Isolation and Characterization of a Peanut Maturity-Associated Protein

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Using reversed-phase high-performance liquid chromatography (RP-HPLC), the peanut protein profile was shown to be related to the maturity, drying time, and drying procedure of the peanut. Differences were seen between (a) immature and mature seeds for untreated and windrow-dried peanuts, (b) untreated and windrow-dried peanuts for immature and mature seeds, and (c) windrow- and stackpole-dried peanuts. The most pronounced HPLC peak that increased in size as the peanut matured and decreased in size with longer drying times was isolated and identified by sodium dodecyl sulfate−polyacrylamide gel electrophoresis and electrospray ionization mass spectrometry to have a molecular weight of 62 500. Since maturity is related to the sensory quality of peanuts, this protein may be a marker for peanuts that will produce a higher quality flavor when roasted.

Keywords: *Peanut; Arachis hypogaia L.; maturity; reversed-phase high-performance liquid chromatography; isolation; electrospray mass spectrometry*

INTRODUCTION

The overall sensory quality of roasted peanuts has been described as intensities of individual flavor descriptors, such as the desirable roasted peanutty and sweet aromatic, or the undesirable fruity fermented, painty, sour, or bitter (Bett et al., 1994). As peanuts mature, it has been shown that the ratio of the desirable to undesirable descriptors increases (Sanders and Bett, 1995). The maturity of peanuts is usually graded on size and color, with larger sizes and darker colors indicating maturity. However, these methods are not always accurate. The flavor quality of roasted peanuts is the sum of a multitude of complex interacting factors. Many factors, such as genetics, processing, and storage, are managed by modern manufacturing processes. However, others, such as the environmental conditions, seed maturity, and drying method cause the flavor quality of the products to vary outside acceptable ranges. Free amino acids and carbohydrates are known to be precursors of the compounds responsible for the flavor of roasted peanuts (Heath and Reineccius, 1986a,b), although the specific mechanisms of peanut flavor and off-flavor are not well understood. The process of roasting peanuts affects the sugar and total protein content equally for all maturities (Rodriguez et al., 1989). This suggests that precursor levels of individual peanut proteins are important to the quality of peanut flavor.

Proteins have been isolated from peanuts (*Arachis hypogaia* L.) since the first report in 1880 where Ritthausen used salt extraction and acid precipitation to obtain crude fractions (Ritthausen, 1880). Later, ammonium sulfate precipitation was used to fractionate different protein mixtures (Johns and Jones, 1916), and more recently, ion exchange chromatography (Dechary, 1961), immunoelectrophoresis (Daussant et al., 1969), cryoprecipitation (Daussant et al., 1969), and gradient centrifugation (Cherry, 1990) have been used. Peanut seed extracts can vary in protein quantities depending on the type and the amount of extraction medium used, but classically, reports have defined two types of globulins, arachin (stored in aleurone grains or protein bodies) and conarachin (present in cellular cytoplasm), and albumin (Dechary, 1961; Daussant et al., 1969). Arachin was separated into two species, a monomer (arachin I) and a dimer (arachin II), both having a monomer molecular weight of 180 000 and the same subunit structure (Yamada et al., 1979a, 1980). Arachin I has been shown to consist of six different subunits with molecular weights from 19 500 to 40 500 (Yamada et al., 1979a,b). Conarachin also has been separated into two species, conarachin I and conarachin II, with different subunit structures. Conarachin II has a molecular weight of 180 000, consisting of three subunits with molecular weights of 65 000 (Yamada et al., 1980). The whole peanut extract contained subunits ranging from 14 000 to 90 000 molecular weight (Rodriguez et al., 1989).

