Effect of Roasting on the Stability of Peanut Proteins

N. J. Neucere, R. L. Ory, and W. B. Carney

The effect of dry roasting on proteins of the peanut was investigated by chromatography on DEAE-cellulose, polyacrylamide gel-electrophoresis, immunoelectrophoresis, and ultracentrifugation. Several basic changes apparently resulted from roasting. The solubility of the proteins in phosphate buffer was reduced to less than one half. The major reserve protein, α-arachin, increased in electrophoretic mobility but maintained its antigenic structure. Both dissociation and association occurred, as evidenced by sedimentation analysis. All other proteins show modified physicochemical properties, some with changes in primary and/or secondary structures.

The use of oilseed proteins for nutritional purposes has come into prominence because of the needs of an expanding world population. Modern research in the food industry is elucidating the biochemical changes affecting taste, odor, texture, and nutritive value of these proteins under various processing conditions.

Basic research on the peanut proteins has been going on for over a hundred years. The two major globulin fractions, arachin and conarachin, were first isolated by Johns and Jones (1916). Subsequent studies by several investigators (Dechary *et al.*, 1961; Johnson and Naismith, 1953; Johnson and Shooter, 1950; Johnson *et al.*, 1950; Tombs, 1965) further characterized these proteins by precise analytical methods. The findings of Dieckert *et al.* (1962) and Daussant *et al.* (1967) suggest that α-arachin (the major component of the arachin fraction) is located in the protein bodies and that α-conarachin (the major component of the conarachin fraction) is dispersed in the cytoplasm.

The effect of heat on plant proteins has been studied in relation to taste, odor, and nutritive value. For example, Newell *et al.* (1967) reported biochemical changes attributing to "typical" and "atypical" roasted peanut flavor. They suggested a possible reaction between sugars and amino acids to produce specific flavor components (pyrazine derivatives). Bensabat *et al.* (1958) found that cooking peanuts in 5.6 to 6.0% moisture for one hour at 232–234° F. caused a drop from 3.4 to 2.8 grams of lysine (per 16 grams of nitrogen). Protein deterioration during processing could be measured by following the decrease in free epsilon-amino groups of lysine. Evans and Bandemer (1967) showed that the nutritive value of sunflower seed proteins decreases after heating. Others have shown that certain limiting amino acids in chickpea proteins are further reduced on autoclaving (Gonzalez del Cueto *et al.*, 1960).

Roasting of whole peanuts is a prerequisite in the manufacture of candies and peanut butter. Normally, one would expect heating to denature irreversibly all of the proteins, especially those having enzymic and/or antigenic properties. The aim of this study was not to elucidate the precise reactions induced by heating *per se*, but to investigate the physicochemical changes in proteins with respect to their structure, function, and intracellular location. Several analytical methods sensitive to modifications in protein structure, size, shape, and charge—chromatography on DEAE-cellulose, polyacrylamide gel-electrophoresis, sedimentation analysis, and immunoelectrophoresis—were employed. As expected, the albumins and conarachin, the major cytoplasmic globulin, were changed. Surprisingly, α-arachin, the major globulin in the protein bodies, was not drastically altered, as evidenced by immunochemical analyses.

Southern Regional Research Laboratory, New Orleans, La. 70119

EXPERIMENTAL

Roasting. Peanuts (Virginia 56-R, 1966 crop) with the shells removed were dry-roasted in an oven for one hour at 145° C. The outer skins were then removed and the cotyledons homogenized.

Extraction of Proteins. Twelve grams of cotyledons from both roasted and unroasted peanuts were homogenized in 100 ml. of phosphate buffer, pH 7.9, ionic strength 0.2 (0.008*M* $NaH_2PO_4 \cdot H_2O$, 0.064*M* Na_2HPO_4), in 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 following protein concentrations were obtained for the two preparations (approximately 40 ml. for each preparation): roasted extract, 10.0 mg. per ml.; unroasted extract, 25 mg. per ml.

Protein Isolation from the Roasted Extract. Chromatography on DEAE-cellulose was performed according to the method of Dechary *et al.* (1961), employing conditions described in Figure 1. Preparative separation was carried out as follows: 200 mg. of protein in 200 ml. of phosphate buffer, pH 7.9, ionic strength 0.03, from the roasted cotyledons was adsorbed on 10 grams of DEAE-cellulose and eluted with a 100-ml. NaCl gradient ranging from 0.0 to 0.4*M* NaCl in the low ionic strength buffer. The eluate was collected in 10-ml. fractions. The fractions corresponding to *A*, *B*, and *C* of the analytical chro-

matogram shown in Figure 1 were isolated for immunochemical studies. Total protein recovery was approximately 75%.

