Paul, Minn., 1971.
- Anderson, R. A., Stringfellow, A. C., Griffin, E. L., Jr., Northwest. Miller 279(2), 10 (1972).
- Anderson, R. A., Stringfellow, A. C., Wall, J. S., Griffin, E. L., Jr., Food Technol. 28(11), 66 (1974).
- Black, L. T., Spyres, G. G., Brekke, O. L., Cereal Chem. 44, 152 (1967).
- Cavins, J. F., Friedman, M., Cereal Chem. 45, 172 (1968).
- Centro Internacional de Mejoramiento de Maiz y Trigo, CIMMYT Report on Wheat Improvement, 1973, El Batan, Mexico, 1974.
- Garcia, W. J., Wolf, M. J., Cereal Chem. 49, 298 (1972).
- Heinrichs, E. R., Hill, R. D., Abstract 103, Cereal Sci. Today 16, 306 (1971).
- Horn, M. J., Fifield, C. C., Blum, A. E., Warren, H., Cereal Chem. 35, 411 (1958).
- Hulse, J. H., Spurgeon, D., Sci. Am. 231(2), 72 (1974).
- Kaltsikes, P. J., Larter, E. N., Wheat Newslett. 17, 30 (1970).
- Lorenz, K., Food Technol. 26(11), 66 (1972).
- Lorenz, K., Welsh, J., Normann, R., Maga, J., Cereal Chem. 49, 187 (1972).
- Madl, R. L., Tsen, C. C., Cereal Chem. 50, 215 (1973).

- Muntzing, A., Proc. Int. Wheat Genet. Symp., 2nd (1963); Hereditas Supp. 2, 291 (1966).
- Rooney, L. W., Gustafson, C. B., Perez, N., Porter, K. B., "Agronomic Performance and Quality Characteristics of Triticale Grown in the Texas High Plains", Texas Agricultural Experiment Station, College Station, Tex., Sept 1969.
- Saunders, R. M., Betschart, A. A., Connor, M. A., Edwards, R. H., Kohler, G. O., "Triticale: First Man-Made Cereal", Tsen, C. C., Ed., American Association of Cereal Chemists, St. Paul, Minn., 1974, p 280.
- Sloneker, J. H., Anal. Biochem. 43, 539 (1971).
- Slotter, R. L., Langford, C. T., Ind. Eng. Chem. 36, 404 (1944).
  Tsen, C. C., Hoover, W. J., Farrell, E. P., Cereal Chem. 50, 16 (1973).
- Unrau, A. M., Jenkins, B. C., Cereal Chem. 41, 365 (1964).
- Vaisey, M., Unrau, A. M., J. Agric. Food Chem., 12, 84 (1964).
- Yasumatsu, K., Sawada, K., Moritaka, S., Misaki, M., Toda, J., Wada, T., Ishii, K., Agric. Biol. Chem. 36, 719 (1972).

Received for review October 30, 1975. Accepted January 26, 1976. Presented at the Division of Agricultural and Food Chemistry, 170th National Meeting of the American Chemical Society, Chicago, Ill., Aug 24–29, 1975. Mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

# Influence of Suspension Medium and pH on Functional and Protein Properties of Defatted Peanut Meal

Kay H. McWatters,\* John P. Cherry,<sup>1</sup> and Mac R. Holmes

Defatted peanut (Arachis hypogaea L. C.V. Florunner) meal was blended with either water, 0.1 M NaCl, or 1.0 M NaCl and the pH of each suspension adjusted to either 1.5, 4.0, 6.7, or 8.2; two-step sequential adjustments from 6.7 to 4.0 to 6.7 and from 6.7 to 4.0 to 8.2 were also included. All suspensions had similar viscosities. Those at pH 4.0 produced soluble extracts with lowest percentage protein and failed to form emulsions. Suspensions at pH 6.7 varied widely in percentage protein, produced the least increase in foam volume, and formed poor emulsions. The most desirable emulsions and foams were produced by peanut meal-water suspensions adjusted from pH 6.7 to 4.0 to 8.2 and from pH 6.7 to 1.5. Gel electrophoresis of soluble proteins and multiple regression analysis showed that functionality of peanut meal was influenced by complex interactions involving suspension medium, pH, and level and character of soluble proteins.

Peanuts have traditionally been consumed in the form of peanut butter and in candies, salted nuts, and snack-type crackers because of their highly acceptable roasted flavor. In recent years, interest has developed in high-protein products such as defatted peanut flour, concentrates, and isolates as potential ingredients having the capacity to perform specific functions in food systems. Oilseed protein products act as emulsifiers and extenders in meat products, fat and water absorption agents in meats and bakery products, thickeners in soups and gravy products, gelling agents in meat products, color control agents in bread products, and whipping agents in toppings, chiffon mixes, and confections (Wolf and Cowan, 1971).

