and polypeptide composition.

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Maturity and Roasting of Peanuts As Related to Precursors of Roasted Flavor

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The effects of maturity, season, and precursor levels on roasting behavior of peanut (*Arachis hypogaea* L.) seed were studied. After the harvest, green pods were classified into five maturity groups by the hull-scrape method, and the seeds from each group were again separated into different commercial categories on the basis of size. From these categories, the medium-size seed category was selected, and a portion of the seed was roasted and another portion used as raw seed. Peanut pastes were prepared with the roasted seeds, and the chemical compositions of raw seeds and pastes were determined. The results showed that the protein and carbohydrate contents of the raw seeds and pastes were similar, but the α-amino nitrogen content of peanut pastes was lower than that in the raw peanuts. Protein and carbohydrates of the seeds of different maturities responded similarly to roasting, but the α-amino nitrogen decreased more (38–55%) in the mature seed than in the immature seed (25–28%). Peanut pastes contained significantly lower amounts of the 90 000, 70 000, 50 000, and 32 000 molecular weight polypeptides than the raw peanuts.

Free amino acids and monosaccharides are known to be responsible for the flavor of roasted peanuts (Newell et al., 1967; Mason et al., 1969). Among the amino acids, aspartic acid, glutamic acid, glutamine, histidine, asparagine, and phenylalanine are precursors of the typical flavor while threonine, tyrosine, and lysine are the precursors of atypical flavor (Newell et al., 1967). In addition to these amino acids, arginine has also been reported as an atypical flavor precursor (Cobb and Johnson, 1973). Sucrose participates in flavor development through its inversion to glucose and fructose during the browning process (Mason et al., 1969; Reyes et al., 1982). It is also known that chemical composition (precursor levels) of peanuts is affected by several factors such as variety, location, climatic conditions, maturity, curing (Oupadissakoon et al., 1980; Mason et al., 1969), seed size, and storage (Pattee et al., 1981, 1982).

In view of the importance of seed precursor levels on the quality of peanut flavor, a study was initiated to determine the effect of maturity and roasting on precursors of roasted

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flavor. For this purpose, raw seeds and peanut pastes of different maturities obtained from two different seasons were subjected to chemical analyses to determine the response of seeds of different maturities containing varying levels of flavor precursors to peanut paste preparation. In this initial report we present the data on variations in total seed components due to maturity and differential response of seeds of varying maturities to roasting.

MATERIALS AND METHODS

Materials. Peanut (*Arachis hypogaea* L. Cv. Florunner) seeds that were grown at the USDA/ARS, National Peanut Research Laboratory, Dawson, GA, during the 1985 and 1986 seasons were used in this study.

Peanuts were dug by hand 142 days after planting and separated into different maturity groups first by placing them in a Pearman peanut blaster to remove the exocarp and then visually separating them into five [yellow 2 (Y2), orange A (OA), orange B (OB), brown (BR), black (BL)] hull-scrape maturity classes, black being the most mature and yellow 2 being the most immature pod (Henning, 1983).

All the maturity classes were dried with ambient air, handshelled, sized, and stored at 4 °C. medium-size category (peanuts fell through a 21/64-in. screen and rode an 18/64-in. screen) peanuts from each maturity stage were selected for chemical analysis (as raw peanuts per se) and for peanut paste preparation.

Sample Preparation. A portion of the medium-size seeds were freeze-dried and ground into a meal, and the remaining portion was roasted (140 °C) in a modified Farberware roaster (Fletcher, 1987). Peanuts were roasted for various lengths of times until a similar Hunter *L* value (50 ± 1.5) was reached for peanuts from each maturity class and season (Sanders et al., 1988). Peanut paste was prepared with use of a Cuisinart food processor and packaged in plastic jars. Raw meals as well as peanut pastes were kept at −20 °C until analyzed. Three samples from each maturity class were subjected to chemical analyses, and the results are the average of three replications.

Methods. *Defatting.* The samples were defatted with hexane until they were essentially free of oil (Basha et al., 1976). The defatted meals were stored at −20 °C until analyzed.

