Effect of Organic Solvents on the Proteins Extracted from Peanuts

Peanuts were defatted with three organic solvents. The proteins in the meals were then extracted with buffer and compared chromatographically with the proteins from nonsolvent-treated peanuts. Several changes in the chromatograms of solvent-treated proteins are noted which could affect their use in artificial milk-type beverages. Some of the proteins become more insoluble, some appear to be partially dissociated, and others seem to form families of proteins with similar chromatographic properties. These observations suggest possible modifications in their molecular conformations. The albumin and the conarachin fractions show the most drastically altered solubility properties. The elution pattern of arachin is affected most by acetone extraction.

Crude oil is normally removed from oilseeds by mechanical pressing, extraction with solvents, or a combination of the two methods (Gastrock *et al.*, 1965). The cake or meal residue from edible oilseeds is further processed for use as a feed or as food products (Pominski *et al.*, 1951). Processing conditions are selected to remove the maximum amount of oil with the least amount of harmful effects on the nutritive value of the meal. One problem in the removal of oil by solvent extraction is a possible adverse effect on the proteins of the seed.

The major reserve proteins of the peanut are arachin and conarachin (Dechary *et al.*, 1961; Johnson and Shooter, 1950; Tombs, 1963). They have been localized at the subcellular level in the cotyledon–arachin in the protein bodies and conarachin in the cytoplasm (Altschul *et al.*, 1964). Both proteins have been well characterized by gel electrophoresis (Tombs, 1963) and by column chromatography (Dechary *et al.*, 1961). These two proteins represent almost 90% of the proteins of the peanut. Thus, any changes in their quantity or quality could affect the protein value of the meal.

The authors have examined the chromatographic patterns of the proteins from peanuts prepared under different conditions. This paper describes the changes in the chromatograms as a result of extraction with several organic solvents.

EXPERIMENTAL

Extraction of Native Tissue. Ten grams of peanut cotyledons (Virginia 56-R) were homogenized in 50 ml. of phosphate buffer, pH 7.9, I 0.2 (0.008M $NaH_2PO_4 \cdot H_2O$, 0.064M Na_2HPO_4) using a Servall Omnimixer for 5 minutes at 0° C. The homogenate was clarified twice by centrifugation at 37,000 × G for 30 minutes and subsequently dialyzed against low ionic strength (0.03) phosphate buffer for 24 hours. The solution was allowed to warm to room temperature (25° C.) and recentrifuged as before. The final supernatant—i.e., the approximate middle half in the centrifuge tubes—was isolated with a syringe and needle. The protein concentration of this portion (20 ml.) was 25 mg. per ml. as assayed by the Lowry method (Lowry *et al.*, 1951).

Preparation and Extraction of Peanut Meals. Five grams of peanut cotyledons were homogenized in 25 ml. of the respective organic solvents (CCl_4, heptane, and acetone) as described above. The homogenate was filtered through a medium-frit glass filter and washed once with equal volumes of solvent at room temperature. In each case, 200 mg. of defatted meal was solubilized in 6.0 ml. of high ionic strength phosphate buffer (I 0.02) and dialyzed against low ionic strength buffer (I 0.03) for 24 hours. The suspension was clarified by centrifugation at 37,000 × G for 30 minutes, isolating the clear solution with a syringe and needle. The protein concentrations ranged from 12 to 15 mg. per ml.

Chromatography on DEAE-Cellulose. In each case, 10 mg. of protein in phosphate buffer, pH 7.9, I 0.03, was adsorbed on 2 grams of DEAE-cellulose. The protein was eluted with a linear sodium chloride gradient ranging from 0.0 to 0.6M NaCl in phosphate buffer and collected in 5-ml. fractions. All experiments were carried out at 25° C. (room temperature). Each fraction was analyzed for protein by the Lowry method (Lowry *et al.*, 1951).

RESULTS AND DISCUSSION

Representative chromatograms of the native cotyledonary proteins and those previously defatted with organic solvents are shown in Figure 1. The two major reserve proteins, α-arachin and α-conarachin, represent the major source of nitrogen within the seed and are therefore metabolically important. Chromatograms B, C, and D obviously show that those proteins eluted between 0.0 and 0.17M NaCl become more insoluble after treatment with organic solvents. Of particular interest is the effect of acetone. There is a decrease in the amount of α-conarachin in the solvent-extracted proteins compared with the native extract. Two changes are observed in the patterns for α-arachin: the concentration of salt at which it is eluted is higher after treatment with acetone, and the protein is partially dissociated into two components. All of the other proteins, after treatment with organic solvents, appear as a family of proteins with very similar ion exchange properties. These observations suggest that modifications in the structures of the proteins have occurred, resulting in changes in their solubilities and ionic properties.

