pulp of Lo Han Kuo fruits contains a sweet principle.

Takemoto and co-workers (1983a) have reported the isolation of two other sweet triterpene glycoside constituents of *T. grosvenori* fruits in addition to mogroside V, namely, mogrosides IV and VI, which have molecular weights of 1124 and 1448 dalton, respectively. While mogroside VI was found to occur in very small quantities, mogroside IV was obtained in a yield almost as high as mogroside V (Takemoto et al., 1983a). During our work on *T. grosvenori* fruits, we have not observed by analytical TLC or HPLC either mogroside IV or any sweet triterpene glycoside less polar than mogroside V. However, since mogrosides IV and V are clearly separable by reversed-phase HPLC, in which a 25 cm × 4 mm Nucleocil C_{18} column was eluted with 42% ethanol (Takemoto et al., 1983a), it is not expected that significant amounts of mogroside IV in *T. grosvenori* samples will affect the validity of the present HPLC assay for mogroside V.

In other work performed in this laboratory on mogroside V, this compound has been shown to be nonmutagenic and to produce no mortality in acute toxicity experiments on mice at doses up to 2 g/kg body weight and to exhibit an equivalent molar sweetness intensity to the *ent*-kaurene glycoside, stevioside, when tested against a standard sucrose solution by a human taste panel (Kinghorn et al., 1985, unpublished results). These attributes, coupled with the high mogroside V levels in dried *T. grosvenori* fruits that are reported here, could serve to stimulate further study as to the suitability of extracts of the fruit of this plant and its constituents as alternative high-intensity sweeteners. It has been suggested already that *T. grosvenori* may be a suitable species for introduction into the United States (Swingle, 1941).

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Changes in the Polypeptide Composition of Peanut (*Arachis hypogaea* L.) Seed during Oil Roasting

Sheikh M. Basha* and Clyde T. Young

Free amino acids and sugars, released during roasting, are known to be major flavor precursors in roasted peanuts and they give rise to pyrazine compounds via Millard sugar–amine type reaction. In order to identify the protein/polypeptide source of these amino acids, peanut (*Arachis hypogaea L.*) seeds of Virginia, Runner, and Spanish market types were roasted in peanut oil for 0–12 min and protein was extracted and examined by gel filtration and gel electrophoresis. Gel filtration studies indicated that roasting caused a decrease in the methionine-rich proteins and aggregation of arachin proteins. Gel electrophoresis studies also showed a decrease in the methionine-rich protein and their polypeptides. In addition, a polypeptide with a molecular weight of 70 000 also gradually decreased during roasting. In contrast, the protein and polypeptide composition of arachin remained relatively unchanged during the 12-min roasting period. It is suggested that the polypeptide/s of methionine-rich protein may be involved in the formation of pyrazine compounds.

The sugar-amine nonenzymatic browning reaction has been shown to be involved in roasted peanut flavor formation (Pickett and Holley, 1952; Newell et al., 1967; Mason et al., 1969). Free amino acids and free sugars which are released during roasting are known to be major flavor precursors in roasted peanuts (Newell et al., 1967) and they give rise to pyrazine compounds via Millard sugar–amine type reaction (Mason et al., 1967; Johnson et al., 1971; Walradt et al., 1971). Koehler et al. (1969) investigated the pathway for the formation of alkylated

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pyrazine compounds in amino acid–carbohydrate model systems of low water content.

The free amino acid content of raw peanut varies among varieties, planting locations, and maturities (Young et al., 1974; Oupadissakoon et al., 1980). Newell et al. (1967) analyzed four Argentine Spanish peanut samples for changes in amino acid and monosaccharide concentrations during roasting, while Young et al. (1974) measured the changes in amino acids during dry, oil, and microwave roasting of peanuts. Aspartate, glutamate, asparagine, glutamine, histidine, and phenylalanine were found to be associated with the production of typical roasted flavor while threonine, tyrosine, lysine, and an unknown compound were considered the precursors of a typical flavor (Newell et al., 1967). The unknown component was later refered to as peptide-2 and is considered to contribute to the typical flavor. During roasting this peptide is hydrolyzed to give the amino acid reactants and appears to be the major contributor to a good roasted flavor (Mason et al., 1967). Although a large amount of this peptide is known to be desirable for better flavor no attempts have been made to identify the source and determine the characteristics of this peptide.