The level or proportion of soluble proteins has been used as a measure of the availability of these components for functional uses (Johnson, 1970; Mattil, 1971; Wolf and Cowan, 1971; Cherry et al., 1975). For example, processing techniques such as moist heat may be applied for the express purpose of modifying the protein components of oilseeds to fit specific product applications. The application of moist heat to alter certain physicochemical and solubility properties of peanut proteins and thus change their functional properties has been discussed by Cherry et al. (1975). These workers found that water-soluble proteins of moist-heated peanut seeds were altered sequentially to various structural components, aggregates, and insoluble forms. Arachin, the major storage globulin of peanut seeds, was altered to these denatured forms at at slower rate than nonarachin proteins. Further studies

Department of Food Science (K.H.M., J.P.C.) and Agricultural Economics (M.R.H.), University of Georgia, College of Agriculture Experiment Stations, Experiment, Georgia 30212.

<sup>&</sup>lt;sup>1</sup>Present address: Southern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, New Orleans, La. 70179.

suggested that arachin and/or the denatured forms of this globulin and nonarachin proteins may play a role in the functionality of peanut components (McWatters and Cherry, 1975).

Meat formulations such as sausage batters have traditionally been described as oil-in-water emulsions (Saffle, 1968). In these systems, salt solubilizes proteins which then act as emulsifying agents having affinity for both water and fat. These proteins are surface active and form emulsions by reducing both the interfacial tension between fat and water and the energy required to form the emulsion. In later studies Brown (1972) suggested that meat proteins and water form a matrix or lattice which binds fat rather than emulsifying it. Although the mechanisms for these phenomena are not clearly understood, proteins are closely associated with solubility, binding, and emulsification properties (Hermansson and Akesson, 1975).

Both pH and salt concentration markedly affect the protein solubility and behavior of peanuts and other oilseeds (Fontaine and Burnett, 1944; Mattil, 1971; Rhee et al., 1972; Fleming et al., 1974; Wu and Inglett, 1974). Rhee et al. (1972) found that salts suppressed the extractability of peanut protein under neutral and alkaline conditions. However, at pH 3.5–5.5, salts in increasing concentrations enhanced protein extraction. Below pH 3.0, protein extractability decreased as salt concentration increased. If functional properties of proteins are in fact dependent upon solubility, the use of salt solution as an extractant, although suitable for meat (Saffle, 1968) and sunflower protein systems (Fleming et al., 1974), would at certain pH levels be undesirable for peanut proteins.

Recently, McWatters and Cherry (1975) observed that aqueous suspensions of peanut paste could form oil-inwater emulsions and foams in model test systems. A two-step pH adjustment from a natural pH of 6.7 to 4.0 to 8.0 was necessary for formation of thick, viscous emulsions having mayonnaise-like consistencies. Interestingly, these pH values were employed by Rhee et al. (1972) for maximum recovery of both protein and oil from raw peanuts in an aqueous system. Moreover, adjusting the pH of water-soluble extracts of peanut flour to 7.0 and adding sugar prior to whipping improved foaming capacity by increasing the viscosity of the resulting foams (Lawhon et al., 1972).

The purpose of this study was to examine the influence of pH and suspension medium on proteins and selected functional properties such as viscosity, emulsification, and foam formation of defatted peanut meal. Gel electrophoretic techniques and multiple regression analysis were used to determine if changes in soluble protein composition relative to pH and suspension medium could be correlated to the functional properties of the treated peanut meal. The extent to which peanut seed proteins are successfully utilized in food formulations will depend upon an understanding of factors which affect their functional properties.

## MATERIALS AND METHODS

Meal Preparation. Florunner peanut seeds used in this investigation were grown in experimental plots at Plains, Ga. in 1974. Skins were removed by hand and without heat. The seeds were first coarsely ground in a Hobart cutter and then partially defatted by extraction with food-grade hexane, and the resulting meal was air-dried overnight at 23 °C. This meal was reground in a pilot-scale stone mill (Morehouse-Cowles, Inc., Los Angeles, Calif.) set at a stone clearance of 0.01 in. and then reextracted with hexane until the oil content was below 1.0%. The

518 J. Agric. Food Chem., Vol. 24, No. 3, 1976

defatted meal was air-dried for 72 h at 23 °C and sifted through a 60 mesh screen. The final product was stored in glass jars at 1 °C until used.

Moisture content of solvent-extracted peanut meal was determined by drying 3-g samples in a forced draft oven at 110 °C for 5 h. Total oil was determined by suspending the dried meal in 25 ml of diethyl ether overnight, removing 10 ml of clarified ether containing dissolved oil, evaporating the ether in a tared vessel, and weighing and calculating the percentage oil originally contained in the meal. Total protein and ash were determined by the American Oil Chemists' Society (1970) methods and total carbohydrate by difference. A factor of 5.46 was used to convert nitrogen content to protein values (Jones, 1931). The proximate composition of the defatted meal was 9.2% moisture, 54.9% protein, 0.9% oil, 4.5% ash, and 30.5% carbohydrate.