Methods used to isolate peanut proteins, to date, have predominately used size exclusion or ion exchange chromatography. These methods separate the multicomponent proteins, but their limited resolution hinders the separation and isolation of individual protein subunits. To obtain a clearer representation of the protein profile of peanuts, we have studied peanut proteins by reversed-phase high-performance liquid chromatography (RP-HPLC) because of its excellent resolving power. With RP-HPLC we have examined the relationship between individual peak height and peanut maturity/sensory quality. In this paper we examine the effects of peanut maturity, drying (curing), and the type of curing on protein content and identify a protein associated with peanut maturity.

MATERIALS AND METHODS

Materials. Peanuts (*Arachis hypogaia* L., var. Florunner; crop years 1990 and 1991) were planted at the USDA, ARS

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National Peanut Research Laboratory (Dawson, GA), dug 120 days after planting, and subjected to windrow drying where samples were taken 0 and 4 days after drying. Stackpole-cured peanut samples were taken after 40 days of slow curing. Peanuts were subjected to gentle abrasion to remove the exocarp, sorted by pod color, and hand shelled, and the seeds were stored at −80 °C. Peanut maturity (defined as yellow, orange, brown, and black) was based on the visual hull-scrape/color method (Williams and Drexler, 1981). Two maturities were used for this study, yellow (immature) and black (mature). Arachin standard was supplied by Joseph Neucere (Neucere, 1969).

Preparation of Peanut Extracts. Extracts were prepared by a modification of the method of Chung et al. (1994). Prior to extraction, the seed coat was removed from the peanuts of different maturities and curing stages, and peanuts (3 g) were defatted by grinding with a Wiley-mill in sequence with cold acetone (20 mL) and hexane (20 mL). After being dried in vacuo, the resultant defatted peanut meals were stored at −20 °C. Extracts were prepared by suspending 0.1 g of defatted peanut meal in 50 mM sodium phosphate buffer, pH 7 (1 mL), and stirring overnight at 4 °C. Crude cell extracts were centrifuged at 11 000 rpm for 20 min at 4 °C. The supernatant was cooled to 4 °C overnight, and the precipitate was removed by centrifugation. The final supernatant was used for HPLC analysis.

HPLC Analysis and Isolation. Chromatographic separation was attained on a Beckman HPLC system with a photodiode array UV detector and Phenomenex Jupiter 300 column (C18, 5 μm, 300 Å, 2.0 × 250 mm). Eluants were 0.1% TFA/acetonitrile using a flow rate of 0.2 mL/min. The chromatograms were obtained on 5 μL injections with a gradient of 0% to 20% acetonitrile in 10 min and a 5 min isocratic elution, followed by a gradient from 20% to 40% acetonitrile in 15 min. Detection was at 214 and 270 nm. Peak isolation was obtained on the same system with multiple 50 μL injections. Collected fractions were lyophilized to a white solid.

Gel Electrophoresis. Proteins were separated using a 10% sodium dodecyl sulfate−polyacrylamide gel electrophoresis (SDS−PAGE) gel, with a 5% stacking gel, according to the method of Laemmli (1970). Molecular weight standards (rainbow markers, Amersham) included myosin (200 kDa), β-galactosidase (116 kDa), phosphorylase B (92 kDa), bovine serum albumin (68 kDa), ovalbumin (45 kDa), and carbonic anhydrase (32 kDa). After electrophoresis, proteins were stained using Coomassie Brilliant Blue R-250.

Electrospray Mass Spectrometry. Protein molecular weight determination was obtained using a Vestec (Perseptive Biosystems) electrospray mass spectrometer. Samples were direct injections of HPLC fractions at 1.6 μL/min (source volt 2.34 kV; source current 235 A; nozzle voltage 235 V; repeller voltage 15 V; block temperature 225 °C; tip heater temperature 50 °C). Masses were acquired from 100 to 2000 for the full scan or 1000 to 1300 for the zoom scan. Deconvolution analysis was performed by a self-written Sigmaplot transform that searches for the smallest standard deviation in the calculated parent mass for several peaks (searching charges from 1 to 100 for each peak).