α-Amino Nitrogen and Soluble Carbohydrates Extraction. Defatted meal (0.20 g) was homogenized with methanol–chloroform–water (MCW) solution (60:25:15, v/v/v) at full-speed setting in a Polytron homogenizer (Brinkman Instruments, NJ). The homogenate was centrifuged at 20000*g* for 10 min (Young et al., 1974). The pellet was reextracted with ethanol–water (80:20, v/v) and ethanol, centrifuged, and added to the previous extract. The supernatants were made up to a known volume, an aliquot of the supernatant was used for α-amino nitrogen and soluble carbohydrate analysis, and the pellet was used for insoluble carbohydrate determination.

α-Amino Nitrogen and Soluble Carbohydrates Analysis. An aliquot of the supernatant (200 μL) from the MCW extract was analyzed for α-amino nitrogen by a colorimetric method, using ninhydrin as a color reagent and leucine as the standard according to the modified method of Yemm and Cocking (1955). Another aliquot from the above supernatant was analyzed for soluble carbohydrates by the method of Yemm and Willis (1954), using anthrone as a reducing agent and glucose as the standard monosaccharide.

Extraction and Analysis of Insoluble Carbohydrates. The pellet (0.025 g) from the MCW extraction was homogenized with 0.04 N H_2SO_4 and heated in a boiling water bath for 1 h. The homogenate was then centrifuged at 20000*g* for 10 min to remove insoluble residue (Watschke and Waddinton, 1974). The resulting supernatant was analyzed for total carbohydrates following the method of Yemm and Willis (1954).

Extraction and Analysis of Total Protein. Protein from the defatted meal (0.025 g) was extracted by homogenizing and then incubating the meal with 1 M NaOH at 37 °C. After overnight incubation the homogenate was centrifuged at 20000*g* for 10 min and reextracted as above. An aliquot (50 μL) of the supernatant was analyzed for soluble protein by the method of Lowry et al. (1951) using bovine serum albumin as the protein standard.

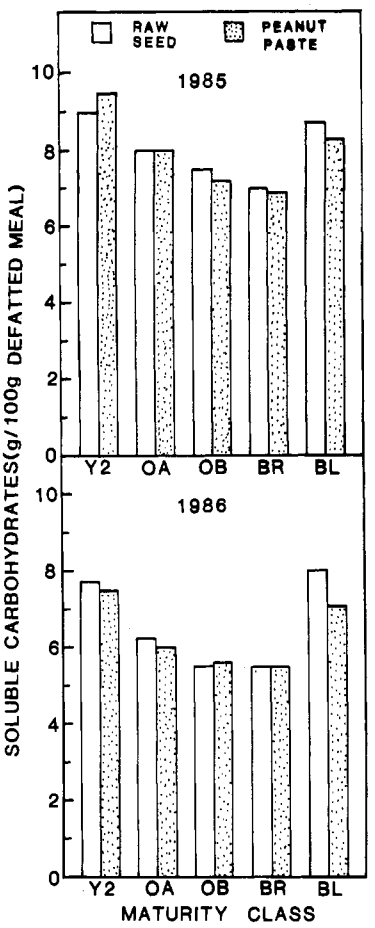

Figure 1. Soluble carbohydrate content (grams/100 g of defatted meal) of raw peanut seeds (RS) and pastes (PP) for five maturity stages from 1985 and 1986 crops: Y2 = yellow 2, OA = orange A, OB = orange B, BR = brown, BL = black.

One-Dimensional Gel Electrophoresis. Defatted peanut meal (0.05 g) was homogined with 2 mL of 0.5 M NaCl and 10 mM Tris-HCl (pH 8.2) buffer, and then the homogenate was centrifuged at 20000*g* for 15 min. The resulting supernatant was subjected to nondenaturing gel electrophoresis in 7.5% (w/v) polyacrylamide gels (Davis, 1964). After electrophoresis, the proteins were fixed and stained with 0.065% Coomassie Blue R-250 in 7% acetic acid and 10% ethanol and destained with 7% acetic acid and 10% ethanol. Another aliquot of the above protein extract was dissociated by boiling with 1.2% Tris, 2% sodium dodecyl sulfate (SDS), and 3% 2-mercaptoethanol and subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) in 10% (w/v) acrylamide gels containing 0.1% SDS (Laemmli, 1970). The proteins were stained with Coomassie Blue R-250 in 7% acetic acid and 40% ethanol and destained with 7% acetic acid and 10% ethanol.