Analytical Methods. Protein content was determined by the method of Lowry *et al.* (1951). Zone electrophoresis was carried out by the method of Evans *et al.* (1962). Sedimentation analyses were made on a Spinco Model E ultracentrifuge; the coefficients were determined according to Schachman (1957). Immunoelectrophoretic analysis (IEA) was performed according to Grabar and Williams (1953), and immunodiffusion according to Ouchterlony (1949).

RESULTS

Chromatography on DEAE-Cellulose. The chromatograms of the total proteins extracted before and after roasting are shown in Figure 1. The total soluble proteins of the mature peanut have been categorized by Dechary *et al.* (1961) into four groups (I, II, III, IV) based on their elution pattern from DEAE-cellulose (Figure 1,*a*). The patterns from roasted and unroasted peanuts are very similar, with one major exception; groups II and III, which are part of the conarachin system, appear as a wide single peak in the roasted extract, *B*—possibly part of group II appears as the small peak at about fraction 15. Also, components *B* and *C* (which are being called α-conarachin and α-arachin for purposes of identification), are eluted at a slightly different salt concentration after roasting. The level of proteins which do not adsorb on DEAE-cellulose, *A*, is slightly higher after heating—i.e., the ratio *A* to (*B* + *C*) is greater than I to (II + III + IV). This evidence does not specify whether these fractions are identical to the ones categorized in unroasted peanuts.

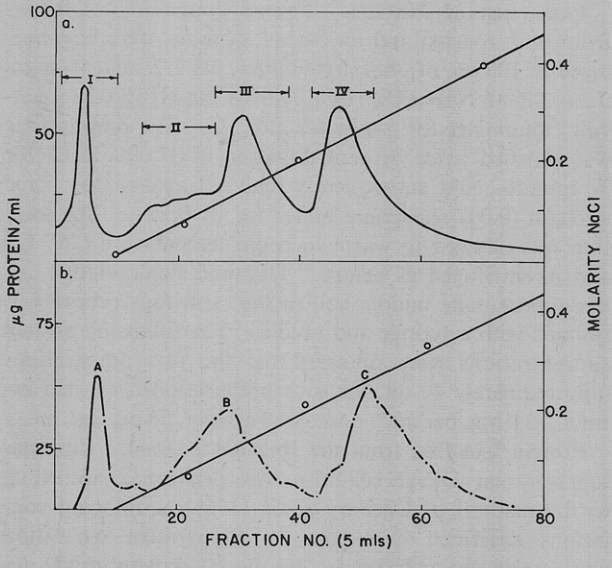

Figure 1. DEAE-cellulose chromatogram of total soluble peanut proteins before, *a* and after, *b*, roasting

Fractions *A*, *B*, and *C* correspond to isolates for immunochemical analysis. Ten milligrams of protein was adsorbed on 2 grams of DEAE-cellulose and eluted with 500 ml. of a linear gradient ranging from 0.0 to 0.6*M* NaCl in phosphate buffer, pH 7.9, ionic strength, 0.03. The straight line represents the NaCl gradient as measured from the eluate

Polyacrylamide Gel Electrophoresis. An electrophoretic study of the arachin system by Tombs (1965) showed that the "arachin group" is dispersed as several zones in different states of aggregation, with some zones possibly related to the conarachin system. The present results (Figure 2) indicate a less complicated pattern, probably owing to the difference in gel concentration. The authors used 5% acrylamide gel, compared to 7.5% by Tombs. Comparison of the two preparations at varying concentrations (1 to 2 *vs.* 3 to 4) clearly shows major differences in the number of components. The first major zone from the origin in the unroasted sample (Figure 2,2) decreases considerably after roasting (Figure 2,4). Also, uncharged or positively charged species—and possibly high polymers remaining at the origin in the unroasted sample—are absent after heat treatment. The two broad zones closest to the anode appear to be identical in both preparations.

Immunoelectrophoresis and Immunodiffusion. An extensive study of the peanut proteins by immunochemical methods has been made by Daussant *et al.* (1967). They identified the two major proteins, α-conarachin and α-arachin, in an extract of the total proteins. The identification of α-arachin in Figure 3 is based on part of this work. The immunoelectrophoretic patterns of the total proteins from the unroasted (Figure 3,1) and the roasted seed (Figure 3,2) are strikingly different. Only two from approximately 14 components remain antigenic after heating. The isolated fractions (3,4,5,6) described in the legend are all antigenically inactive except the one corresponding to α-arachin (group IV of Dechary *et al.*). Thus, α-arachin is the only protein of the peanut not undergoing drastic structural changes after heating. Part 8 in Figure 3 shows an increase in the diffusion coefficient of α-arachin after