RESULTS AND DISCUSSION

The RP-HPLC profile of raw (day 0 windrow-cured) peanuts is shown in Figure 1A for both immature and mature seeds. The predominant change as the peanut matures is the loss of the peak at 10.5 min (peak A) and an increase in the peaks at 33.5 (C), 35.5 (D), and 36 (E) min. Thus, there is a change from more hydrophilic compounds to those with a more hydrophobic nature. Figure 1B shows the similar HPLC maturity-difference profile of windrow-dried peanuts. Peak A, seen in Figure 1A, is not seen in these samples, prohibiting its use as a maturity marker. The major change with maturity for these samples is an increase

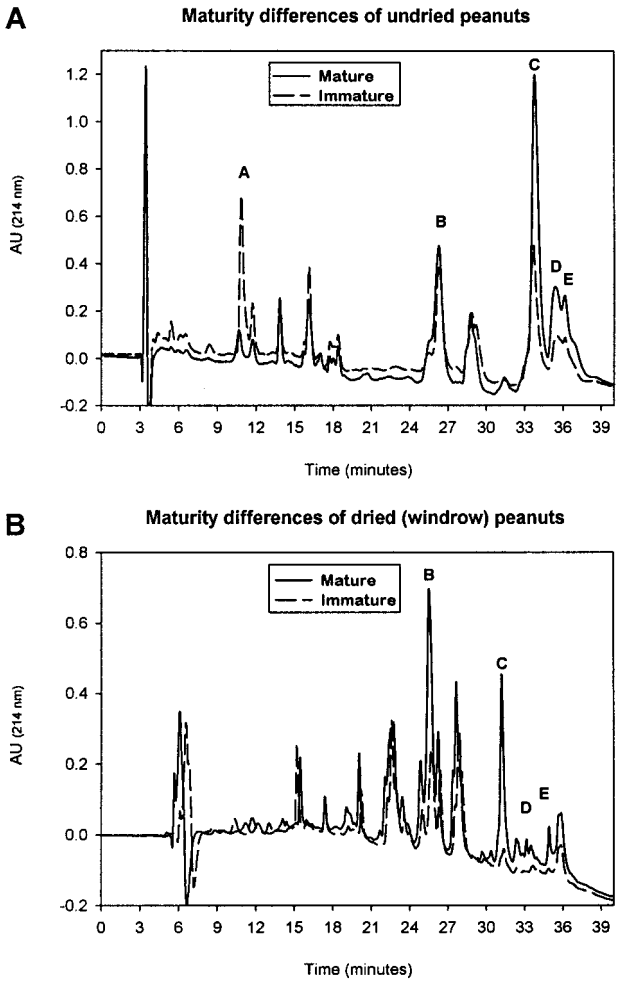

Figure 1. RP-HPLC protein profile of defatted peanut phosphate extract (yellow vs black peanuts): (A) undried (windrow day 0) and (B) dried (windrow day 4) peanuts. Peaks denoted with letters are associated with maturity and are described in the text.

in the peaks at 26 (B) and 31.5 (C) min (there was a variability for peak retention times; all peak-letter correlations have been verified). The change in peak height for peak B was found to be variable because it was associated with the cryoprecipitated material. This is characteristic of the multicomponent protein known as arachin, which has been found to precipitate from solution at 2 °C overnight. However, the retention time of arachin standard was similar to those of peaks D and E (data not shown). Overall, peak C exhibited the greatest increase in height in association with maturity and was not deleteriously affected by cryoprecipitation as were peaks B, D, and E.

In addition to comparing the differences in maturity for a certain amount of drying, the data can also be examined as the difference in drying time for a certain maturity. Figure 2A shows the effect of drying on immature peanuts. Drying the peanuts results in an obvious decrease in the 27 (B), 34.5 (C), 36 (D), and 37 (E) min peak heights, while two new small peaks are formed at 22 and 24 min. Figure 2B, shows the effect of drying on mature peanuts, where the pattern seen for immature peanuts is repeated, except for the loss of the decrease in peak B, which, as stated above, exhibits variability in peak height because of cryoprecipitation.