Molecular Weight Estimation. The 10% acrylamide gel was calibrated against protein standards of known molecular weight. The protein standards were dissociated with 1% (w/v) SDS, 1.5% (w/v) dithiothreitol, and 1.2% (w/v) Tris by boiling for 3 min. Twenty micrograms of each sample was loaded on the gel and electrophoresed (Laemmli, 1970). The protein standards used for gel calibration were thyroglobulin (334 500), β-galactosidase (130 000), phosphorylase *b* (94 000), bovine serum albumin (67 000), ovalbumin (43 000), carbonic anhydrase (30 000), soybean trypsin inhibitor (20 000), and lysozyme (14 000).

RESULTS AND DISCUSSION

Defatted meals prepared from raw seeds (RS) and peanut pastes (PP) were analyzed to determine their compositional differences in order to asses the response of seeds of varying maturities to paste preparation.

Soluble and Insoluble Carbohydrates. The soluble carbohydrate content was generally higher (Figure 1) in

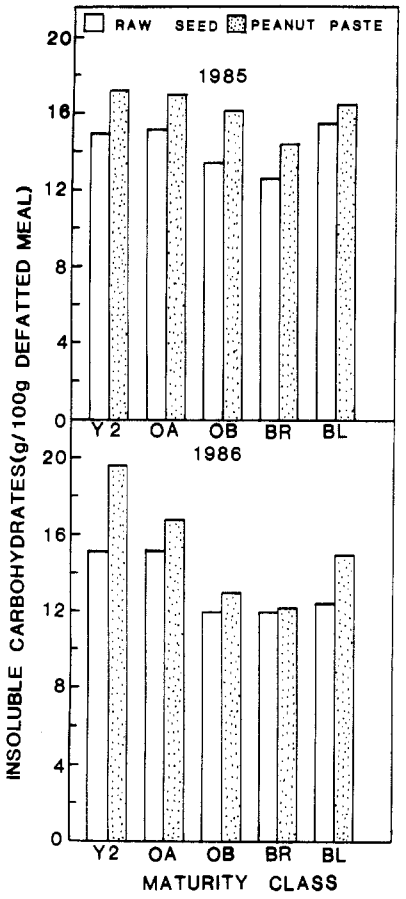

Figure 2. Insoluble carbohydrate content (grams/100 g of defatted meal) of raw peanut seeds (RS) and pastes (PP) for five maturity stages from 1985 and 1986 crops: Y2 = yellow 2, OA = orange A, OB = orange B, BR = brown, BL = black.

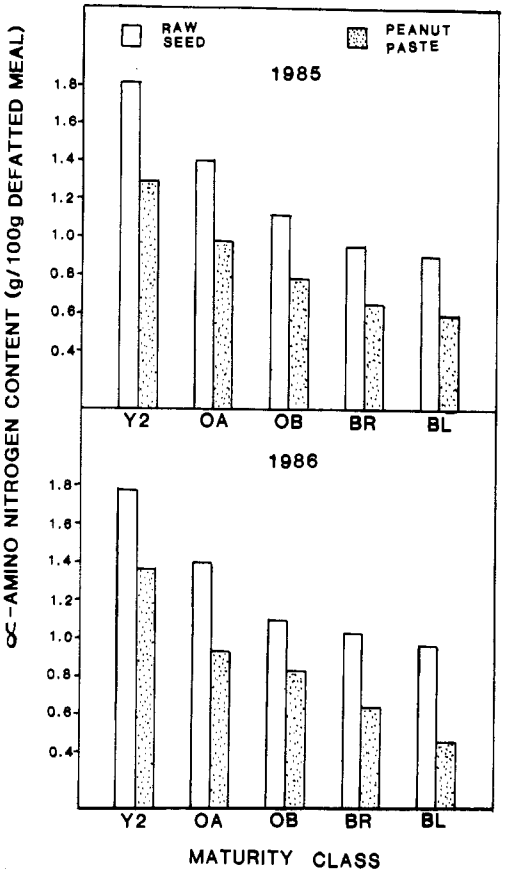

Figure 3. α-Amino nitrogen content (grams/100 g of defatted meal) of raw peanut seeds (RS) and pastes (PP) for five maturity stages from 1985 and 1986 crops: Y2 = yellow 2, OA = orange A, OB = orange B, BR = brown, BL = black.