Suspension Preparation and pH Adjustment. Defatted peanut meal was blended with either distilled water, 0.1 M NaCl, or 1.0 M NaCl (8% suspensions; w/v) in an Osterizer blender operated at low speed. Blending was conducted in four 30-s intervals, allowing the contents to remain undisturbed for 5 min between each mix to ensure protein solubilization. The pH of each suspension was then adjusted to either 1.5, 4.0, 6.7, or 8.2. Two additional pH adjustments included two-step sequential changes from 6.7 to 4.0 to 6.7 and from 6.7 to 4.0 to 8.2. The desired pH was attained by adding 1 N HCl or NaOH solution to suspensions with continuous stirring.

**Viscosity.** A Brookfield viscometer (Model RVT) equipped with a No. 1 spindle was used to measure the viscosity of suspensions at 23 °C in centipoises (cP). Following pH adjustment, each suspension was transferred to a 600-ml beaker and the viscosity was measured at 100 rpm after a 30-s rotation.

**Emulsion Capacity.** Oil-in-water emulsions were prepared as described by McWatters and Cherry (1975) with the exception that all oil added was from a buret. Emulsion capacity was considered to be the point at which a sudden drop in viscosity occurred due to separation of oil and water into two phases. These data were reported as milliliters of oil emulsified per 25 ml of suspension sample.

Foam Capacity and Stability. Samples of peanut meal suspensions (100 ml) were prepared for foam capacity measurements as described by McWatters and Cherry (1975). The volume of foam after 1 min was measured in a graduated cylinder and reported as total milliliters of foam. The volume of foam remaining after standing for 60 min was recorded as a measure of foam stability.

**Protein Solubility and Gel Electrophoresis.** A 5-ml sample of each suspension was centrifuged at 43 500g to separate soluble and insoluble protein fractions. Quantitative and qualitative changes in soluble proteins of peanut meal suspensions relative to extraction medium and pH were determined by the procedure of Lowry et al. (1951) and polyacrylamide gel electrophoretic techniques described by Cherry et al. (1975). In addition, defatted meal and soluble and insoluble fractions from the suspensions were lyophilized and examined for protein content using the macro-Kjeldahl technique and a conversion factor of 5.46. Salt levels in extracts were calculated and appropriate adjustments were applied to the protein data.

**Statistical Analysis.** A multiple regression analysis was used to quantify the relationships between functional properties of defatted peanut meal in the suspension media and various independent variables. A step-down computer

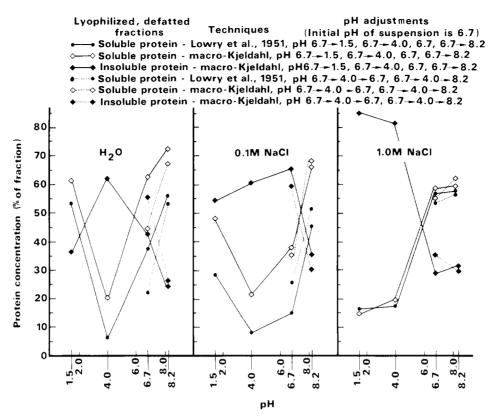


Figure 1. Effect of pH and suspension medium on the solubilization of protein in defatted peanut meal.

program was used in the selection of the equation of best fit as described by Cherry et al. (1975) and McWatters and Cherry (1975).

Variables evaluated in statistical (multiple regression) models included pH, signed deviations of pH from 4.0 and from 6.7, absolute deviations of pH from 4.0 (or 6.7) for pH values above 4.0 (or 6.7), absolute deviations of pH from 4.0 (or 6.7) for pH values below 4.0 (or 6.7), suspension medium (0, 0.1, and 1.0 M NaCl), and percentage soluble and insoluble protein. Other variables evaluated included dummy (0–1) variables for treatments in which the pH was lowered from 6.7 to 4.0 and then raised to either 6.7 or 8.2 and the squares and cubes of changes in percentage protein in soluble and insoluble fractions for pH values ranging from 1.5 to 8.2.

Measurements of the functional characteristics (emulsion capacity, foam capacity, and foam stability) were used directly as dependent variables and also after conversion to their natural logarithms.

## **RESULTS AND DISCUSSION**

Protein Solubility. The effects of various suspension media and pH adjustments on protein solubility of defatted peanut meal are presented in Figure 1. In addition, percentage protein in lyophilized soluble and insoluble fractions determined by the macro-Kjeldahl and Lowry et al. (1951) methods are also shown in Figure 1. Percentages of protein in lyophilized extracts of water and 0.1 M NaCl were higher at each pH when determined by the macro-Kjeldahl method than by the Lowry et al. (1951) procedure. On the other hand, similar results were obtained with both methods when extracts contained 1.0 M NaCl. Evidently, nitrogen in 1.0 M NaCl extracts was derived from protein whereas preparations made with water or 0.1 M NaCl had high quantities of nonprotein nitrogen-containing material and/or proteins low in tyrosine.