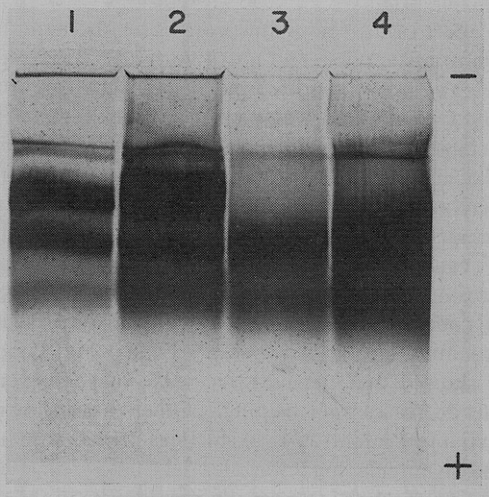

Figure 2. Zone electrophoresis of total cotyledonary proteins in phosphate buffer, pH 7.9, ionic strength 0.03, before and after roasting

1,2. Unroasted extract containing 0.05 and 0.1 mg. protein in each reservoir, respectively
3,4. Roasted extract, same quantities as in 1 and 2
samples were dialyzed against phosphate buffer, pH 7.9, ionic strength 0.03, before electrophoresis. Separation conducted in this buffer for 3 hours in a vertical apparatus at 17.0 volts/cm. at 5° C.; gel concentration, 5% cyanogum-41 gelling agent

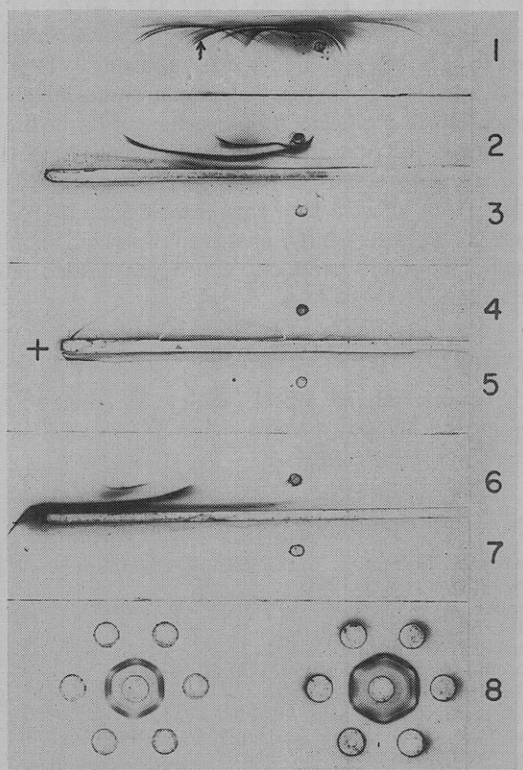

Figure 3. Immunoelectrophoresis and immunodiffusion of total roasted and unroasted peanut proteins and of fractions from the roasted extract (1.5% agar)

1. Total proteins from unroasted peanuts (arrow indicates α-arachin)
2. Total proteins from roasted peanuts
3. Dialyzate from roasted peanut extract
4. Fraction *A* from chromatogram described in Figure 1, *b*
5. Fraction *B* from Figure 1, *b*
6. Fraction *C* from Figure 1, *b*
7. No sample, empty reservoir. The troughs in the above were filled with immune serum against total proteins from the unroasted seed
8. Left side, outer wells contain total proteins from roasted seeds, 0.1 mg. in each well. Right side, outer wells contain total proteins from unroasted seeds, 0.1 mg. in each well. The central wells of the immunodiffusion gels were filled with immune serum to α-arachin. Migration of antigen toward the center well provides a measure of the diffusion coefficient

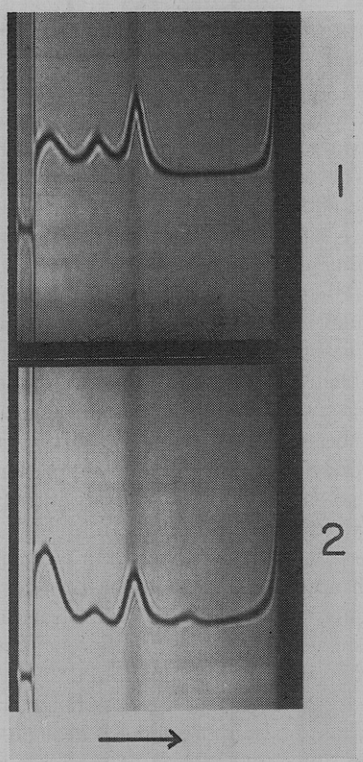

Figure 4. Sedimentation patterns of the total proteins extracted before and after roasting. Migration is from left to right

1. Total proteins from unroasted seeds: sedimentation coefficient from left to right, 2.2 *S*, 8.8 *S*, and 13.8 *S*
2. Total proteins from roasted seeds: sedimentation coefficient of the two major fractions from left to right, 1.8 *S* and 13.6 *S*

Experiments were made in phosphate buffer, pH 7.8, ionic strength, 0.2. No corrections were made to reduce sedimentation coefficients relative to the viscosity and density of water at 20° C. and for zero concentration; the photographs were taken 28 minutes after reaching top speed, 59,780 r.p.m. *S* refers to the Svedberg unit.

roasting, suggesting a possible release of subunits, all or part of which remain antigenic, but merely dissociated.