The method used to cure peanuts may also affect its protein profile. Figure 3 shows the difference between

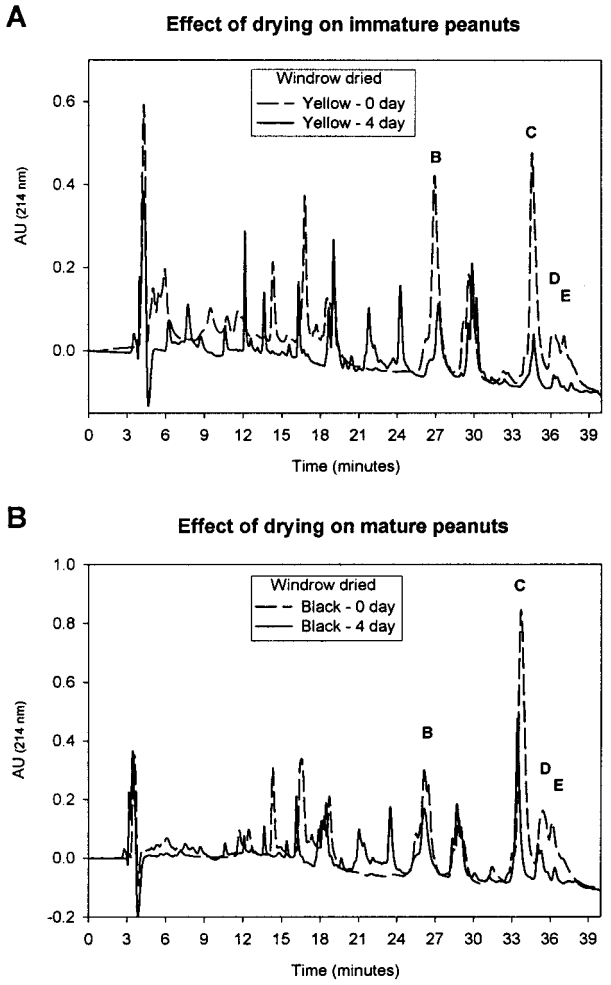

Figure 2. RP-HPLC protein profile of defatted peanut phosphate extract (windrow day 0 vs windrow day 4): (A) immature (yellow) and (B) mature (black) peanuts. Peaks denoted with letters are associated with maturity and are described in the text.

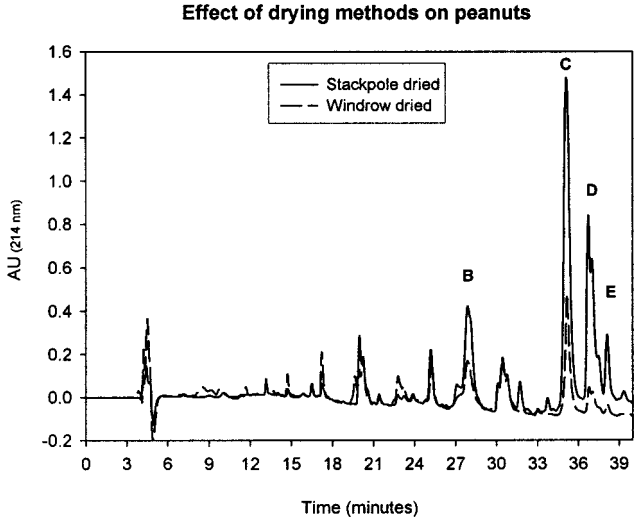

Figure 3. RP-HPLC protein profile of defatted peanut phosphate extract (windrow day 4 vs stackpole day 40). Peaks denoted with letters are associated with maturity and are described in the text.

stackpole and windrow drying on mature peanuts. Besides the variable peak B at 28 min, peaks C, D, and E at 35, 36.5, and 38 min are much larger in stackpole-dried peanuts than for the windrow-dried peanuts.

