boiling temperature of the liquid in the still pot will be high, and an excessive amount of pitch formation will result. It is therefore important to keep the pressure drop as low as possible.

When the two columns are compared on the basis of pressure drop per theoretical plate, the data show that the bubble-cap column is more satisfactory in distilling fatty acids than is the fibrous-glass packing. A comparison of the data in Table IV with that in Table VI shows that for the same vapor velocity, the pressure drop per theoretical plate is about five times as great for the fibrous glass packing as for the bubble-cap column.

Although the allowable vapor velocity is lower for the bubble-cap column used, this should be improved by increasing the plate spacing. It was pointed out by Neureuther (7) that the H.E.T.P.'s on small laboratory columns are generally lower than on commercial columns. Therefore as the size of the column increases to commercial size, the H.E.T.P. and therefore the pressure drop per theoretical plate would be expected to increase. On the other hand, an increase in size of a bubble cap column might even increase the plate efficiency, due to better plate design and to liquid cross-flow. For larger sizes, the comparison should therefore be even more favorable to the bubble-cap column.

Conclusions

1. Vapor-liquid equilibrium data are presented for the systems lauric acid-myristic acid, myristic acid-palmitic acid, and palmitic acid-stearic acid at four mm. pressure. Deviations from Raoult's law are observed.

2. The performance of a small glass column packed with fibrous glass was studied. The H.E.T.P. was 30 to 35 inches and showed no consistent variation with composition. Above a vapor velocity of about seven feet per second, the H.E.T.P. increased.

3. The performance of a small bubble-cap column was studied. The overall plate efficiency was about 35%. The allowable vapor velocity was between four and five feet per second.

4. For the distillation of fatty acids at four mm. of Hg pressure the bubble-cap column was more satisfactory than the packed column containing fibrous glass since the pressure drop per theoretical plate was about one-fifth as great at comparable capacities. The comparison should be even more favorable to the bubble-cap column with larger sizes.

Nomenclature

G.....pounds of vapor per square foot of cross sectional area per hour.
H.E.T.P...height equivalent to a theoretical plate.
K_V.....a constant.
P.....total pressure, atmospheres.
p_1.....vapor pressure of substance "1," atmospheres.
ΔP.....pressure drop, inches of water per foot of packing.
u.....allowable vapor velocity, feet per second.
x_1.....mol fraction of substance "1" in the liquid phase.
y_1.....mol fraction of substance "1" in the vapor phase.
γ.....activity coefficient.
ρ_L.....density of the liquid, pounds per cubic foot.
ρ_V.....density of the vapor, pounds per cubic foot.
Φ.....(density of vapor/0.075.)$^{1/2}$

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Evaluation of Peanut Protein for Industrial Utilization. A Review[1]

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Introduction

THE properties of peanut protein which are important for its industrial utilization as adhesives, sizes, water-thinned paints, and fibers are primarily physico-chemical in nature. To date the specific amino acid composition of the protein has not been shown to affect to any marked degree the industrial applications of the protein. The following discussion of the evaluation of peanut protein for industrial utilization is concerned therefore only with the physico-chemical characteristics of the components or fractions of peanut protein.

In 1913 Lichnikov (24) reported the separation and hydrolysis of albuminous substances from peanuts. The proteins were extracted from oil-free meal by means of water, 70% alcohol, 10% solution of sodium chloride, and 0.25% solution of potassium hydroxide in that order, thereby dividing the whole protein fractions into albumin, glutin, and globulin components.

In 1916 Johns and Jones (18-21, 23) reported and named the two major fractions of peanut protein, based on differences in solubility properties. They suspended 500 grams of oil-free peanut meal in 2.5 liters of a 10% solution of sodium chloride, clarifying the suspension by filtration. The protein fraction isolated by precipitation and filtration of the clarified solution by addition of ammonium sulfate to produce

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0.2 saturation was designated as arachin. The filtrate resulting after the separation of the arachin fraction was saturated with ammonium sulfate whereupon a second protein precipitated which was designated as conarachin. These authors also reported that the filtrate from the second precipitation contained an albumin which coagulated on heating the solution to 65°-70°C.

Data obtained by ultracentrifugal sedimentation of peanut protein reported by Eirich and Rideal (11) in 1940 showed that the protein mixture contained five fractions with molecular weights of 600,000, 400,000, 140,000, 30,000, and 20,000. Application of the same method to ovalbumin indicated it had a molecular weight of 49,000.