This study was initiated to identify the seed polypeptides that undergo structural modifications during roasting for use in detecting the flavor polypeptide/s.

MATERIALS AND METHODS

Seed Material. Peanut (*Arachis hypogaea l.*) seed belonging to the Virginia, Runner, and Spanish market type obtained from a commercial sheller were used in this study.

Roasting Peanut Samples. Pure peanut oil (420 mL, Galandie, Inc., Norfolk, VA), which had been stored at 4 °C was heated to 147 °C in a deep fryer (Presto Fry Baby). Peanuts (200 g each) were roasted for various intervals between 0 and 12 min. During roasting the oil level was maintained at a constant level by adding new oil. After roasting for the desired length the samples were allowed to drain for 1 min and cooled for 5 min in a bin equipped with a small fan. The peanuts were rubbed with a paper towel to remove the excess oil and then blanched by hand to remove the skins and hearts.

The above sequence was repeated on all the replications of each sample. After blanching, a 25-g sample was ground with a Krup KM-45 grinder (Robert Krups) and stored at −18 °C until analyzed.

Preparation of Defatted Peanut Meals. Roasted peanut seeds were thoroughly blotted with paper towel to remove excess oil and then the cotyledons were ground into a meal. The meals were defatted with hexane (1:5, v/v) and the defatted meals were stored at −18 °C.

Protein and Nitrogen. Protein content was determined by extracting the defatted meal with 1 M NaOH (1:20, meal–buffer) at 37 °C and analyzing an aliquot of the extract by the Lowry et al. (1951) method, using bovine serum albumin as the protein standard. Nitrogen content of the defatted meal was analyzed by the micro-Kjeldahl method (AOAC, 1970).

Gel Filtration. Defatted meal (3 g) was extracted with 12 mL of 2 M NaCl, 10 mM Tris-HCl (pH 8.2), 0.002% (w/v) sodium azide (NaN_3), and 2 mM phenylmethanesulfonyl fluoride (a protease inhibitor) by using a mortar and pestle. The homogenate was centrifuged at 20000*g* for 20 min at 15 °C. The clear supernatant was then placed on a Sephacryl S-300 column (125 × 2.5 cm) which had been equilibrated at room temperature with 0.5 M NaCl, 10 mM Tris-HCl (pH 8.2), and 0.002% NaN_3. The column was eluted with the equilibration buffer in 5-mL fractions by using a peristaltic pump. Protein content of each fraction was monitored by measuring the absorption at 280 nm.

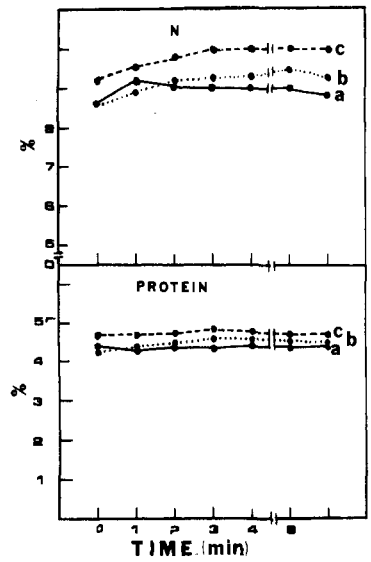

Figure 1. Changes in the total nitrogen and protein content of seed during various periods of roasting. (a) Virginia market type, (b) Runner market type, (c) Spanish market type.