Data obtained with these methods show that percentage protein in either water or 0.1 or 1.0 M NaCl soluble fractions was highest at pH 8.2, intermediate at 6.7, and lowest at 4.0. The isoelectric point of peanut seed proteins is between pH 3.0 and 5.0 depending on the type of extraction medium used to prepare these components (Basha and Cherry, 1976; Quinn and Beuchat, 1975; Ayres et al., 1974; Rhee et al., 1972). The percentage protein in lyophilized extracts of water or 0.1 M NaCl was higher at pH 1.5 than at 4.0. The percentage protein decreased slightly in 1.0 M NaCl as the pH was lowered from 4.0 to 1.5. The changes in percentage protein in insoluble preparations after each pH change were inversely related to the soluble extracts (Figure 1).

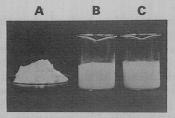
Adjusting the pH from 6.7 to 4.0 and then back to either 6.7 or 8.2 showed that percentage protein in lyophilized water-soluble extracts as determined by both methods was less than those after a one-step pH change. In lyophilized extracts containing 0.1 M NaCl, the macro-Kjeldahl method showed little difference in protein composition after a one- or two-step adjustment, whereas data from the Lowry et al. (1951) procedure suggested that the two-step adjustment caused an increase in percentage protein in the soluble fraction. On the other hand, variations in protein percentages were small whether the extract was adjusted to pH 6.7 or 8.2 by the one- or two-step adjustment. This was also true when protein evaluations were determined by either the macro-Kjeldahl or Lowry et al. (1951) methods in which suspensions from seed meal were made with the 1.0 M NaCl solution. Moreover, greater percentages of protein were found in high-salt extracts than in those containing no or low salt at pH 6.7.

Viscosity. Data in Table I show that peanut seed meal suspended in either water or a salt solution resulted in low viscosities. Neither variations in type of medium (water or 0.1 or 1.0 M NaCl) nor pH produced substantial changes in suspension viscosities. This behavior is in contrast to that exhibited by soy flour dispersions which showed

Table I.	Effect of	pH and Suspensio	on Medium on	<b>Functional Properties</b>	of Defatted Peanut Meal
----------	-----------	------------------	--------------	------------------------------	-------------------------

	Suspension medium	Viscosity, cP	Foam			Emulsion	
Treatment, pH			Capacity, ml, 1 min	Stability, ml, 60 min	% change	Capacity, ml of oil	Туре
$6.7 \rightarrow 1.5$	Water	23.0	228.7	145.3	36.5	119.1	Mayonnaise-like
	0.1 M NaCl	19.0	198.0	110.7	44.1	92.4	Mayonnaise-like
	1.0 M NaCl	18.7	180.7	104.7	42.1	38.6	None formed
$6.7 \rightarrow 4.0$	Water	22.8	143.0	86.0	39.9	38.7	None formed
	0.1 M NaCl	18.3	140.0	85.0	39.3	41.9	None formed
	1.0 M NaCl	20.5	145.0	83.0	42.8	49.9	None formed
6.7	Water	17.3	76.0	58.0	23.7	57.4	Pourable suspension
	0.1 M NaCl	18.0	82.0	58.0	29.3	42.4	Pourable suspension
	1.0 M NaCl	20.8	108.7	60.0	44.8	62.2	Pourable suspension
$6.7 \rightarrow 4.0 \rightarrow 6.7$	Water	20.7	90.0	72.0	20.0	62.0	Thick salad dressing
	0.1 M NaCl	18.3	103.0	72.0	30.1	47.0	None formed
	1.0 M NaCl	19.3	138.0	85.0	38.4	57.3	Sl. thickening, pourable
6.7  ightarrow 8.2	Water	17.7	102.7	78.0	24.1	74.9	Thick salad dressing
	0.1 M NaCl	19.8	96.7	68.0	29.7	61.3	Sl. thick salad dressing
	1.0 M NaCl	22.5	125.3	79.3	36.7	57.9	None formed
6.7  ightarrow 4.0  ightarrow 8.2	Water	22.3	141.3	95.3	32.6	94.8	Mayonnaise-like
0.1 1.0 0.2	0.1 M NaCl	21.5	136.0	90.7	33.3	66.3	Salad dressing
	1.0 M NaCl	25.0	127.3	87.3	31.4	68.3	Salad dressing

Emulsions



A: Mayonnaise-like

**B:** Salad dressing

C: Pourable-None

Figure 2. Types of emulsions produced by defatted peanut meal as influenced by pH and suspension medium.

marked increases in viscosity as pH increased from 7.0 to approximately 10 or 11 (Johnson, 1970).

These data also suggest that the percentage protein in soluble extracts of peanut meal was not related to viscosity. For example, specific suspensions adjusted to pH 4.0 (water and 0.1 and 1.0 M NaCl), 1.5 (1.0 M salt), or 6.7 (0.1 M salt) had lower protein percentages in soluble fractions than those at other pH values and yet they produced equally viscous solutions (cf. Figure 1, Table I). Thus, it would be difficult to predict viscosity properties of peanut meal suspensions based solely on percentage protein in the soluble fraction.