The major proteins of the peanut globulins are primarily anionic species—i.e., they migrate toward the anode when separated by electrophoresis (Tombs, 1963) and are displaced from ion exchange resins by nucleophilic reagents (Dechary *et al.*, 1961)—e.g., the displacement of a negatively charged protein from an anion exchanger by the more nucleophilic chloride ion. Conversely, the minor proteins

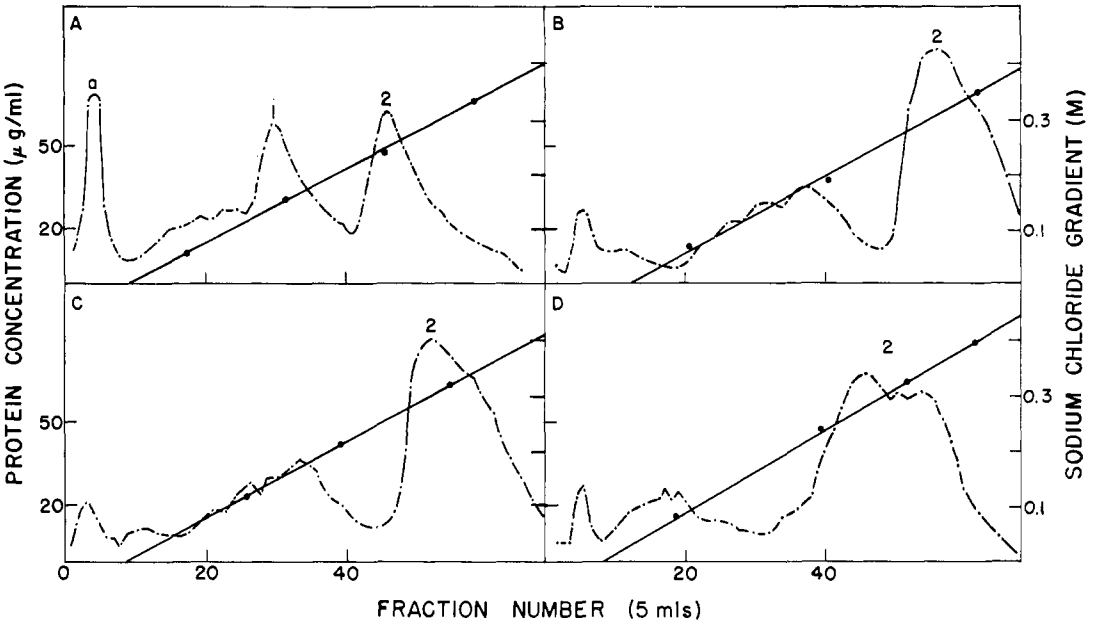

Figure 1. DEAE-cellulose chromatograms of total cotyledonary extract from native and organic-solvent treated samples

A. Native extract
B. Heptane-extracted sample
C. Carbon tetrachloride-extracted sample
D. Acetone-extracted sample
a. Albumins
1. α-Conarachin
2. α-Arachin
For each chromatogram, 10 mg. of protein was adsorbed on 2 grams of DEAE-cellulose and eluted with 500 ml. of a linear NaCl gradient ranging from 0.0 to 0.6*M* in phosphate buffer, pH = 7.9, μ = 0.03. The straight line represents the gradient as measured from the eluate

comprising 10% or less of the seed are water soluble and could be considered "albumins." On the basis of DEAE-cellulose chromatography, however, the fact that they do not adsorb does not imply that they are totally uncharged species. Some of these proteins more than likely are cationic in nature and may have similar but reverse properties of the globulins. Further studies employing a cation exchanger such as CM-cellulose may show this.

The present results show that the albumins and the conarachin fraction are the most drastically altered in solubility properties. Apparently the isoelectric point has changed to a pH nearer neutrality after extraction with organic solvents. The distinct separation of arachin into two closely related components on DEAE-cellulose after solvent extraction is not easily explained. The authors observed that the lipid layer of a peanut homogenate after centrifugation contained much arachin (Neucere, 1967). This would imply the possibility of arachin having the character of a lipoprotein. If this is the case, then polar solvents such as acetone may selectively rupture the weak linkage between lipide and protein, releasing subunits with varying degrees of polarity. The effect of high concentrations of polar solvents such as alcohol or acetone on lipoproteins was reported by McFarlane (1942).

The fact that some of the peanut proteins undergo changes in their physical properties does not imply that their nutritive value has been impaired. These findings are not designed to assess nutritive qualities. Evans and Bandemer (1967) recently reported on the nutritive value of hexane-extracted peanut meal with growing rats. They found that rats fed extracted peanuts supplemented with methionine, isoleucine, and lysine did not grow any better than rats fed the unsupplemented peanuts.

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