Irving, Fontaine, and Warner (15, 17) analyzed solutions of peanut protein in ammonia buffer by means of electrophoresis (0.2 M ammonia and 0.1 M hydrochloric acid, pH 9.26, at 4°C., 0.1 ionic strength) and reported that arachin is composed of two fractions and conarachin of one major and several minor components.

In 1944 Compbell and Johnson (10) and in 1946 Johnson (22) reported new investigations of the properties of peanut protein, using ultracentrifugal, diffusion, and electrophoretic methods of analysis. They fractionated peanut protein by means of solubility methods, using a 10% solution of sodium chloride to peptize the protein contained in peanut meal. The sodium chloride-protein solution was clarified, and the proteins were precipitated as fractions by means of dilution of the solution with water, acidification of the solution to pH 5, or addition of ammonium sulfate to the solution until it was 40% saturated with the salt. The three fractions obtained in this manner had sedimentation constants of 14.6, 9.5, and 6.5 Svedberg units. By means of diffusion methods the molecular weight of the 14.6-fraction was determined to be equal to about 396,000. Utilizing a 0.05 M phosphate buffer as a solvent, it was found by means of electrophoretic analysis that each protein fraction contained at least two components when analyzed in solutions having 0.5 to 1.5% protein concentration.

It may be concluded on the basis of the aforementioned observations that peanut protein is composed of several different components or fractions. The physico-chemical properties of these components when they are dispersed in colloidal solutions determine the industrial applications of peanut protein.

The properties of these colloidal solutions of protein are complex, and up to the present time no definitive explanation of these properties has been proposed. However certain procedures have been established to evaluate peanut protein for use in different products. These procedures may be grouped as follows: 1. determination of the solubility of the protein contained in oil-free peanut meal in different solvents; 2. analysis of peanut protein isolated from peanut meal to determine its nitrogen and ash contents, solubility in different solvents, and color of the protein when dispersed in sodium hydroxide solutions: 3. determination of the viscosity characteristics of concentrated colloidal solutions of peanut protein, for example, viscosity-time changes, tackiness, and adhesiveness; 4. specific product testing.

Solubility of Protein of Oil-Free Meal

The solubility of the protein constituents contained in oil-free peanut meal in different solvents is important in the industrial utilization of the meal and protein in two ways, namely, with respect to the type of reagents required in isolating the protein and the yield of isolated protein. It has been reported that good yields of protein can be obtained from peanut meals prepared by removing the oil either by mechanical methods of expression or by solvent extraction methods (1, 5, 6, 12). However there is a wide variation in the pH of the solutions used to prepare the protein, depending on the previous history of the peanut meal. If solutions having very high or very low pH values are required for protein isolation, it is necessary to construct the equipment used for extraction with acid-alkali resistant material. This structural material is more expensive than that required for equipment where solutions having nearly neutral pH values may be used.

In order that peanut meals may be produced which contain proteins which are very soluble in water or nearly neutral salt or alkali solutions, the oil must be removed from peanut flakes with organic solvents at low temperatures. The color and solubility of the isolated protein are also affected by the modification of the protein during the removal of the oil from the peanut flakes.

The solubility of the protein contained in peanut meal in different solvents is determined as follows: 2.5 grams of meal are suspended in 100 ml. of the solvent and allowed to stand for 3 hours at 25°C. with occasional shaking; the suspension is clarified by centrifugation for 15 minutes at 900 times gravity. The percentage of the total nitrogen of the meal in the clear centrifugate is determined by the macro-Kjeldahl method (12). More than 80% of the nitrogenous constituents of solvent-extracted peanut meal should dissolve in water (pH 6.2-6.8); 90 to 95% of these constituents should dissolve in solutions of sodium hydroxide (pH 7.5) or 0.5 M sodium chloride (pH 6.2-6.8).

Analysis of Isolated Protein

The nitrogen and ash contents of isolated protein are reliable criteria of the quality of the product. Peanut protein, containing more than 16% nitrogen and less than 2% ash, has satisfactory properties for the production of fibers and adhesives which require the use of raw materials containing more than 95% protein (4, 7, 8, 16, 25). The nitrogen content of the protein may be determined by means of the macro-Kjeldahl method, and the ash content by burning a 2- to 5-gram sample of the isolated protein at 600°C. for four hours.

The solubility of the isolated protein in different solvents is determined, as follows: 1.25 grams of isolated protein is suspended in 100 ml. of solvent and allowed to stand for 3 hours at 25°C. with occasional shaking. The suspension is clarified by centrifugation, and the percentage of the total nitrogen of the protein in the clear centrifugate is determined by the macro-Kjeldahl method (14). This test indicates the effect of the pH and ionic strength of the solvent on the solubility of the protein. However in the preparation of more concentrated solutions of protein the amount of dispersing reagent used per unit weight

of protein is also a factor in determining the solubility of the protein.