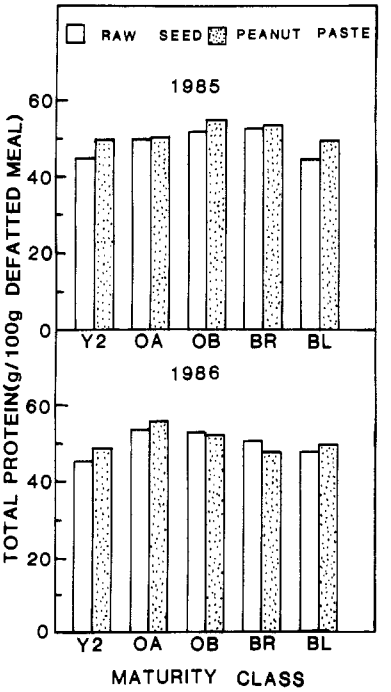

Figure 4. Total protein content (grams/100 g of defatted meal) of raw peanut seeds (RS) and pastes (PP) for five maturity stages from 1985 and 1986 crops: Y2 = yellow 2, OA = orange A, OB = orange B, BR = brown, BL = black.

the raw seeds and peanut pastes from the 1985 crop than from the 1986 crop. The soluble carbohydrate content of the 1985 crop ranged between 7.14% and 8.92% for the raw seeds and 6.92% and 9.35% for corresponding pastes, while the 1986 crop ranged between 5.49% and 7.82% in the seed and 5.58% and 7.44% in pastes. Yellow 2 and black categories from both years contained higher amounts of soluble carbohydrates than the orange A, orange B, and brown categories. The decrease in the amount of soluble carbohydrates with increasing maturity could be due to their utilization in the synthesis of seed components such as starch, lipid, and protein (Basha et al., 1976). As the seeds become more mature, seed metabolic activity decreases accompanied by smaller precursor pools.

Peanut paste contained higher amounts of insoluble carbohydrates (Figure 2) compared to the raw seeds. The insoluble carbohydrates content ranged from 12.35% to 19.70% for the 1986 peanut paste samples and 14.52% to 17.17% for the 1985 peanut pastes, while the insoluble carbohydrate content of the corresponding raw seeds varied from 12.18% to 15.43% for the 1986 seeds and 12.79% to 15.67% for the 1985 seeds. Higher amounts of insoluble carbohydrates in the pastes than in the raw seed may be attributed to the roasting effect and the fine particle size of the peanut pastes, which might have facilitated efficient extraction of the insoluble carbohydrates. In addition, the amount of insoluble carbohydrates was higher in the 1985 crop than in the 1986 crop. Observed seasonal variations in the carbohydrates content may probably due to the effect of temperature, moisture, and cultural practices, which are known to affect peanut seed composition (Oupadissakoon et al., 1979).

α-Amino Nitrogen. The α-amino nitrogen content of the seed decreased with increasing maturity (Figure 3). A decrease in α-amino nitrogen content with increasing maturity is to be expected because of their utilization in

