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Characterization of Peanut Proteins during Roasting As Affected by Initial Moisture Content

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Dry peanut kernels (Tainan 9, a Spanish cv.) were moisture-conditioned, and their internal temperature profiles roasted at 150 °C were monitored. Higher initial moisture content resulted in a slower rate of internal temperature increase. Phosphate buffer extracts of proteins from splits roasted to internal temperatures of 80, 100, 120, and 150 °C varied with regard to initial moisture content and tendency to denature. When splits were roasted at temperatures up to 140 °C, the amount of extracted protein declined with an increase in moisture content from 3.2 to 23.2%. Gradient PAGE analysis of proteins extracted from splits and meals subjected to roasting at 150 °C and splits heated in phosphate buffer in a boiling water bath for 5, 15, 25, and 60 min was done. The thermal behavior of arachins and non-arachins varied depending upon the means of heat treatment, extent of heating, and initial moisture content.

Roasting is the most common method of processing peanuts. Moisture measurement of raw peanuts using instruments or by subjective estimation before roasting is usually a prerequisite step in establishing the roasting process, i.e., roasting temperature and time, in order to optimize the roasted peanut quality. The functions of water in this process are multiple, and the reactions involved are complicated. One of the most important effects of water on chemical reactivity in foods is its ability to mobilize and act as a solvent for food components. Chemical reaction rates generally accelerate with increasing moisture content due to increased reactant mobility. However, at elevated moisture content, chemical reactivity may decrease with increasing moisture because of the dilution effect of excessive water on reactant concentrations. A reactant in foods must dissolve in water before the reactivity can be initiated. Consequently, optimal reactivity can be eventually achieved when the reactants are dissolved in a specific amount of water to reach the critical concentration for the reaction.

One of the most important reactions during peanut roasting is the unique change in proteins. The effect of heat on such proteins has been studied. Newell et al. (1967) reported that biochemical reactions between sugars and amino acids produced specific roasted peanut flavor components. The effect of roasting on stability and nitrogen solubility of peanut proteins has been intensively studied (Neucere et al., 1969; Ory et al., 1970; Labib et al., 1977). The effect of oil cooking on changes in the peanut seed polypeptide composition was studied (Basha and Young, 1985). However, attention was directed toward the effect of heat on changes in protein at one or two moisture levels. Knowledge concerning how water functions directly or indirectly to change peanut protein properties is limited. In other foodstuffs and constituents such as myoglobulin, apples, fababean protein, oat globulin, strawberries, and soybean lipoxygenase, the effect of water on thermal be-

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havior has been intensively studied (Hagerdal and Martens, 1976; Lozano et al., 1979; Arntfield et al., 1985; Ma and Harwalkar, 1988; Roos, 1987; Wang and Toledo, 1987).

In this study, internal temperature changes in peanuts containing various moisture contents were monitored during roasting. Nitrogen solubility of peanuts subjected to various time/temperature roasting conditions was quantitatively studied. Physicochemical changes in peanut proteins during roasting and cooking in a water bath were investigated with use of gradient PAGE.

MATERIALS AND METHODS

Raw Materials and Moisture Conditions. Peanut kernels (Tainan 9, a Spanish cv.) containing 7.6% moisture (dry weight basis) were nitrogen flushed and stored in laminated polyethylene/nylon bags at 5 °C until use in this study. For moisture conditioning, the salt hygroscopicity method (Rockland, 1960) was modified. Sound peanut kernels of the same size were stored in desiccators over various salt solutions, which equilibrated at different relative humidities. Before peanuts were subjected to experiments, the vessels were removed from 5 °C storage and held for 24 h at room temperature.