One-Dimensional Polyacrylamide Gel Electrophoresis. Defatted meal (100 mg) was extracted with 2 mL of 0.5 M NaCl and 10 mM Tris-HCl (pH 8.2) and centrifuged (20000*g*, 20 min, 15 °C), and the supernatant (125 μg) was subjected to nondenaturing gel electrophoresis in 7.5% acrylamide gels (Davis, 1964). For sodium dodecyl sulfate (SDS) gel electrophoresis, the above protein extracts were boiled for 3 min in a buffer containing 2% (w/v) SDS, 1.5% (w/v) dithiothreitol, and 1.2% (w/v) Tris. The dissociated proteins (125 μg) were electrophoresed in 10% (w/v) acrylaminde gels containing 0.1% SDS (Laemmli, 1970). Following electrophoresis, the proteins were stained with Coomassie Blue R-250 and destained with 7% acetic acid and 10% ethanol. The gels were scanned in a Beckman Model 25 spectrophotometer equipped with a gel scanner at 600 nm and 0.05-mm slit.

Two-Dimensional Gel Electrophoresis (2-D PAGE). Protein from 200 mg of defatted meal was extracted with a solution (3 mL) containing 9.3 M urea, 5 mM K_2CO_3, 0.5% (w/v) dithiothreitol, and 2% (v/v) nonidet p-40 (a nonionic detergent, BDH Chemicals, Poole, UK) and subjected to 2-D PAGE by a modified method of O'Farrell (1975) as described by Basha (1979). The first dimension consisted of isoelectric focusing (IEF) in 4% acrylamide gel containing 9.3 M urea, 2% nonidet P-40, and 2% (v/v) ampholine mixture (pH 3.5–10, pH 5–7, pH 9–11 ampholines, 10:7:3). About 500 μg of protein was loaded on each gel and focused for 18 h toward the anode. After IEF, the gels were equilibrated for 10 min with a buffer containing 1% SDS, 1% 2-mercaptoethanol, and 65 mM Tris-HCl (pH 6.9). The equilibrated gels were transferred onto 10% acrylamide slab gels and subjected to SDS-PAGE following the method of Laemmli (1970). After electrophoresis, the slab gels were stained with Coomassie Blue R-250 for 18 h and destained with 7% acetic acid containing 10% ethanol.

RESULTS AND DISCUSSION

Total Nitrogen and Protein Content. The seed nitrogen content of Virginia and Runner lines remained stable during the 12-min roasting period (Figure 1). However, there was a slight increase in the nitrogen content of Spanish seed during roasting. Like nitrogen, the

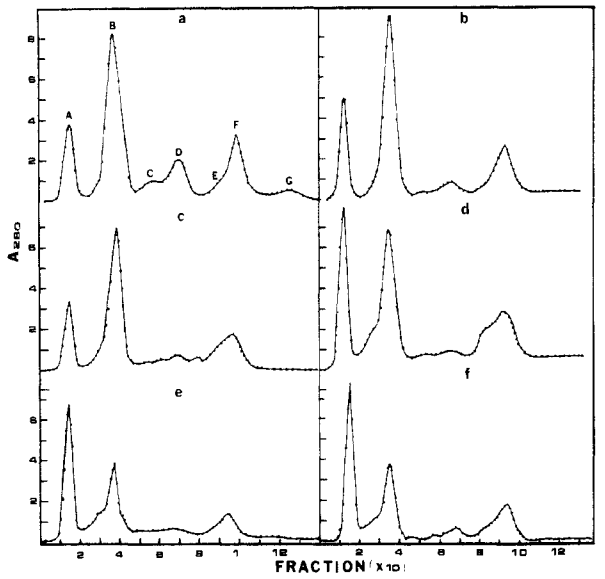

Figure 2. Gel filtration profiles of protein from peanut seed roasted for 0 (a), 2 (b), 4 (c), 6 (d), 8 (e), and 10 min (f). Seed proteins were extracted from the defatted meal (3 g) with 2 M NaCl and 10 mM Tris-HCl buffer, pH 8.2 (12 mL), and applied on a Sephacryl S-300 column (2.5 × 135 cm).

seed protein content also remained unchanged during the roasting (Figure 1), indicating that during the 12-min roasting period total seed protein content is not greatly affected.