If the pigments present in the skins or testa of the peanut kernel are not removed or rendered insoluble in alkali prior to the extraction of the oil and protein, they will separate partly or completely with the protein. Alkaline solutions of protein containing the pigments that are present in the skin have a brown to red color.

The evaluation of the color of peanut protein is difficult and in general subjective. A method has been proposed for determining the color of the protein by analyzing the light absorption of a solution of 100 mg. of protein dissolved in 25 ml. of 0.02 *N* sodium hydroxide (pH 12.2). The dominant wave length of the pigments in the solutions ranges from 570 to 575 millimicrons. The luminous transmittance of light-colored protein solutions at these wave lengths is better than 90% (13).

A rapid evaluation of the color of the protein may be made by visually comparing alkaline solutions of peanut protein with casein solutions or standard decolorized peanut protein solutions. Peanut protein for use as a standard may be extracted from peanut meal from which the skins have been removed by hand. The lightest-colored peanut proteins give a yellow-tinted alkaline solution.

Viscosity Characteristics of Solutions of Peanut Protein

The physical properties of protein solutions have been of major importance in determining their industrial applications. Proteins for use in adhesives must, when dispersed in solution, have relatively stable and low viscosities and high tack, and they must produce a strong adhesive and water-resistant bond. The viscosity of the solutions should be uniform throughout a given gluing operation in order that the amount of adhesive applied to a unit area of the material being bonded is a constant value. The glue solution should develop sufficient tack to bond the material as rapidly as possible for continuous gluing operations. If sufficient tack is developed on initial application of the adhesive to the material being bonded, final conditioning of the bond can take place after the glued product has been removed from the production line. Protein-adhesive bonds must be more water resistant than starch-adhesive bonds in order that the former can compete economically with the lower priced starch-adhesive.

Proteins from oil seeds are usually globular in nature, and it has been reported that their solutions have higher initial viscosities than comparable casein solutions and that the viscosity of the protein solutions tends to increase as the solutions are matured. To produce vegetable proteins which will yield solutions having stable and low viscosities, it has been a common practice to modify the protein by means of partial hydrolysis with alkali, various salts, or enzymes or by means of heat. These modified proteins when dispersed in solution usually have a lower and more stable viscosity than unmodified proteins. However the modified proteins have less tack and give a weaker adhesive bond than the unmodified proteins.

A consideration of the reactions by which the proteins are dispersed in solution has made it possible to prepare solutions having low and stable viscosities containing unmodified vegetable proteins. Three steps are involved in preparing a colloidal solution of protein, namely, 1. reducing of the particle size of the protein to the colloidal range, 2. hydrating of the protein molecules, and 3. altering the charge on the molecules so that it differs from the isoelectric charge. It has been observed at the Southern Regional Research Laboratory that the order in which these steps are accomplished affects the viscosity characteristics of the resulting solution. For example, if the protein is wetted with water momentarily and a dispersing reagent, such as sodium hydroxide, is added, the protein solution has a higher initial viscosity, and this value usually increases as the solution is matured. However if the protein is thoroughly wetted with water and the particle size of the protein is reduced, for example by vigorous agitating of the protein-water mixture, followed by addition of the dispersing reagent, this protein solution has a relatively low and stable viscosity.

A typical preparation of a low viscosity dispersion is made as follows: 1. 44 grams of protein is mixed with 200 ml. of water in a Waring Blendor at room temperature (23°-27°C.) for about 15 minutes to subdivide and thoroughly hydrate the protein particles, 2. stirring the protein-water suspension, and adding 2.5 grams of sodium hydroxide (preferably as a 50% solution), 3. followed by stirring to disperse completely the protein within 15-20 minutes. The resulting protein solution has a pH of 12.0 and a stable viscosity of 30 centipoise.

A protein solution prepared by omission of the first step which involves vigorous agitation of the protein-water suspension prior to addition of alkali may have a viscosity of 10 to 20 poise one hour after protein is dispersed, and the viscosity of the solution may increase to several hundred poise as it is matured (9).

The relative tackiness of a protein solution can be measured by extruding the solution through an orifice into a coagulating bath in a manner similar to that used in the production of synthetic fibers and determining the maximum elongation which the filament will take before breaking (2).