Roasting and Heat-Transfer Determination. Moisture-conditioned peanut kernels were manually split, and hearts were removed. A copper-constantan thermocouple (No. 36) was inserted into one cotyledon (split), which was then roasted in a forced-air convection oven at 150 °C. The internal, surface, and air temperatures were simultaneously measured and recorded. The roasted splits were removed from the oven when an internal temperature of 140 °C was reached, cooled in a desiccator, weighed, and subsequently used for protein extraction. The moisture content of the counterpart cotyledon was measured with a moisture balance (Ohaus MB 301) at 110 °C to indicate the exact moisture content of the kernel roasted.

Protein Extraction and Quantitation. Each split peanut was combined with sodium phosphate buffer (0.2 M, pH 7.9) at a ratio of 1:20 (w/v, based on the dry weight of peanut) and ground with a homogenizer at 5000 rpm for 3 min at room temperature. After homogenization, the suspension was centrifuged (30000g) for 30 min at 15 °C. The protein content in the middle layer of the centrifuge tube was quantitated by the method of Lowry et al. (1951).

Peanut Protein Denaturation. Three lots of sound kernels of the same size were moisture-conditioned in three desiccators as described above. After moisture equilibration, a portion of the kernels was subjected to moisture analysis. The average moisture contents were 3.2 ± 0.5%, 7.6 ± 0.8%, and 23.2 ± 1.2%, respectively. Split peanuts and peanut meals prepared by grinding manually blanched splits with a cyclone mill (0.5 g of meal spread on 4-cm^2 aluminum foils) were roasted at 150 °C for 5, 15, 25, and 60 min. Concurrently, peanut splits were immersed in phosphate buffer in a test tube at a ratio of 20:1 (buffer volume to dry solid weight of splits) and cooked in a boiling water bath for 5, 15, 25, and 60 min. After cooking, the test tubes were cooled in an ice bath, buffer volume was replenished with distilled water, and the mixture was homogenized in situ to facilitate protein extraction and further characterization.

Nitrogen Solubility Index (NSI) Variation of Peanuts during Roasting. Various lots of peanut kernels were moisture-conditioned, and their average equilibrated moisture contents were determined. Seven kernels in each lot were split and roasted at 150 °C according to Figure 1. The roasting time to reach a predetermined internal temperature was measured. After roasting, splits were weighed and their moisture contents were estimated. The proteins were extracted and quantitated according to the procedure described above. The NSI was expressed as a percentage and calculated by dividing the amount of extracted proteins from peanuts after roasting by that of raw peanuts at the same basis of dry solid content and multiplying by 100. NSI variation during roasting was stepwise calculated in terms of differences in protein extractability between two splits from the same kernel subjected to different heat treatments. The overall protein denaturation rate (ODR) at a specific internal temperature was expressed by dividing the NSI decreases by the time of roasting. The stepwise protein denaturation rate (SDR) between two consecutive temperatures was expressed by dividing the NSI differences by the time intervals.