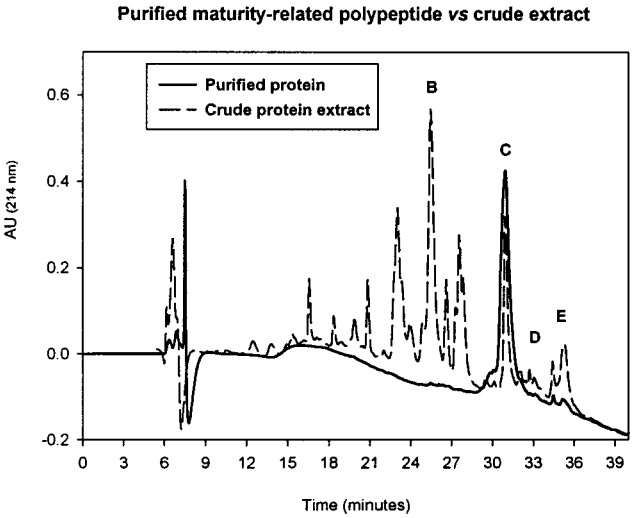

Figure 4. RP-HPLC purified peak C compared to the crude phosphate extract of defatted peanuts. Peaks denoted with letters are associated with maturity and are described in the text.

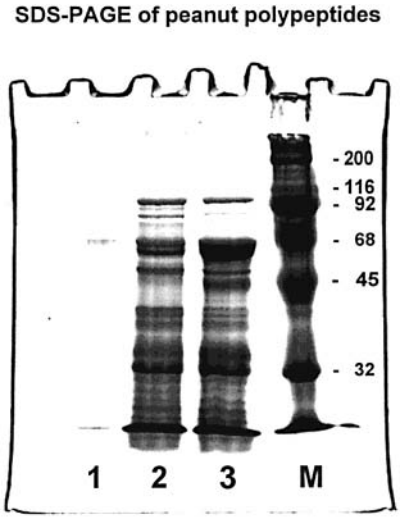

Figure 5. SDS−PAGE protein profile of defatted peanut phosphate extract. Lane 1 is the purified HPLC peak C, lane 2 is the dried (windrow day 4) immature (yellow) peanut extract, lane 3 is the dried mature (black) peanut extract, and lane M is the protein standards (molecular weights are given to the right of each band).

Thus, besides those peaks associated with arachin, peak C was found to be the predominant peak to increase in relation to maturity and decrease in relation to drying. This peak was also more prominent in the higher quality stackpole-dried peanuts compared to the windrow-dried peanuts. For these reasons and because peak C was abundant and showed stability in peak height from sample to sample, it was chosen as the best marker for peanut maturity. Peak C was isolated by RP-HPLC for characterization. Figure 4 shows the isolated peak C compared to the crude peanut extract. Figure 5 shows an SDS−PAGE gel of the purified peak C in lane 1 compared to the crude extract from windrow-dried immature (lane 2) and mature (lane 3) peanuts (lane M contains protein standards as molecular weight markers). Comparisons of lanes 2 and 3 show an enrichment in the proteins at approximately 65, 45, and 35 kDa in the mature peanuts as compared to the immature peanuts, with a concomitant reduction in proteins between 116 and 68 kDa. Lane 1 shows the