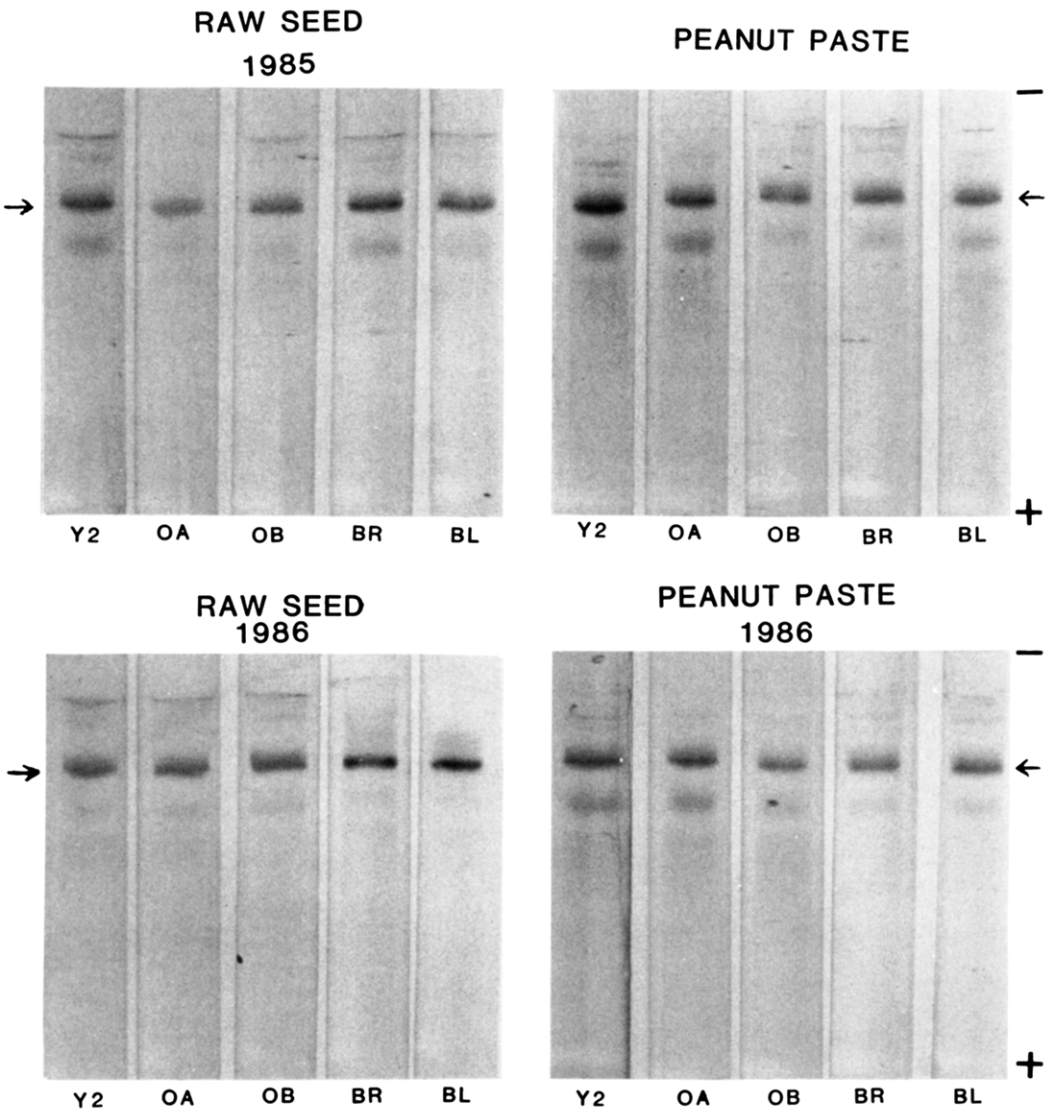

Figure 5. Electrophoretic profiles from raw peanut seeds (RS) and pastes (PP) for five maturity stages from 1985 and 1986 crops: Y2 = yellow 2, OA = orange A, OB = orange B, BR = brown, BL = black.

protein synthesis. Comparison of response of different maturities to paste preparation showed a greater decrease of α-amino nitrogen in the mature seeds than in the immature seeds. For example, the peanut pastes from the 1985 and 1986 crops, respectively, contained 38% and 55% less α-amino nitrogen in the black category and 28% and 25% less in the yellow 2 category than that in the raw seed. Among the maturities, the 1985 yellow 2 raw seed category had the highest α-amino nitrogen (1.81%) while the corresponding paste contained 1.29%. Likewise, the 1986 yellow 2 raw seed contained 1.77% α-amino nitrogen while the paste had 1.36%. In each case pastes prepared from more mature seeds contained less α-amino nitrogen than the immature seeds. The decrease in the amounts of α-amino nitrogen in the paste may be due to the reaction between free amino acids and sugars resulting in pyrazine compounds via the Maillard reaction (Mason et al., 1966; Newell et al., 1967; Maga, 1982). Alternatively, the loss may be attributed to the breakdown or modification of the amino acids into nonamino acid form.

Protein. No major differences were found in the protein content of the raw seed and their pastes, indicating that paste preparation did not affect the total seed protein content (Figure 4). This finding is consistent with our previous report showing no change in seed protein content during roasting (Basha and Young, 1985). In general, the seed protein content varied between 45% and 52% while that of pastes ranged between 50% and 55%. Higher protein values in the pastes than in the raw seed may be due to increased extractability of the protein from the pastes than the raw seed meals.

One-Dimensional Gel Electrophoresis. In order to determine the differences in proten composition between raw seed and peanut paste, the samples were subjected to polyacrylamide gel electrophoresis under nondenaturing and denaturing conditions.