Gel Filtration. Following gel filtration on Sephacryl S-300 column, proteins from unroasted control seed resolved into four major (A, B, D, and F) and three minor (C, E, and G) peaks (Figure 2). However, roasting appears to cause major changes in the protein profiles. As seen in Figure 2 the amount of protein increased in peak A (void volume), while in peak B it decreased. In our earlier studies (Basha and Pancholy, 1981a) we have identified peak B as the arachin monomer (*Mr* 380 000). An increase in peak A protein content therefore sugggests that during roasting arachin proteins (peak B) aggregate to form high molecular weight polymers. There was also a major reduction in peak D proteins between 0 and 4 min of roasting. This peak has been previously identified as the methionine-rich protein (Basha and Pancholy, 1981b). This would indicate that methionine-rich proteins disappeared during early periods of roasting. Except for these, no other major changes were observed in the protein content of the remaining peaks, suggesting that the other proteins may not be greatly affected during roasting.

One-Dimensional Polyacrylamide Gel Electrophoresis. Gel electrophoresis of protein extracts from Florigiant (a Virginia market type peanut) under nondenaturing conditions showed (Figure 3-A) one protein band (a) for arachin and a broad band (c) for methionine-rich proteins (Basha and Pancholy, 1981). Electrophoresis data indicated that the amount of protein in arachin peak (a) remained relatively constant during roasting. Thus the ratio of peaks a/b was 2.0 between 0 and 8 min of roasting. However, the protein content in the methionine-rich protein band (c) decreased dramatically during roasting with major reduction occuring between 0 and 4 min of roasting. The ratio of peaks a/c which was 2.9 at 0 time increased to 5.0, 7.1, and 10.0 after 2, 4, and 10 min of roasting, respectively. The electrophoresis data thus showed that while the arachin content remained relatively constant, the methionine-rich protein decreased significantly during roasting. Similar changes in protein composition were observed in roasted seeds of Runner (Figure

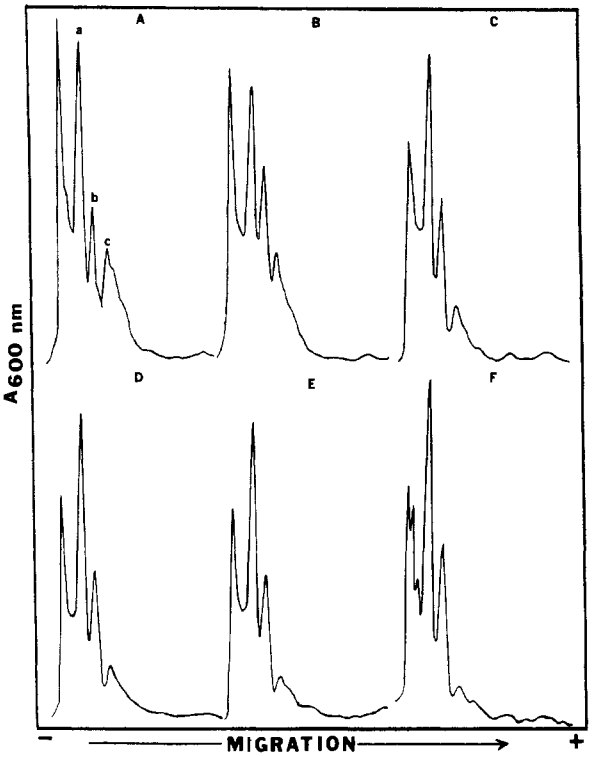

Figure 3. One-dimensional nondenaturing gel electrophoretic patterns of protein extracted from Virginia market type peanut seed roasted for 0 (A), 1 (B), 2 (C), 3 (D), 4 (E), and 8 min (F). About 125 μg of protein was loaded on each gel.