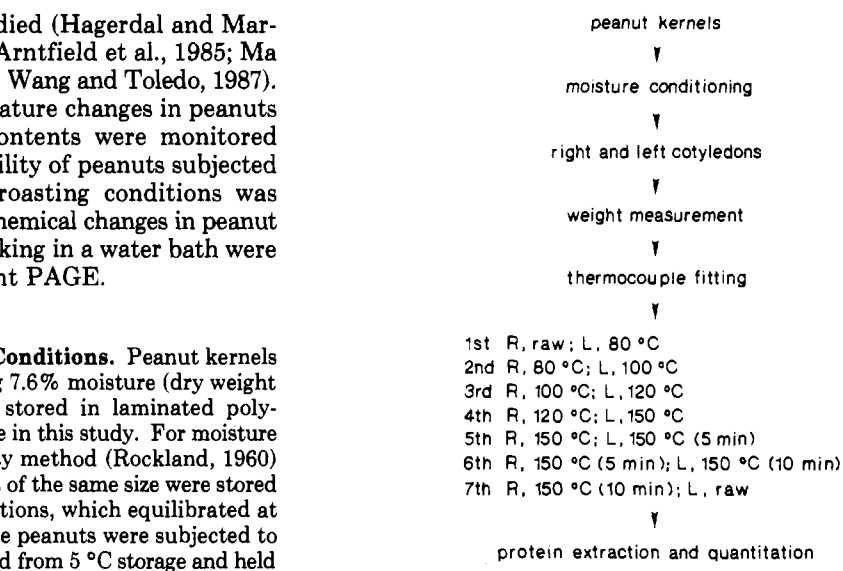

Figure 1. Flow sheet for NSI determinations of peanuts with various initial moisture contents and different times of roasting: R = right and L = left cotyledon of a peanut kernel.

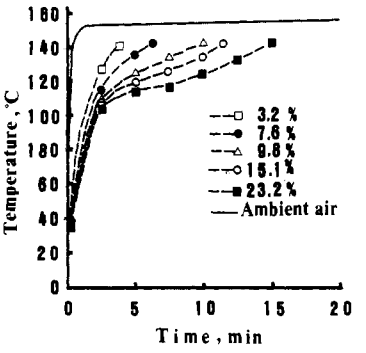

Figure 2. Internal temperature profiles of peanut splits containing various initial moisture contents when roasted at 150 °C.

Electrophoretic Examination of Peanut Proteins. Native extracted peanut proteins were separated by gradient PAGE (polyacrylamide gel electrophoresis) (5–15% gel, 1-mm thickness, 20 × 20 cm slab) run at 40 mA/slab for the initial 30 min and at 60 mA until finished (Bio-Rad Protein II and Pharmacia EPS 500/400). The protein in the gel was denatured by 10% trichloroacetic acid, stained with coomassie blue, and destained with an aqueous solution containing acetic acid and methanol. Protein extracts from treated peanuts were applied at the same dry solid basis as extracts from raw peanuts to enable quantitative comparison among samples.

RESULTS AND DISCUSSION

Internal Temperature Changes in Peanut during Roasting. Peanut splits containing various initial moisture contents were roasted in an oven at 150 °C, and internal temperature changes during roasting were recorded (Figure 2). The time required to reach the predetermined final temperature of 140 °C increased with an increase in initial moisture content of splits from 3.2 to 23.2%. The initial rate of temperature increase also decreased with an increase of initial moisture content. For those splits with initial moisture contents higher than 9.8%, plateaus occurred at about 110 °C. A similar phenomenon was observed by Wang and Toledo (1987) who heated soybeans with microwaves. They found that plateaus occurred between 100 and 105 °C for soybeans with moisture content higher than 8.7%. In general, when a material is heated, its specific heat is considered the major factor governing the rate of temperature increase. The fact that higher

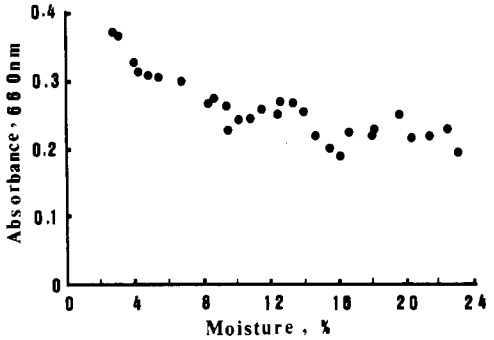

Figure 3. Extracted peanut proteins determined by the Lowry method and expressed as absorbance units in relation to the initial moisture content of peanut splits roasted at 150 °C until an internal temperature of 140 °C was reached.

moisture content was correlated with higher specific heat for peanuts is undoubtedly related to variations in the rate of temperature increase. However, during roasting, water plays a unique role. In addition to the specific heat effect, mass transfer, i.e., evaporation, and other enthalpy-related reactions, e.g., heat transition such as endothermic or exothermic reactions of the constituents are also involved. For a simplified case, if moisture transfer during roasting is considered separately, the continuous vapor evaporation associated with heat absorption will cause a delay in the internal temperature increase. Evaporation is definitely moisture-dependent. In this study, a temperature plateau at about 110 °C was particularly observed for peanuts with initial moisture contents higher than 9.8% (Figure 2). The roasting process is considered as a heat-transfer controlled period in which water transfer in a split from the inner part to the outer surface for evaporation is unlimited and temperature remains at the boiling point, which is slowly increasing due to an increase in solid content with regard to water evaporation. Consequently, the plateau period was prolonged, and the temperature was lower for peanuts with higher moisture content.