**Emulsion Capacity.** The types of emulsions produced by peanut meal suspensions are illustrated in Figure 2. Suspensions which exhibited excellent emulsifying properties produced thick, viscous mayonnaise-like products (A) while those with fair emulsifying capacity formed emulsions which resembled thick, pourable salad dressings (B). Suspensions which exhibited poor emulsifying properties failed to form emulsions or showed only slight thickening (C).

Data in Table I indicate that the capacity of peanut meal to form an emulsion was highly sensitive to changes in pH and suspension medium. The best emulsions having viscous, mayonnaise-like consistencies were produced by suspensions at pH 1.5 in water (119.1 ml of oil) and 0.1 M NaCl (92.4 ml) and by the water suspension (94.8 ml) adjusted from pH 6.7 to 4.0 to 8.2. The high-salt level (1.0 M) evidently interfered with emulsion formation at pH 1.5. Interestingly, percentage protein in soluble fractions was progressively lowered at pH 1.5 by increasing salt levels; in water it was highest at 55.0–60.0%, 0.1 M NaCl was intermediate with 30–50%, and 1.0 M NaCl was lowest containing approximately 16% (Figure 1).

At pH 4.0, no emulsions could be formed, regardless of suspension medium. Poor emulsifying properties were also noted at pH 6.7 (samples receiving minor or no pH adjustment) where mixtures with thin, pourable consistencies were produced by both water and salt preparations. Highly variable levels of soluble protein occurred among the suspensions at this pH. The two-step pH adjustment from 6.7 to 4.0 to 6.7 improved emulsification properties over that of samples receiving minor or no pH adjustment for only the water suspension. The presence of salt even at a low concentration (0.1 M NaCl) suppressed emulsion formation with this pH treatment.

Adjusting the pH of peanut meal suspensions to 8.2, either directly from 6.7 or by the two-step treatment (6.7 to 4.0 to 8.2), resulted in high percentages of soluble protein (45.6 to 70.0%) and except for the 1.0 M NaCl suspension, aided emulsion formation. The consistencies of emulsions ranged from thick salad dressings (61.3 to 74.9 ml of oil) to a mayonnaise-like product (94.8 ml). Emulsion formation was evidently inhibited by the presence of 1.0 M NaCl at pH 8.2, even though the percentage protein in the soluble fraction was among the highest (60.0%) observed in this study. These data indicate that suspending peanut seed meal in either water or 0.1 M NaCl solution and adjusting the pH to 1.5 or 8.2 (directly or preferably by the two-step procedure) significantly improved their capacity to form emulsions.

Peanut meal suspensions receiving the two-step pH adjustment from 6.7 to 4.0 to 8.2 exhibited better emulsifying properties than suspensions adjusted directly from 6.7 to 8.2, though percentage protein in the soluble fraction

FUNCTIONAL PROPERTIES OF PEANUT PROTEINS

Foam Capacity (1 min) A B C C A B C C A B C C A B C C A B C C A B C C A B C C A B C C A B C C A B C C A B C C A B C

- B: Intermediate
- C: Low

Figure 3. Types and stability of foams produced by defatted peanut meal as influenced by pH and suspension medium.

did not differ extensively between the two treatments (cf. Figure 1, Table I). Possibly, exposure of peanut meal components to pH 4.0 and then to pH 8.2 may have caused a structural rearrangement in proteins which increased the number of binding sites to interact with oil and water without appreciably affecting protein solubility.

Foam Capacity and Stability. The types and stability of foams produced by defatted peanut meal suspensions are shown in Figure 3. Foams are representative of high, intermediate, and low foaming capacity. Adjusting the pH of suspensions prior to whipping significantly affected foam formation and stability (Table I). The largest increase in foam volume (180 to 228 ml) occurred at pH 1.5; at this pH, increasing the salt concentration significantly reduced the percentage protein in soluble fractions and tended to depress foam formation. At pH 4.0, percentage proteins in soluble fractions were among the lowest observed in this study (6.6 to 17.4%), yet increases of 140 to 145 ml in foam volume occurred in peanut meal suspended in both water and salt solutions. These data as well as those of viscosity (Table I) and whippability of sunflower meal products (Lin et al., 1974) suggest that constituents other than protein may contribute to the functional characteristics of peanut meal.

At pH 6.7, the smallest increases in foam volume due to whipping occurred (76 to 108 ml). Percentage protein in the soluble fraction was highly varied at this pH, ranging between 15.0 and 40.0% in 0.1 M NaCl and 55.0 to 60.0% in 1.0 M NaCl solutions. Foam volume tended to increase as salt concentration increased at this pH. Changing the pH from 6.7 to 4.0 to 6.7 improved foam formation over that of the unadjusted (pH 6.7) suspension despite variation in percentage protein of soluble fractions, i.e., percentage protein in these fractions was reduced in water suspensions, increased in 0.1 M NaCl (Lowry et al. (1951) method; the macro-Kjeldahl technique showed a small decrease), and was relatively unchanged or slightly lowered in 1.0 M NaCl mixtures.

