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Effect of Salt Concentration and Duration of Boiling on Peanut Seed Composition

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Green peanuts (*Arachis hypogaea* L. Cv. Florunner) were boiled in water containing various amounts of salt (0–5%) for periods of 0–2 h to determine the effect of wet heat on seed composition. The results showed that boiling caused a reduction in soluble carbohydrates, soluble protein, and free amino acids, while total protein, insoluble carbohydrates, and oil remained unaffected. Gel filtration and gel electrophoresis data showed the disappearance of major protein and polypeptide entities, indicating that while total seed protein content is unaffected during boiling, the protein and polypeptide composition are greatly altered.

In the United States, about 70% of the peanuts produced are consumed locally as food and the remaining is used as peanut byproducts. The aroma, texture, and flavor of raw peanuts can be improved by several methods. All methods of peanut preparation for food involve heating, which in turn alter or destroy various seed components such as protein, carbohydrates, vitamins, and natural antioxidants (Woodroof, 1983).

Liardon and Hurrel (1983) reported that the proteins are the most reactive of the major food components and during food processing they react with sugars, fats, and their oxidation products, polyphenols and various food additives. Previous studies have shown conformational changes in seed proteins (Neucere, 1974; Jacks et al., 1975; Srikanta and Rao, 1974; Kumar et al., 1980; Yamada et al., 1983) due to heating. Recently, Basha and Young (1985) reported changes in protein and polypeptide composition following oil roasting of peanut seeds for periods greater than 4 min.

Most of the previous reports were concerned with roasted peanut products. Although large amounts of peanuts are consumed as roasted products, freshly dug, unshelled, immature peanuts boiled in a brine are consumed as a delicacy in certain areas of the United States and various peanut growing regions of the world. However, little information exists on the effects of boiling on seed composition and its nutritional value. The objective of this study was to determine the effects of boiling periods and the presence of different concentrations of salt during boiling on peanut seed composition.

MATERIALS AND METHODS

Peanut (*Arachis hypogaea* L. Cv. Florunner) seeds were grown under recommended cultural conditions at the University of Florida Agricultural Experiment Station, Marianna, FL. After harvesting, green peanuts were pulled from the plant, washed, and then divided into 150-g batches. In the salt concentration study, peanuts were boiled for 45 min in 700 mL of water containing 0, 1, 2, 3, 4, and 5% (w/v) salt. For the study of the effect

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Table I. Effect of Boiling (45 min) on Peanut Seed Composition in the Presence of Various Amounts of Salt

salt concn, g/100 mL water	seed component, %					
	oil[a]	α-amino N[b]	sol protein[b]	total protein[b]	sol carbohydr[b]	insol carbodr[b]
unboiled	49.0	0.67	15.2	40.1	9.2	16.9
0	49.5	0.33**[c]	13.8*	44.9	6.3*	16.6
1	49.0	0.38**	13.3*	45.1	6.5*	17.1
2	48.4	0.42**	13.0*	45.3	6.3*	17.2
3	47.6	0.40**	12.5*	45.2	6.4*	17.3
4	47.2	0.40**	12.3*	43.2	6.3*	17.0
5	47.0	0.37**	12.5*	40.2	6.2*	17.1

[a] Grams/100 g of meal. [b] Grams/100 g of defatted meal. [c] Key: ** = significantly different from unboiled seed at $P = 0.01$; * = significantly different from unboiled seed at $P = 0.05$.

Table II. Effect of Duration of Boiling with 4% Salt on Peanut Seed Composition

duration, min	seed component, %					
	oil[a]	α-amino N[b]	sol protein[b]	total protein[b]	sol carbohydr[b]	insol carbohydr[b]
unboiled	49.0	0.67	15.2	40.1	9.2	16.9
10	49.1	0.39**	12.4*	38.9	6.2*	17.0
20	48.8	0.41**	12.6*	43.5	6.6*	16.9
30	47.3	0.43**	12.3*	4.34	6.8*	17.0
40	47.7	0.40**	12.4*	42.5	6.8*	16.5
50	47.2	0.37**	12.4*	43.7	6.9*	16.4
60	48.0	0.37**	12.6*	43.0	6.9*	16.2
75	46.4	0.35**	12.4*	44.8	6.7*	16.2
90	46.0	0.28**	12.1*	43.6	5.5*	16.2
105	44.5	0.28**	11.6*	43.7	5.1*	16.3
120	45.0	0.27**	11.5*	43.0	4.8*	16.3

[a] Grams/100 g of meal. [b] Grams/100 g of defatted meal. [c] Key: ** = significantly different from unboiled seed at $P = 0.01$; * = significantly different from unboiled seed at $P = 0.05$.

of boiling time, the peanuts were boiled in 4% (w/v) salt solution for various times from 0 to 120 min. All the treatments were conducted in triplicate with unboiled green peanuts serving as the control.