Thermal Denaturation of Peanut Proteins. Peanut proteins in splits with various initial moisture contents roasted at 150 °C until an internal temperature of 140 °C was reached were extracted with phosphate buffer and quantitated (Figure 3). Due to inevitable natural variation among kernels, the moisture contents of peanuts after moisture equilibration in a relative humidity controlled vessel were randomly distributed within a narrow range. Moisture content in various samples ranged from 3.2 to 23.2%, depending upon the saturated salt solution used in the equilibration vessel. For each kernel, the initial moisture content in the counterpart split was accurately measured to address the effect of moisture on protein denaturation. After roasting, the amounts of extracted proteins were closely dependent on initial moisture content. In general, a higher initial moisture content resulted in a lower protein extractability after roasting. In addition, it was noted that protein extractability of splits decreased more rapidly and was more markedly influenced in the moisture content range from 3.2 to about 10% than at higher moisture contents.

Since a decrease in protein solubility is one of the most general properties of protein denaturation, the decrease in peanut protein extractability during roasting is mainly due to heat denaturation. The dependency of peanut protein denaturation on moisture during roasting, as indicated by a change in extractability, is in agreement with other reports. For instance, as early in 1933, Barker reported that the denaturation temperature for egg albumin, as determined from the degree of solubility, was a linearly

Table I. Nitrogen Solubility Index (NSI) and Denaturation Rates (ODR, SDR) of Peanuts Containing Various Initial Moisture Contents and As Influenced by Roasting at 150 °C

moisture, %	item[a]	temperature, °C control	80	120	150	150	150
3.2	time	0	0.7	2.0	7.1	12.1	17.1
	NSI	100	100	99.2	84.1	79.4	72.6
	ODR	0	0	0.4	2.2	1.7	1.6
	SDR	0	0	0.6	3.0	0.9	1.4
7.6	time	0	0.9	3.3	10.5	15.5	20.5
	NSI	100	98.8	97.4	69.2	66.0	54.3
	ODR	0	1.3	0.8	2.9	2.2	2.2
	SDR	0	1.3	0.6	3.9	0.6	2.3
11.1	time	0	1.1	4.3	13.5	18.5	23.5
	NSI	100	95.6	89.2	66.7	48.3	36.7
	ODR	0	4.0	2.5	2.5	2.8	2.7
	ADR	0	4.0	2.0	2.5	3.7	2.3
23.2	time	0	1.4	8.1	19.3	24.3	29.3
	NSI	100	91.5	61.5	51.2	40.6	36.4
	ODR	0	6.1	4.8	2.5	2.4	2.2
	SDR	0	6.1	4.5	0.9	2.1	0.8

[a] Key: time, roasting time required to reach the specific temperature, min; NSI, nitrogen solubility index; %; ODR, overall denaturation rate (NSI of control − NSI after roasting)/time; SDR, stepwise denaturation rate (NSI of former − NSI of latter)/time difference.