At pH 8.2, percentage protein values for soluble extracts were among the highest observed in the study, yet increases in foam volume (96 to 125 ml) were not as great as those occurring at pH 1.5 or 4.0. Peanut meal suspended in salt solutions and water adjusted from pH 6.7 to 4.0 to 8.2 produced soluble fractions with high percentage protein and exhibited good foaming properties (127 to 141 ml increases). A slight inhibition of foam formation was noted as salt concentration increased with this latter pH adjustment although lesser than that observed at pH 1.5.

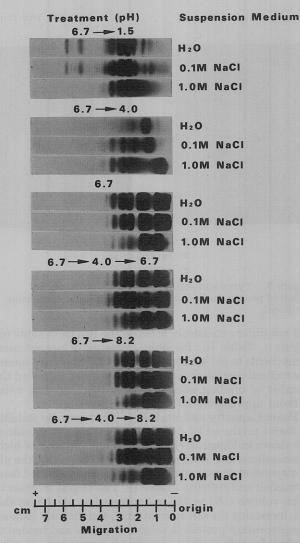
The improved foaming capacity of peanut meal in acidic suspensions may be related to a molecular stabilizing effect which has been shown to occur at acid pH (Griswold, 1962). This mechanism is employed in preparation of angel food cakes where cream of tartar is added to egg white foams. The acidity developing as a result of added cream of tartar coagulates some of the protein that surrounds the air cells of the foam, preventing its collapse, thereby stabilizing the foam. The foams produced at pH 1.5 were relatively stiff and peaked with small air bubbles; those produced at the neutral pH (6.7) were frothy, lacked stiffness, and had large air bubbles. The acidic foams and those prepared from salt suspensions were in most instances the least stable, as shown by the larger percentage decrease in volume after standing for 60 min (Table I). The pH of a suspension is evidently an important consideration in determining the type, volume, and stability of foam produced with whipping. Adjusting the pH to levels above or below the natural pH (6.7) would appear to be an effective means of improving the foaming properties of peanut meal suspensions.

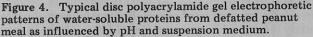
**Multiple Regression Analysis.** Stepdown elimination was used to select the final equation, or model, for each functional characteristic. These models are presented in Table II. The relative importance of the variables included in any one model was determined by comparisons of either the  $\beta$  values and/or the squared partial correlation coefficients. A squared partial correlation coefficient indicates the percentage relative reduction in unexplained variation  $(1.0 - R^2)$  obtained by introducing the corresponding variable into a particular model (Ezekiel, 1941).

The models based on absolute deviations above and below pH 4.0 and below 6.7 and the dummy variables on the two-step pH adjustments consistently produced better results than those based on logarithmic values or other measures of pH. The multiple regression models for prediction of functional properties of defatted peanut meal that produced the highest percentage multiple  $R^2$  (or adjusted R<sup>2</sup>) values of 93.02 (or 90.11), 94.32 (92.57), and 92.30 (89.09) for emulsion capacity, foam capacity, and foam stability, respectively, are presented in Table II. If there had been several additional pH values included in the analyses, other equations such as those including the logarithmic function could possibly have been effective in these models. Also, use of variables based on measurements of soluble protein percentages as determined using the technique of Lowry et al. (1951) consistently improved the fit of the models as judged by  $R^2$ . Interactions involving suspension medium only appeared in the models evaluating emulsion capacity and foam stability.

According to the  $\beta$  value or the square of the partial correlation coefficient, the ability of defatted peanut meal to form an emulsion was suggested to be dependent mainly on the type of pH adjustment, suspension medium (low or no salt), and percentage protein in the soluble fraction. Interactions of suspension medium and pH adjustments above and below pH 4.0 were highly important variables for this particular functional property. Foam capacity of defatted peanut meal was dependent mainly on changing the pH to values below 6.7 and the percentage protein in soluble preparations. The two-step pH adjustments to 6.7 and 8.2 also influenced the foam capacity of defatted meal. The variables which accounted for the stability of defatted peanut meal foams were similar to those determining foam capacity. However, statistical analysis suggested that pH