Figure 5 shows the electrophoretic profiles of total protein from raw seeds and their pastes for 1985 and 1986 crops. The major band (shown with an arrow) represents the arachin (major storage protein of peanut) while the minor bands represent the non-arachin proteins. Except for a slight increase in the non-arachin band of peanut pastes, no apparent differences were evident in the protein profiles between the raw seed and pastes, indicating that protein mobility was not significantly altered due to paste preparation.

In order to determine possible structural alterations, proteins were dissociated into polypeptides and subjected to SDS–polyacrylamide gel electrophoresis. Under this system the peanut proteins were resolved into several polypeptides with molecular weights ranging between 14 000 and 90 000. The polypeptide profiles of raw seeds

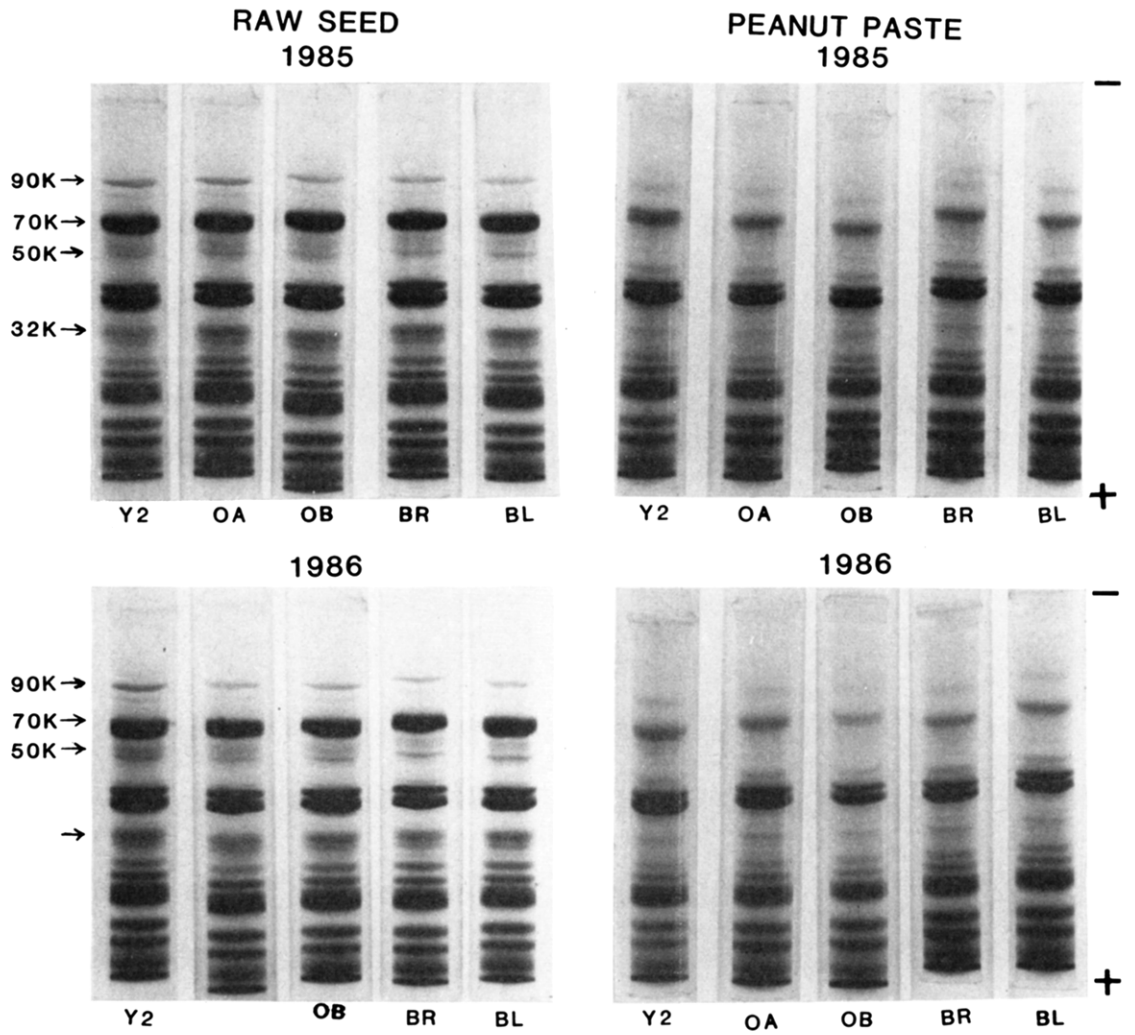

Figure 6. SDS gel electrophoretic profiles from raw peanut seeds (RS) and pastes (PP) for five maturity stages from the 1985 and 1986 crops: Y2 = yellow 2, OA = orange A, OB = orange B, BR = brown, BL = black.