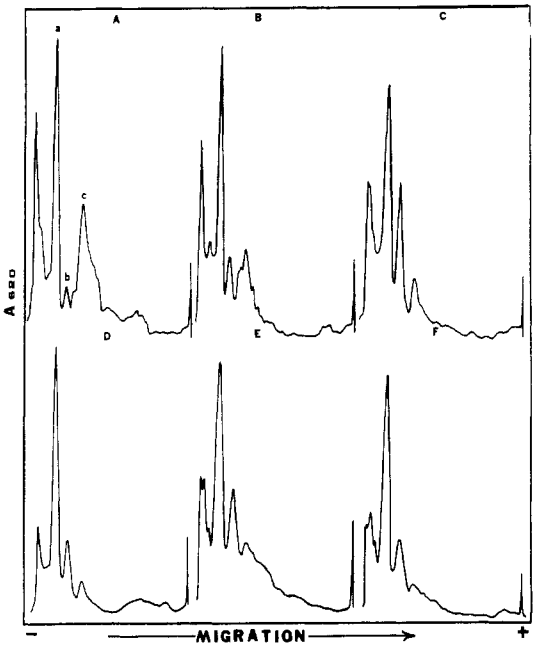

Figure 4. One-dimensional nondenaturing gel electrohoretic patterns of protein from Runner market type peanut seed during roasting: (A) 0, (B) 1, (C) 2, (D) 3, (E) 4, and (F) 8 min. About 125 μg of protein was loaded on each gel.

4) and Spanish (Figure 5) market types. It is interesting to note that the ratio of a/c was lowest (2.09) for the Spanish line compared to the Virginia (2.9) and Runner (2.3) lines. This would indicate that the Spanish type peanuts are rich in band c proteins compared to the other market types and hence, may yield a better flavor upon roasting.

In addition to the protein composition, the polypeptide composition of the seed proteins was also monitored by sodium dodecyl sulfate (SDS) gel electrophoresis (Figure

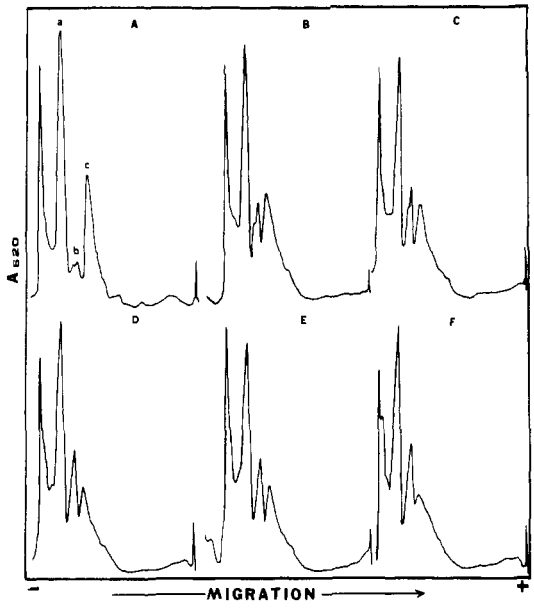

Figure 5. One-dimensional nondenaturing gel electrophoretic profiles of protein from Spanish market type peanut seed: (A) 0, (B) 1, (C) 2, (D) 3, (E) 4, and (F) 8 min. About 125 μg of protein was loaded on each gel.