Grinding and Defatting. After treatment, boiled peanuts were shelled, the seed coats were removed, and the nuts were freeze-dried. The freeze-dried samples were ground to a meal. The resulting meal was then extracted with hexane until it was fat-free (Basha et al., 1976), and the weight of the extracted oil was recorded. The resulting defatted meal (DFM) was allowed to dry at room temperature in a hood and stored at −20 °C until used for chemical analysis.

α-Amino Nitrogen, Soluble Carbohydrates, and Insoluble Carbohydrates. Defatted meal (0.20 g) was homogenized for 1 min with methanol–chloroform–water (60:25:15, v/v/v) in a Polytron homogenizer. The homogenate was centrifuged at 20000*g* for 15 min. The resulting pellet was reextracted and centrifuged as above. A third extraction with ethanol was then done (Young et al., 1974), and the supernatants from the above three centrifugations were pooled and made up to 15 mL with ethanol. An aliquot of the supernatant was analyzed for α-amino nitrogen by the method of Yemm and Cocking (1955) with leucine as the standard. Another aliquot was analyzed for soluble carbohydrates according to the method of Yemm and Willis (1954) with anthrone as a reducing agent and glucose as the standard.

The pellet from the methanol–chloroform–water extraction was air-dried, and a portion (0.05 g) was homogenized with 0.04 N H_2SO_4 and heated in boiling water for 1 h (Watschke and Waddington, 1974). The homogenate was then centrifuged at 20000*g* for 15 min, and the resulting supernatant was analyzed for sugars by the method of Yemm and Willis (1954).

Protein. Protein was extracted by homogenizing defatted meal (0.05 g) with 1 M NaOH in a Polytron homogenizer at 37 °C and then centrifuging at 20000*g* for 20 min (Basha et al., 1976). Soluble protein was extracted by grinding 0.05 g of defatted meal in 0.01 M Tris-HCl, pH 8.2 (15 mL), with a Polytron homogenizer. The homogenate was centrifuged at 20000*g* for 20 min. The supernatants obtained from the above centrifugations were analyzed for protein following the method of Lowry et al. (1951) with bovine serum albumin as the standard.

One-Dimensional Gel Electrophoresis. Protein was extracted from the defatted meal with 0.5 M NaCl and 0.01 M Tris-HCl, pH 8.2, and subjected to electrophoresis under nondenaturing (Davis, 1964) and denaturing (Laemmli, 1970) conditions in 7.5% and 10% (w/v) acrylamide gels, respectively. After electrophoresis, proteins were stained with Coommassie Blue for visualization.

Molecular Weight Estimation. The gel was calibrated against protein standards of known molecular weight. The protein standards were dissociated with 1% (w/v) SDS and 1.5% (w/v) dithiothreitol and 1.2% (w/v) Tris by boiling for 3 min. Twenty micrograms of each sample was loaded on a 10% acrylamide 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).

High-Performance Liquid Chromatography (HPLC). Protein was extracted by grinding 50 mg of the defatted meal with a Polytron homogenizer and 2 mL of 0.02 M sodium phosphate buffer, pH 7.0, containing 0.5 M sodium chloride. The homogenate was centrifuged at 20000*g* for 20 min, and a 20-μL aliquot of the supernatant was analyzed by HPLC (Basha, 1988). The HPLC system (Waters, MA) consisted of a Model 510 pump, a UV/vis detector, a Model 840 data station, a U6K injector, and a PROTEIN PAK 300 SW column (7.8 mm × 20 cm). The mobile phase was 0.02 M sodium phosphate buffer, pH 7.0, containing 0.4 M sodium chloride and 0.05% sodium azide. Flow rate was 1.0 mL/min, isocratic, and the compounds were detected at 280 nm; the range was 1 AUFS.

RESULTS AND DISCUSSION

Oil. Boiling caused a slight reduction (2–4%) in the oil content of the peanut (Tables I and II). Presence of various amounts of salt during boiling had no significant ($p < 0.05$) effect on the oil content of the peanut seed (Table I). Likewise, increasing the duration of boiling also showed no significant ($p < 0.05$) changes in the oil content of the peanut seed even after 2 h. However, after 2 h of boiling oil content of the seed was decreased of about 4%.