decreasing function of the moisture content. Bull and Breese (1968) found that there was an increase in solubility for ovalbumin when heat-treated at a lower moisture content than 30%. More recently, Hagerdal and Martens (1976) used the technique of differential scanning calorimetry to examine the effect of heat treatment on a model protein of sperm whale myoglobulin at various water contents. At a water content below 30%, a certain fraction of the protein underwent irreversible transitions with close dependence on the amount of water present during the heat treatment. Arntfield et al. (1985) examined the effect of moisture content on the thermal behavior of legumin and vicilin, two major fababean proteins, by means of differential scanning calorimetry. They observed that, at water levels lower than 1.5 g of water/g of protein for legumin and 0.9 g of water/g of protein for vicilin, the denaturation temperature increased as the moisture content was lowered. However, since these investigations dealt with considerably purified protein compared to peanut proteins, extrapolations and comparisons of similarities are tenuous. In addition, as shown in Figure 3, the moisture range of ca. 10–15% in peanut splits resulted in substantial fluctuation in extracted proteins. This may be a result of a complex involvement of peanut components during roasting.

To further investigate the behavior characteristics of peanut proteins during roasting, proteins were extracted from splits roasted at 150 °C. Extracts were made over an internal temperature range of 80–150 °C (Figure 1; Table I). Cotyledons with higher moisture content required a longer heating time to reach the desired internal temperature. This can be reasonably illustrated by examining the internal temperature profiles shown in Figure 2. At any moisture content, the NSI decreased with time as the internal temperature increased.

The initial protein denaturation temperatures during roasting of splits with various moisture contents can be illustrated by the initial changes of NSI (Table I). For instance, at an internal temperature of 80 °C, the NSI values were 100, 98.8, 95.6, and 91.5% for splits with moisture contents of 3.2, 7.6, 11.1, and 23.2%, respectively. This indicates that lower initial moisture contents resulted in higher initial denaturation temperatures. This observation was in agreement with data reported on fababean

proteins and sperm whale myoglobulin (Arntfield et al., 1985; Hagerdal and Martens, 1976). A hypothesis proposed by Bull and Breese (1968) stated that during successive water removal from the protein, inter- and intramolecular electrostatic interactions and hydrogen bonds must be established in order to satisfy the sites earlier occupied by water. These inter- and intramolecular interactions created the increased thermostability of the proteins. In this study, the inter- and intramolecular interactions might have commenced during the period of moisture conditioning of the peanut kernels with lower moisture contents and resulted in having higher thermal stabilities.

Since the rate of internal temperature increase is governed by the moisture content (Figure 2), the effect of various heat treatments on NSI was further expressed by ODR (overall denaturation rate) and SDR (stepwise denaturation rate) (Table I). When peanuts were heated up to 120 °C, ODR increased significantly with an increase in the initial moisture content. However, when an internal temperature of 150 °C was reached and held for 10 min, the ODR range was small (1.6–2.8), even when heating times were quite different. This agrees with the observation that extensive heating of proteins can result in similar effects as heating at higher temperature during a shorter period of time (Pence et al., 1953).

The highest SDR for peanuts with 3.2 and 7.6% of moisture content occurred during the period of internal temperature ranged from 120 to 150 °C. For peanuts containing 11.1 and 23.2% moisture, the highest SDR occurred during the initial roasting period at 80 °C. For instance, in splits containing 23.2% moisture, a sharp decrease in NSI from 100 to 61.5% occurred during the initial 8.1 min before an internal temperature of 80 °C was reached. This was followed by a slow decrease in NSI from 61.5 to 36.4% in the subsequent heating period of about 21 min (from 8.1 to 29.3 min).

The internal temperature increase to a plateau during roasting (Figure 2), which is influenced largely by water evaporation, occurred at temperatures below 120 °C. Therefore, most proteins, as influenced by moisture content, were denatured when the internal temperature was below 120 °C. However, in splits containing very low moisture, e.g., 3.2%, or during the latter periods of roasting peanuts with higher initial moisture content in which most moisture had evaporated, proteins still continued to be denatured with time. This suggests that some peanut proteins are susceptible to denaturation only at high temperatures and that denaturation occurs without moisture dependency only when a specific temperature is reached.