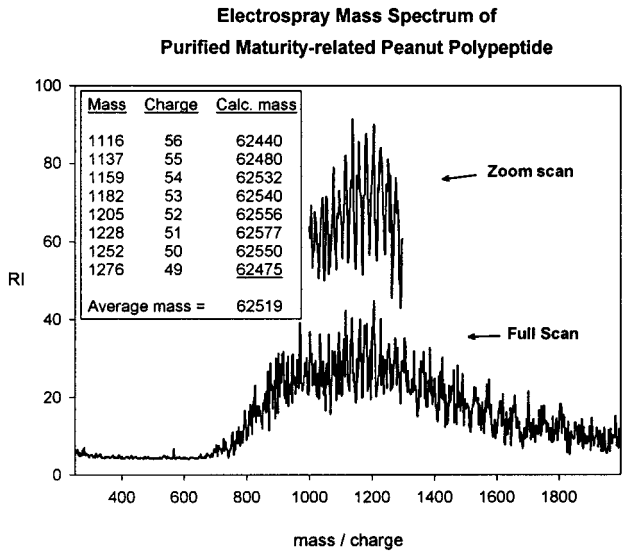

Figure 6. Electrospray mass spectrum of HPLC purified peak C. Full mass scan and zoom scan from 1000 to 1300 *m*/*z*. The inset contains *m*/*z* associated with peaks from the zoom scan and the charge and calculated molecular weight of the polypeptide. The average molecular weight is also shown.

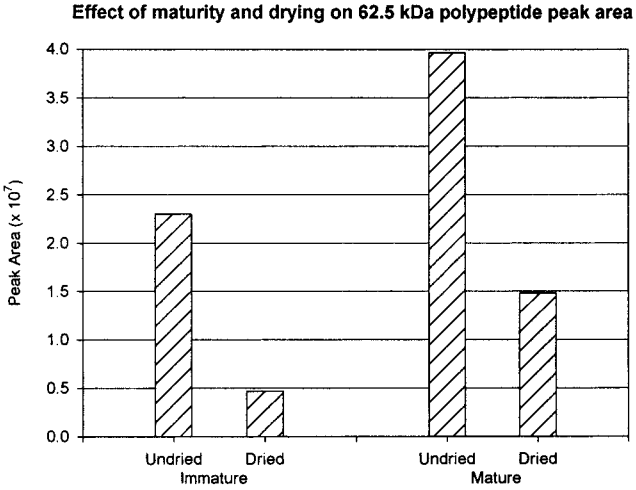

Figure 7. HPLC peak area of peak C (62.5 kDa) of undried (windrow day 0) and dried (windrow day 4) peanuts from both immature (yellow) and mature (black) samples.

purified peak C is associated with the 65 kDa protein. Electrospray mass spectroscopy was used to further characterize the maturity-related protein (peak C). As seen in Figure 6, multiply charged species were observed from 700 to 2000 *m*/*z*. A zoom scan gave a better defined portion of the spectrum, where peak masses could be deconvoluted (Figure 6, inset), giving a parent molecular weight of 62 519 with a standard deviation of 48.

Sensory quality has been associated with maturity, but this was after drying, curing, and roasting procedures. Considering the data presented above, sensory quality could be based on the level of the 62.5 kDa protein present (a) before drying (72% more in mature than immature) or (b) after drying (217% more in mature than immature) (see Figure 7). Since drying has a negative effect on the amount of 62.5 kDa protein present, the amount lost during drying could be responsible for high sensory quality, instead of the actual amount present after drying. The loss of this protein could increase the amount of certain amino acids or peptide fragments that then contribute to the Maillard reaction and give an increased sensory quality. The amount of 62.5 kDa protein lost during drying is 35% more in mature peanuts than in immature peanuts.

Therefore, we have identified a 62.5 kDa protein associated with peanut maturity. From literature reports, it does not correspond to an α-arachin subunit protein but may correspond to a subunit protein of α-conarachin. Although not specifically reported, it has been shown that the conarachin fraction has a band near 60 kDa on SDS−PAGE (Cherry, 1990). A protein found to be associated with peanut allergenicity has also been shown to have a SDS−PAGE band near 65 kDa (Burks et al., 1995). Future work will determine if there is a connectivity between the isolated 62.5 kDa protein and these reported proteins. Since maturity is related to the sensory quality of peanuts, this maturity-associated protein may prove to be a marker for peanuts that will produce a higher quality flavor when roasted.

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