Product Evaluation

The only way to evaluate the properties of peanut protein for a specific end use is by making the desired products containing the protein and measuring their properties. The adhesive strength of peanut protein when used in coating formulations may be determined by preparing coated paper using the protein as the bonding agent between the mineral matter and the paper and by using the standard Dennison waxes to evaluate the strength of the bond (3). The adhesive strength of peanut protein for use in plywood glues may be evaluated by gluing wood and measuring the strength of the joint obtained (7). Similarly, other adhesive products containing peanut protein can be evaluated.

The properties of peanut protein for use in the production of a synthetic fiber can be determined by analyzing the protein with respect to quality and color as suggested above, by preparing spinning solutions and extruding of fiber, and by measuring the physical properties of the fiber.

Summary

The properties of peanut protein have been briefly reviewed, and procedures for the evaluation of this protein for different industrial uses have been indi-

cated. The determination of the solubility of the protein in different solvents, analysis of the isolated protein to determine its nitrogen and ash contents and its color when dispersed in sodium hydroxide solutions, determination of the viscosity characteristics of concentrated peanut protein solutions, and specific product testing are used to evaluate peanut protein for industrial utilization.

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Determination of Free Gossypol in Cottonseed Materials

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SEVERAL spectrophotometric methods (1, 2, 3) have been proposed for the determination of free gossypol in cottonseed products. They require extensive manipulation and employ either lengthy or tedious extraction procedures or reagents lacking in specificity. Difficulties have been experienced with them in the hands of independent investigators (4).

A method is proposed in which the gossypol is extracted with 70% aqueous acetone and determined by the color developed with *p*-anisidine. In preparing pure gossypol for another investigation (5), it was observed that gossypol is quite stable in acetone. This stability appears to be due to the formation of a "loose" acetone-gossypol compound which is easily dissociated and in which the aldehyde groups of gossypol are stabilized. This compound was mentioned briefly by Carruth (6) and is being investigated further.

Aniline is usually employed for the colorimetric determination of gossypol because it reacts with gossypol to form dianilino gossypol (1, 2). In order to avoid the repeated distillation of aniline, several solid aromatic amines were investigated, namely, *o*-dianisidine, *p*-aminobenzoic acid, α-napthylamine, β-napthylamine, benzidine, *o*-tolidine, 2,4-diaminophenol, and *p*-anisidine. All of these compounds were found to give yellow-colored solutions when reacted with gossypol. In all cases the presence of a trace of acetic acid led to the development of a more intense color. *p*-Anisidine was selected because it is a white crystalline solid (m.p. 57°C.), easily purified, and stable in the solid state.

Reagents

1. *70% Aqueous Acetone (by Volume).* 700 ml. A.C.S. grade acetone plus 300 ml. distilled water.

2. *95% Ethyl Alcohol, Aldehyde Free.* Reflux U.S.P. 95% ethyl alcohol over potassium hydroxide and aluminum (10 grams KOH plus 5 grams aluminum per liter) for one hour and distill.

3. *Glacial Acetic Acid.* A.C.S. reagent.

4. *p-Anisidine.* Prepare a saturated solution of technical grade *p*-anisidine in hot water and filter through paper. Upon cooling in a water bath with stirring at room temperature the black oxidation products settle out on the sides of the beaker. Decant the slightly yellow supernatant into a clean beaker and keep overnight in a refrigerator. The crystalline product is usually pure. If slightly yellow, recrystallize. Dry in a desiccator over phosphorus pentoxide and store in a brown bottle. *p*-Anisidine in the solid state has proven to be stable for at least several months.

5. *p-Anisidine-Acetic Acid-Ethyl Alcohol Reagent.* Dissolve 0.500 gram recrystallized *p*-anisidine in purified ethyl alcohol. Add 1 ml. of glacial acetic acid and make to 50-ml. volume with the ethyl alcohol. Use 3 ml. of this reagent for each determination. Since *p*-anisidine is not stable in solution, this reagent must be made fresh each day.

6. *Ethyl Alcohol-Acetic Acid Reagent.* Dilute 1 ml. glacial acetic acid to 50-ml. volume with purified ethyl alcohol. Use 3 ml. of this reagent for each gossypol blank.

7. *Standard Gossypol Solutions.* Weigh accurately 25 mg. of pure gossypol, dissolve in 70% aqueous acetone and make to 200-ml. volume with 70% aqueous acetone. This stock solution of gossypol contains 0.125 mg. gossypol per ml. Dilute 2, 5, 10, 15, 20, 25, 30, 35, and 40 ml. of this stock solution to 50 ml. with 70% aqueous acetone. Two-milliliter aliquots of each solution are used for developing the standard curve as outlined below.

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