Sedimentation Analysis. A sedimentation study of peanut proteins by Johnson and Naismith (1953) showed a complex reversible association-dissociation system dependent upon pH and ionic strength. Sedimentation patterns of the two present preparations at high ionic strength (0.2) are shown in Figure 4. The heavy component in the untreated sample (Figure 4,1) has a sedimentation coefficient of 13.8 *S*, compared with 13.6 *S* for the comparable component in the roasted extract (Figure 4,2). The value for arachin determined by Johnson and Shooter (1950) is 14.6 *S* at 0.1 ionic strength (14.0 corresponds to approximately 380,000 molecular weight). The roasted extract shows a larger quantity of low molecular weight fragments, 1.8 *S*. A trace component larger than α-arachin is also observed. The overall results are quite complicated; however, both association and dissociation seem to occur as a result of roasting.

DISCUSSION

The results of this study show three basic observations as a consequence of heat treatment. First, the concentration of total soluble protein is decreased considerably—25 mg. per ml. for unroasted peanuts, 10 mg. per ml. after roasting. Second, the antigenic structure of the major reserve protein, α-arachin, is unchanged. The third observation is that other proteins have modified physicochemical properties, some with an apparent maintenance of primary structure.

Plant proteins are among the most complex and diverse substances in biological systems. The complexity is related to their structure and function, enzyme activity, immunological activity, cell morphology, etc. In view of

this, it is surprising that some can withstand 145° C. heating for an hour. Lenarskii (1951) and Pence *et al.* (1953) have reported the effects of heat on wheat proteins. They showed that the rate of denaturation was increased by increased moisture content of the grain and/or heating temperature. The peanuts in this study were dry roasted to minimize the effect of moisture. Protein solubility is related to the isoelectric point. The total soluble proteins before roasting was 25 mg. per ml.; after roasting this dropped to 10 mg. per ml. Hence, the loss in protein solubility could be attributed to irreversible denaturation, a change in the isoelectric point, or both.

Some problems remain in trying to correlate results obtained using two different physicochemical methods. Analysis of the two preparations on DEAE-cellulose, for example, shows fewer differences in the patterns than the comparable gel-electrophoretic analysis on polyacrylamide gel. The patterns of the proteins from both roasted and unroasted seeds (Figure 2) have two broad bands near the anode which appear identical in the two samples; these more than likely belong to the arachin fraction. Medvedeva (1965) showed that the electrophoretic pattern of heat-treated gliadin was almost unaffected by mild heating (40° C., for 15 minutes), but the proportions of four fractions were changed by more intense heat (70° C. for 60 minutes); an apparent merging of the most mobile fractions was observed. A loss of free epsilon-amino groups of lysine by heating as reported by Bensabat *et al.* (1958) could alter the charge on the surface of the proteins, influencing electrophoretic migration. The question then arises whether a given peak for column chromatography can always be identified with a particular zone from gel-electrophoresis because of the association-dissociation properties involved. Because components *A* and *B* from Figure 1 do not give the typical precipitin reaction (Figure 3), it is suggested that they are not subunits of α-arachin.

The fact that α-arachin increases in electrophoretic mobility in agar after purification is not easily explained. Daussant *et al.* (1967) have suggested a possible deamination of glutamine and/or asparagine which could produce a net increase in negative charge of this protein (increased number of carboxyl groups). This effect was obtained within an hour by the action of trypsin. In the present study, however, one would expect proteolytic enzymes to be inactivated by the heating. Consequently, the actual cause may be due to induced charges by ionic groups on the DEAE-cellulose itself. Weak ionic bonds on proteins can be altered by this adsorbant. This occurred with castor bean proteins separated by chromatography on DEAE-cellulose and subsequently analyzed by gel-electrophoresis (Ory *et al.*, 1967).

α-Arachin is located in the aleurone grains of the cell (Daussant *et al.*, 1967; Dieckert *et al.*, 1962). The membrane around the particles may protect the molecules to a certain extent, since light microscopy showed spherical bodies which are still intact after roasting. The large amount of oil surrounding the protein bodies might also have a protective influence on α-arachin. For the present, only qualitative interpretation of the data is possible. However, these results indicate that this major storage protein is not affected to a significant degree by roasting (Figure 3). Consequently, unaltered α-arachin should be present in peanut products which are heated during processing.

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