tion in lained ion <sup>b</sup>	90 55 93 93	97 26	95 13 19 89 69	59 96 ance at duction of dard error Standard
% reduction in unexplained variation <sup>b</sup>	31.90 71.92 83.55 73.47 44.93	93.97 30.26	46.95 64.13 91.19 39.89 55.69	58.59 20.96 tes significan from introdu 011. Standa 0.8909. Str
Partial corr coeff	$\begin{array}{c} 0.5648\\ 0.8481\\ 0.9141\\ -0.8571\\ -0.6703\end{array}$	0.9694 0.5501	0.6852 0.8008 0.9550 0.6316 0.7463	$\begin{array}{l} 0.7655 \\ -0.4578 \\ \text{terisk deno} \\ \text{a resulting} \\ \text{ed } R^2 = 0.9 \\ \text{justed } R^2 = \end{array}$
β value	0.204 27 0.595 38 0.589 28 -0.588 88 -0.306 64	1.137 66 0.174 58	0.257 89 0.374 03 1.204 25 0.251 53 0.358 67	0.398 46 -0.177 80 = 5% level; double as nnexplained variatio $R^2 = 0.9302$ ; adjust riple $R^2 = 0.9230$ ; ad
Partial <i>t</i> value <sup>a</sup>	2.371* 5.544** 7.807** -5.764** -3.129**	14.235**2.375*	3.392** 4.821** 11.147** 2.822* 3.883**	4.121** -1.784 s significance at the o a percentage) in 1 1941). c Multiple 10.966 31. e Mult
Regression coeff.	$\begin{array}{c} 35.822 & 29\\ 11.396 & 01\\ 0.669 & 53\\ 18.059 & 25\\ -21.398 & 90\\ -4.413 & 55\\ \end{array}$	65.227 13 22.273 52 18.315 91	27.056 13 0.790 97 47.432 65 12.405 16 13.884 15 19.798 44	0.443 36 -2.810 87 vel; asterisk denote ction (convertible t he model (Ezekiel, r of the estimate =
Description	Constant (0-1) variable for pH $6.7 \rightarrow 4.0 \rightarrow 8.2$ Soluble protein percentage (Lowry) Absolute value of (pH -4.0) for pH <4.0 ( $X_3 \times$ suspension medium) interaction (Absolute value of [pH -4.0] for pH >4.0 $\times$ enconsion medium) interaction	Constant value of $(pH - 6.7)$ for $pH < 6.7$ (0-1) variable for $pH 6.7 \rightarrow 4.0 \rightarrow 6.7$	(0-1) variable for pH $6.7 \rightarrow 4.0 \rightarrow 8.2$ Soluble protein percentage (Lowry) Constant Absolute value of $(pH - 6.7)$ for pH $< 6.7$ (0-1) variable for pH $6.7 \rightarrow 4.0 \rightarrow 6.7$	$ \begin{array}{llllllllllllllllllllllllllllllllllll$
Variable	$egin{array}{c} X_1 \\ X_3 \\ X_5 \\ X_5 \end{array}$	X' X	X <sub>4</sub> X <sub>4</sub> X <sub>4</sub>	$X_{s}^{3}$ $X_{s}^{3}$ ession coeffi the partial c the model, ultiple $R^{2} = ($
Functional property	Emulsion capacity <sup>c</sup>	Foam capacity <sup>d</sup>	Foam stability <sup>e</sup>	<sup>a</sup> All t values for partial regret the 1% level. <sup>b</sup> The square of the corresponding variable into of the estimate = $6.729$ . <sup>d</sup> Mi





adjustments to values less than 6.7 interacting with the type suspension also affected foam stability.

For emulsion capacity, it is noted that the multiple regression model included deviations from pH 4.0 whereas foam capacity and stability had deviations from pH 6.7. These data add further support to the hypothesis that molecular changes associated with subjecting a defatted peanut meal suspension briefly to pH 4.0 (the isoelectric point of most peanut storage proteins) enhance its capacity to form an emulsion. Changes in pH from 6.7 improved foam capacity and stability of defatted peanut meal suspensions.

**Gel Electrophoresis of Proteins.** At pH 4.0, few protein components were distinguished on electrophoretic gels; however, more were present as the salt level increased from 0.1 to 1.0 M (see Figure 4). No improvement in emulsion capacity occurred at this pH even though percentage protein in the soluble fraction increased in the presence of salt. When the pH was lowered to 1.5, levels of nonarachin proteins (region 2.5–4.5 cm) increased and the arachin components (region 0.5–2.5 cm) became difficult to discern in the gels. Cherry et al. (1973) and Basha and Cherry (1976) have described these peanut protein components using gel electrophoretic techniques. Two components in region 4.5–5.5 cm in gels of water and low salt extracts were not present in those containing 1.0 M NaCl. Moreover, only the former two preparations formed

92

error of the estimate = 7.556

#### MCWATTERS, CHERRY, HOLMES

Multiple Regression Models for Prediction of Functional Properties of Defatted Peanut Meal

Table II.

mayonnaise-like emulsions and produced high levels of foam. At pH 6.7, 8.2, and the two-step pH treatments, water and low salt mixtures resulted in soluble proteins having similar electrophoretic properties. These gels showed that suspensions with 1.0 M NaCl had lower amounts of the nonarachin proteins in region 2.5-5.5 cm which could be related to the poor emulsions from these preparations. Moreover, the arachin components were diffuse and difficult to discern in these gels. No specific protein properties detectable by gel electrophoresis could be related to the unique ability of the water suspension subjected to the two-step pH adjustment of 6.7 to 4.0 to 8.2 to form a mayonnaise-like emulsion.