Electrophoretic Examination of Peanut Proteins. A gradient PAGE technique was used to investigate changes in the peanut proteins during heat treatment. Three moisture levels, namely 3.2, 7.6, and 23.2%, were studied. The peanut splits and meals that were dry roasted at 150 °C and splits cooked in phosphate buffer solution for 5, 15, 25, and 60 min were monitored (Figure 4). According to other reports (Tombs, 1965; Neucere et al., 1969; Dawson, 1971; Basha and Cherry, 1976), proteins with migration distances from 0 to 4 cm are arachins, including arachin and conarachin, and those localized between 5 and 10 cm are designated as non-arachins. During roasting of splits with initial moisture contents of 3.2 and 7.6%, arachins were relatively stable when roasted for up to 25 min. However, significant heat denaturation of arachins occurred when splits were heated for 25–60 min. Neucere et al. (1969) dry roasted whole peanut kernels at 145 °C for 1 h and reported that solubility of

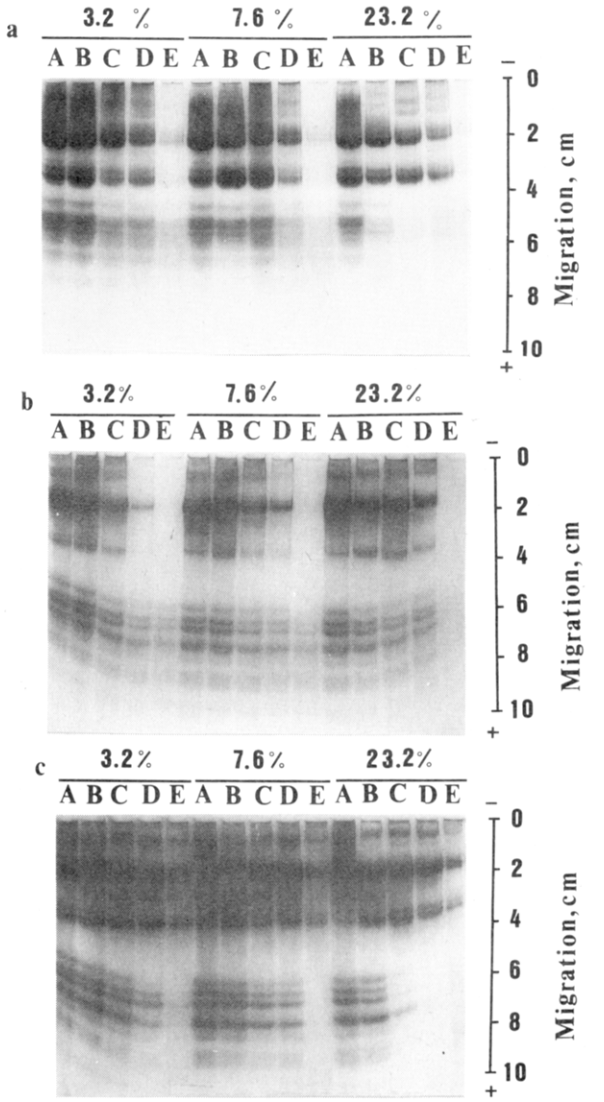

Figure 4. Photographs of peanut proteins run on gradient PAGE: a, splits roasted at 150 °C; b, split meals roasted at 150 °C; c, splits in phosphate buffer cooked in a boiling water bath. Conditions: (A) 0, (B) 5, (C) 15, (D) 25, and (E) 60 min of heat treatment.

the total proteins was reduced to less than half of the control, but α-arachin was not significantly altered. Basha and Young (1985), using peanut kernels oil-cooked at 147 °C, indicated that arachin remained relatively unchanged during a 12-min roasting period. In this study, splits were used and a roasting temperature of 150 °C resulted in more extensive denaturation: i.e., when peanuts were roasted for 60 min, more arachin denaturation was observed. This is in agreement with observations on splits and peanut meals roasted at 150 °C (Figure 4a,b). The heat effect was of course more severe for meals than splits. The average internal temperature of peanuts containing 3.2% moisture was higher than those with 23.2% moisture during the initial 25 min of roasting (Figure 2); more arachins denatured in the latter peanuts, indicating the moisture dependency of arachin on heat denaturation. In addition, the observation that arachins were almost completely denatured when heated from 25 to 60 min, regardless of the initial moisture content, indicates that at an elevated temperature, e.g., 150 °C, denaturation occurs independent of water.