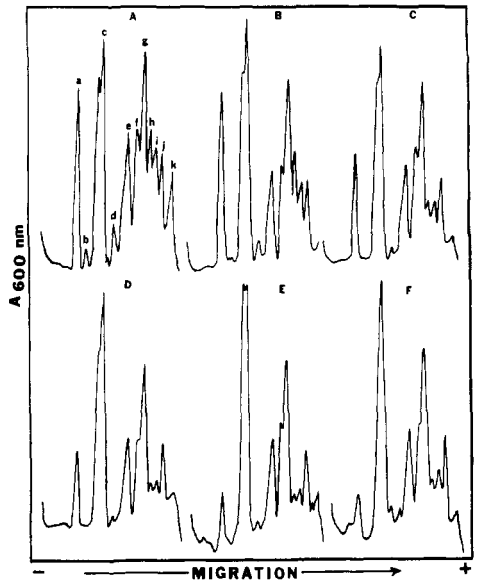

Figure 6. Sodium dodecyl sulfate gel electrophoretic patterns of proteins from Virginia market type peanut roasted for 0 (A), 1 (B), 2 (C), 3 (D), 4 (E), and 8 min (F). About 125 μg of proteins was loaded on each gel.

6). Under this system proteins are dissociated into polypeptides and then they are separated based on their molecular weight. Unlike the nondenaturing gel system, the SDS gel system showed more complex changes in the polypeptide composition. It is found that during roasting the polypeptide components a, d, h, and i decreased while the components c, e, and j remained relatively stable. It should be noted that components h and i belonged to the methionine-rich protein (Basha and Pancholy, 1981a,b). Thus, the SDS gel electrophoresis indicated that the seed proteins undergo selective degradation of certain polypeptide components to various degrees during roasting. Analysis of seed proteins from Runner (Figure 7) and Spanish (Figure 8) lines also showed a decrease in components a, d, h, and i implying that only these components are affected during roasting.

Two-Dimensional Gel Electrophoresis (2-D PAGE). Compositional changes in the seed polypeptide compo-

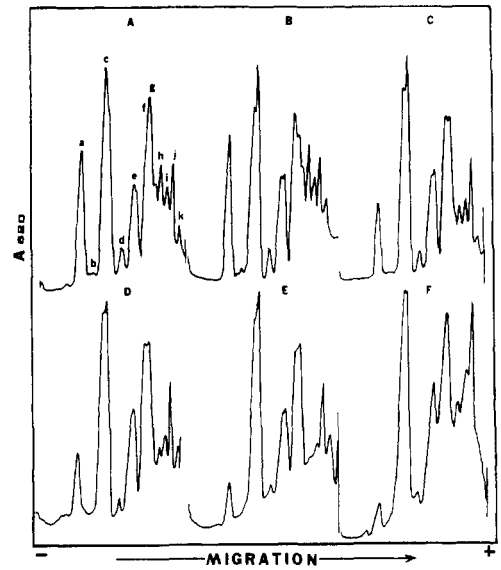

Figure 7. Sodium dodecyl sulfate gel electorphoretic patterns of proteins from Runner market type peanut during roasting: (A) 0, (B) 1, (C) 2, (D) 3, (E) 4, and (F) 8 min. About 125 μg of protein was applied on each gel.

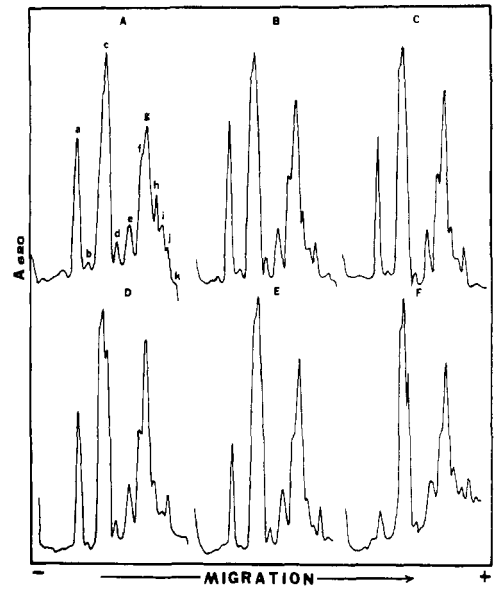

Figure 8. Sodium dodecyl sulfate gel electrophoretic profiles of protein from Spanish market type peanut during roasting: (A) 0, (B) 1, (C) 2, (D) 3, (E) 4, and (F) 8 min. About 125 μg of protein was loaded on each gel.