and peanut pastes for the 1985 and 1986 crops are shown in Figure 6. Compared to the raw seeds, four polypeptides with apparent molecular weights of approximately 90 000, 70 000, 50 000, and 32 000 were greatly affected in the peanut paste samples. All five maturity categories behaved alike in paste preparation, indicating that irrespective of the maturity status roasting had a similar effect on seed proteins. Basha and Young (1985) also found a decrease in the 70 000 molecular weight polypeptide following oil roasting of peanut seed. This decrease may be attributed to the breakdown of these polypeptides into smaller polypeptides and free amino acids during roasting, which may then react with free sugars via Maillard sugar–amine reactions (Newell et al., 1967) to yield roasted flavor components. The data suggest that protein and carbohydrates of peanut seeds of all maturities generally react similarly during peanut paste preparation while the α-amino nitrogen decreases more in the mature seed than in the immature seed. Thus, variation in the peanut seed roast characteristics may be attributed to the inherent quantitative and qualitative differences in the seed components (mainly soluble carbohydrates and free amino acids) among different maturities and differential response of free amino acids to roasting depending upon the maturity status of the seed. Pattee and Young (1987) reported that the ratio of amino acids reported to produce typical roasted flavor to amino acids producing atypical roasted flavor increases with seed size. In addition, considering larger size as most mature, they have suggested that, in general, mature peanuts contain more of the amino acids associated with typical roasted flavor and less of those associated with atypical flavor.

In view of the observed variations in the response of seeds to roasting, the seeds and pastes from different maturities are being analyzed for individual sugars and amino acids. In addition, sensory data are also being studied to correlate with the biochemical data in order to determine the relationship between seed component level and flavor characteristics.

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HPLC Separation and Comparison of the Browning Pigments Formed in Grapefruit Juice Stored in Glass and Cans

Russell L. Rouseff,* James F. Fisher, and Steven Nagy

Pigments associated with browning in grapefruit juice stored in cans and glass containers were separated by an HPLC procedure employing a ternary solvent gradient with a C_{18} column. The solvent system consisted of water, acetonitrile, and tetrahydrofuran. The types and amounts of pigments are container-dependent. Greater numbers of browning pigments were formed in bottles than in cans, and fewer but more intense browning pigments were formed in cans. Some pigments were unstable and diminished or disappeared at longer storage times, whereas others increased with increasing storage time. Amounts of late-eluting, nonpolar browning pigments increased with increasing storage time. These pigments were a major source of browning in juices stored both in cans and in glass bottles. The number and quantities of browning pigments observed increased dramatically with increasing storage temperature.

Under nonrefrigerated storage conditions, processed grapefruit juice darkens with increasing storage time due to the formation of browning pigments. This nonenzymatic browning has been a visual defect of processed grapefruit juice for many years and is one of the factors determining shelf life. Browning in grapefruit juice is accompanied by an increase in off-flavors, which also limits shelf life.

Browning in citrus juices has been studied by a number of investigators (Joslyn, 1957; Wolfrom et al., 1974; Lee and Nagy, 1988), and the subject has been reviewed recently by Handwerk and Coleman (1988). The general consensus of this review is that citrus browning compounds require the interaction of reducing sugars and/or sugar degradation products with non-sulfur-containing amino acids. The vast majority of citrus browning studies have attempted to investigate the initial reactants associated with the production of browning pigments. By adding specific components to juice or model systems, the compounds that accelerate or inhibit browning have been identified. However, virtually nothing is known about the actual browning compounds formed in citrus juices. In addition, while it is generally known that browning is more of a problem in glass containers than in tin-plated steel cans, the number or types of browning pigments formed in each case are unknown.

Therefore, the purpose of this study was to develop a procedure that would allow characterization of the browning pigments formed in grapefruit juice. Secondary

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