For splits containing 23.2% moisture, arachins were quantitatively denatured from 0 to 5 min and 15 to 60 min as shown on the gel (Figure 4a). This is in agreement with data in Table I showing that the initial NSI decrease and

the SDR were highest in peanuts containing 23.2% moisture. For comparison, non-arachins, except at 23.2% moisture, were considerably more heat stable, even in peanuts roasted for 60 min. In kernels with 23.2% moisture, significant heat denaturation was noted after 15 min of roasting. This revealed that denaturation of non-arachins was moisture-dependent.

In peanut meals (Figure 4b), the thermal behavior of arachins was affected differently compared to the behavior in splits (Figure 4a). Since splits were finely ground to prepare meals and thinly spread on aluminum foil for roasting, the heating temperature of 150 °C was undoubtedly reached in a much shorter time than in splits, and the moisture effect was consequently less significant among samples due to rapid vapor evaporation during the initial stage of roasting. In addition, the effect of oxygen may have played a greater role in denaturing arachin in meals than in splits during roasting at an elevated temperature. Non-arachins were heat-stable during roasting up to 25 min, regardless of the initial moisture content. After 60 min of roasting, non-arachins in meals containing 23.2% moisture were denatured. This supports the previous conclusion that denaturation of non-arachins was influenced more by moisture than by temperature.

When splits were cooked in phosphate buffer in a water bath at 100 °C (Figure 4c), vapor evaporation from peanuts did not occur and the internal temperature was held constant. Arachins were comparatively the most stable in all three heat treatments. This is in agreement with the observation that arachins heated at 100 °C for 15–90 min remained unchanged (Cherry and McWatters, 1975). However, these researchers did not compare the effect of initial moisture content. Similar to dry roasting of splits (Figure 4a), arachins in splits with an initial moisture content of 23.2% were more labile to cooking than in splits with lower moisture content. This may also be related to the effect of initial moisture content in splits acting as a better thermal-transfer medium. The internal temperature increase in splits containing 23.2% moisture during cooking in buffer would be more rapid than that in splits with lower moisture contents. Consequently, even with the water uptake by splits during cooking, proteins in peanuts originally characterized by a drier state had higher thermal stability. This is in agreement with Bull and Breese (1968), who stated that protein hydration state was related to its thermal stability.

A comparison of non-arachins in cooked splits with three moisture levels reveals that thermal denaturation is dependent upon initial moisture content (Figure 4c). In splits containing 23.2% moisture, after 15 min of cooking, non-arachins essentially disappeared. After 60 min of cooking, regardless of their initial moisture content, non-arachins were completely denatured and replaced by hydrolyzed polypeptides which were distributed on gels at migration distances of 5–10 cm or more. This is in agreement with the observation that peanuts moist-heated for 30 min in an autoclave had decreased non-arachin content (Cherry and McWatters, 1975). Therefore, it seems reasonable to suggest that thermal sensitivity of non-arachins is strictly moisture dependent.

In conclusion, the thermal behavior of arachins and non-arachins during heat treatments is different. The initial moisture content of peanuts plays an important role in governing heat transfer and physicochemical changes of proteins. However, since heat-related reactions in peanuts during roasting or cooking are complex and the interchanges among peanut protein molecules, e.g., association or dissociation, are frequent, depending upon surrounding conditions (Neucere et al., 1969; Basha and Young, 1985), a simplified model system in accordance with and in consideration of real food states needs to be studied further.

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