nents during roasting was also monitored by subjecting the seed proteins to two-dimensional gel electorphoresis. In this system proteins are separated by isoelectric focusing in the first dimension and SDS gel electrophoresis in the second dimension. The results showed (Figure 9) no significant changes in the arachin polypeptide content during the first 4 min of roasting. After 4 min there was a gradual decrease in the content of a high molecular weight polypeptide "A". In our earlier studies (Basha and Pancholy, 1981a) we have characterized this polypeptide as a high molecular weight (70 000) polypeptide with an isoelectric point around pH 6.5. In addition to this polypeptide there was also a significant decrease in six, low molecular weight polypeptides between 0 and 6 min of roasting. (Shown with arrows). Similar decreases were found with the Runner and Spanish lines (not shown). Previously we have identified these polypeptides as components of the methionine-rich protein (Basha and Pancholy, 1981b). These components have a molecular weight between 16 000 and

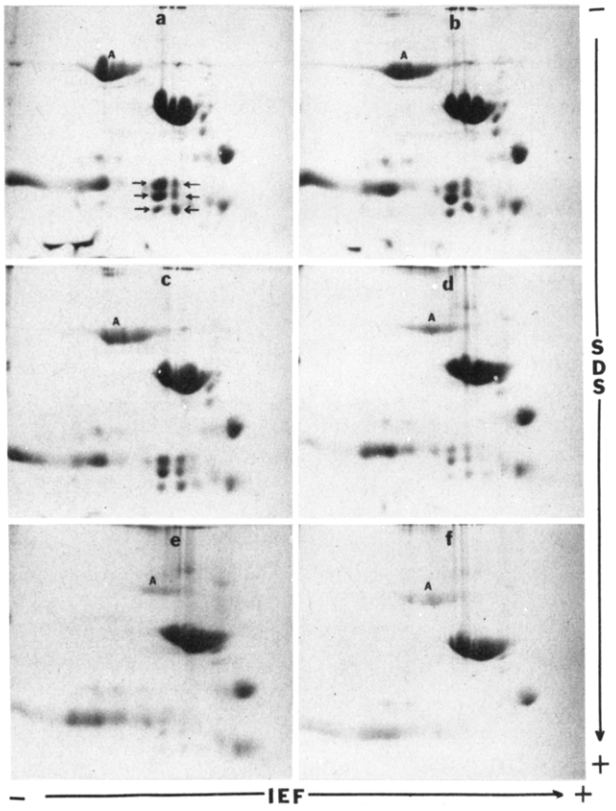

Figure 9. Two-dimensional polyacrylamide gel electrophoretic patterns of seed proteins from Virginia market type peanut roasted for 0 (A), 2 (B), 4 (C), 6 (D), 8 (E), and 10 min (F). About 500 μg of protein was applied on each gel.

20 000, and isoelectric points between pH 5.6 and 6.2 (Basha and Pancholy, 1981a).

Thus based on the gel filtration data and electrophoretic analysis it appears that the methionine-rich polypeptides may be degraded first during early stages of roasting, and the released amino acids may then react with the sugars to yield the typical flavor (pyrazine) compounds. Additional studies are in progress to identify and characterize the breakdown products of the above polypeptides that disappear during roasting to determine their fate during roasting and the pathway for the formation of pyrazine and carbonyl compounds.

CONCLUSION

During oil roasting of peanuts, methionine-rich proteins are degraded rapidly between 0 and 6 min of roasting. Since the methionine-rich protein is high in sulfur-containing amino acids (methionine 2.9%, cystine 10.7) (Basha and Pancholy, 1981b) and H_2S evolved during roasting is believed to be related to the roasted flavor, it is suggested that the amino acids released from the methionine-rich polypeptide/s may be involved in the development of roasted peanut flavor via the Millard Reaction.

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