## CONCLUSIONS

In meat systems, the quantity as well as the quality of aqueous salt-soluble protein is an excellent measure of certain functional properties of these products in foods (Saffle, 1968). Our data suggest that predicting the functional properties of defatted peanut meals cannot be done on the basis of proteins alone. Water, proteins, carbohydrates, oil, and a number of unknown constituents in oilseed meals evidently interact differently than in meat systems depending on pH and salt levels (Lin et al., 1974; Cherry et al., 1975; McWatters and Cherry, 1975). Studies on interactions of processing conditions and protein composition such as those explored in this study should lead to an understanding of their effects on the functionality of peanut protein products. Processors are now beginning to develop some of the new and expanded areas for peanut utilization dealing with high protein meals. flours, concentrates, and isolates. Furthermore, the behavior of specific peanut components must receive more research emphasis if the potential of peanut products as functional ingredients is to be fully realized. One example of the significance of such studies is presented in this paper in that adjusting the pH of peanut meal suspensions in water from 6.7 to 4.0 to 8.2 (or water and low salt mixture from pH 6.7 to 1.5) dramatically improved their functional behavior in emulsion formation.

## ACKNOWLEDGMENT

The authors gratefully acknowledge the capable technical assistance of Linda Garrison and Margree Ector and the photography of Leroy Hicks.

## LITERATURE CITED

- American Oil Chemists' Society, "Official and Tentative Methods", 3rd ed, Chicago, Ill., 1970.
- Ayres, J. L., Branscomb, L. L., Rogers, G. M., J. Am. Oil Chem. Soc. 51, 133 (1974).
- Basha, S. M. M., Cherry, J. P., J. Agric. Food Chem. 24, 359 (1976).
- Brown, D. D., Ph.D. Thesis, University of Georgia, Athens, Ga., 1972
- Cherry, J. P., Dechary, J. M., Ory, R. L., J. Agric. Food Chem. 21,652 (1973)
- Cherry, J. P., McWatters, K. H., Holmes, M. R., J. Food Sci. 40, 1199 (1975).
- Ezekiel, M., "Methods of Correlation Analysis", Wiley, New York, N.Y., 1941.
- Fleming, S. E., Sosulski, F. W., Kilara, A., Humbert, E. S., J. Food Sci. 39, 188 (1974).
- Fontaine, T. D., Burnett, R. S., Ind. Eng. Chem. 36, 164 (1944).
- Griswold, R. M., "The Experimental Study of Foods", Houghton Mifflin Co., New York, N.Y., 1962.
- Hermansson, A.-M., Akesson, C., J. Food Sci. 40, 595 (1975).
- Johnson, D. W., J. Am. Oil Chem. Soc. 47, 402 (1970).
- Jones, D. B., U.S. Dep. Agric. Circ. No. 183 (1931). Lawhon, J. T., Cater, C. M., Mattil, K. F., Cereal Sci. Today 17, 240 (1972).
- Lin, M. J. Y., Humbert, E. S., Sosulski, F. W., J. Food Sci. 39, 368 (1974).
- Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., J. Biol. Chem. 193, 265 (1951).
- Mattil, K. F., J. Am. Oil Chem. Soc. 48, 477 (1971).
- McWatters, K. H., Cherry, J. P., J. Food Sci. 40, 1205 (1975). Quinn, M. R., Beuchat, L. R., J. Food Sci. 40, 475 (1975).
- Rhee, K. C., Cater, C. M., Mattil, K. F., J. Food Sci. 37, 90 (1972).
- Saffle, R. L., Adv. Food Res. 16, 105-160 (1968).
- Wolf, W. J., Cowan, J. C., Crit. Rev. Food Technol. 2(4), 81 (1971). Wu, Y. V., Inglett, G. E., J. Food Sci. 39, 218 (1974).

Received for review December 15, 1975. Accepted February 9, 1976

# An Enzymic Assay for Acetate Fruit Juices and Wines

# Leo P. McCloskev

A rapid enzymic method for the determination of acetate anion in juices is described. It utilizes three coupled enzyme mediated reactions so that the spectrophotometric determination of NADH is stoichiometrically related to acetate anion levels. The method is described for use with vegetable and fruit juices, and wine. The recovery of acetate anion was 97-102%, with standard deviations as good as 0.25mg/100 ml (2.5 ppm) in an apple juice with 22 mg/100 ml of acetate anion. The procedure was compared to a steam distillation procedure, showing that the enzymic assay was vastly superior as to accuracy and recovery of acetate. The procedure should be of advantage in agricultural areas involving the processing of fruits and their products, including fermented beverages. One operator can perform 200 assays per day manually and more with some automation.

There are various enzymatic assays for the carboxylic acids, including acetic acid, described in the literature (Bergmeyer, 1963, 1974b; Holz and Bergmeyer, 1970; Postel and MacCagnan, 1971). The availability of enzymes commercially at reasonable prices is a limiting factor in the feasibility of the use of some of these assays (Bergmeyer, 1974a; Lundquist et al., 1961).

A practical and popular method used for the measurement of acetate in biological fluids is that of Rose

Ridge Vineyards Inc., 17100 Montebello Rd., Cupertino, California 95014, and Santa Cruz Agricultural Chemistry, 339 Stanford Ave., Santa Cruz, California 95062.