α-Amino Nitrogen. The α-amino nitrogen, which indicates the amount of total free amino acids, was significantly ($p < 0.01$) lower in the boiled peanuts than in the unboiled peanuts (Tables I and II). The presence of dif-

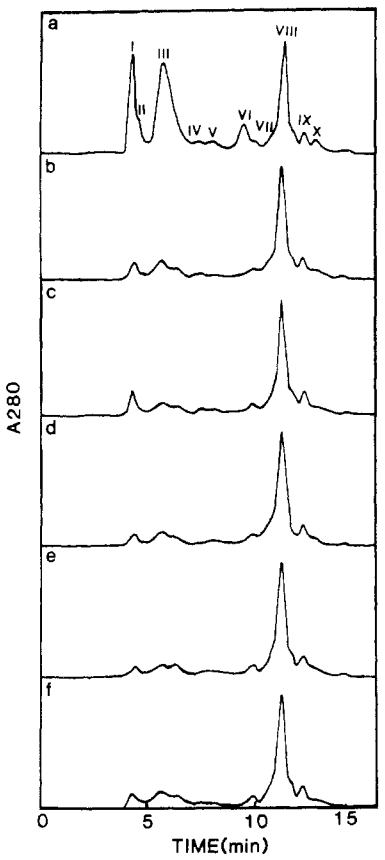

Figure 1. Changes in the protein composition of peanut seed following boiling in the presence of various amounts of salt as observed by HPLC: a = unboiled, b = 0%, c = 1%, d = 2%, e = 3%, f = 5%.

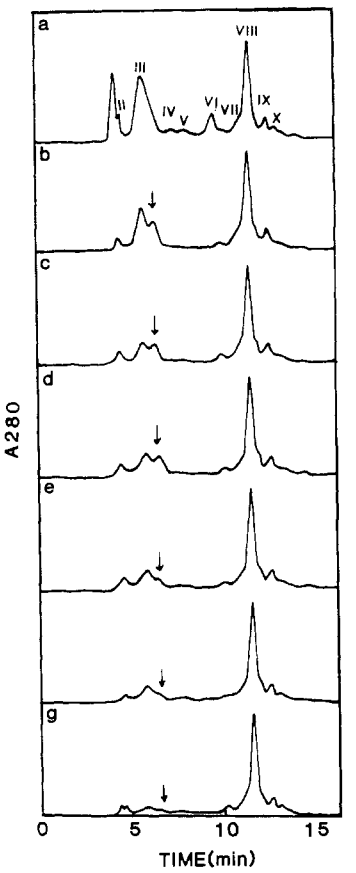

Figure 2. HPLC profiles of peanut seed proteins obtained following boiling for 0 min (a), 10 min (b), 20 min (c), 30 min (d), 60 min (e), 90 min (f), and 120 min (g).

ferent amounts of salt had no significant effect on the levels of α-amino nitrogen (Table I). The α-amino nitrogen content of the peanuts decreased by 40% within the first 10 min of boiling and then remained unchanged up to 75 min of boiling (Table II). Additional boiling caused slight reduction in the α-amino nitrogen content of the seed. The observed loss in the α-amino nitrogen content of the seed by boiling may be attributed to the leaching of this component into the boiling media.

Soluble Proteins. The soluble protein content of the boiled peanuts was lower than in the unboiled peanuts. Although boiling caused a significant ($P < 0.05$) decrease in the soluble protein, salt treatments had no effect on the protein (Table I). Boiling for 10 min caused a significant ($P < 0.05$) decrease in the soluble protein, and boiling for up to 75–90 min had no further effect. The observed decrease in the soluble protein content of peanut seed may be attributed to the heat denaturation of proteins, which would affect their solubility.

Total Protein. Unlike the soluble proteins, the total protein content of the seed remained unaffected by boiling. As seen in Tables I and II no significant ($P < 0.05$) differences were observed between the total protein content of the boiled and unboiled seeds, indicating that the observed reduction in the soluble protein content may be due to decreased protein solubility resulting from heat denaturation and/or protein aggregation (Neucere et al., 1969; Yamada et al., 1983; Basha and Young, 1985). Salt treatments and different boiling periods had no significant effect on seed protein content.

Soluble Carbohydrates. Boiling caused a significant ($P < 0.05$) decrease in the soluble carbohydrate content of the seed (Tables I and II). Peanuts boiled in the presence of various salt concentrations contained similar soluble carbohydrate concentrations (Table I), indicating that the salt had no effect on the loss of soluble carbohydrates from the seed. There was a major loss (30%) of soluble carbohydrates in the first 10 min of boiling, and then the concentration remained unchanged for up to 75 min (Table II). Boiling for more than 75 min caused further loss in the soluble carbohydrates of the seed. The decrease in the soluble carbohydrate content of the seed is attributed to their leaching into the boiling media.

Insoluble Carbohydrates. Insoluble carbohydrates, which represent the structural components, remained unaffected by boiling. The presence of various amounts of salt (Table I) and different boiling periods (Table II) had no significant ($P < 0.05$) effect on the insoluble carbohydrate content of the seed, suggesting that boiling did not cause major structural changes in the peanut seed.

High-Performance Liquid Chromatography. Changes in the seed protein composition due to boiling were monitored by high-performance liquid chromatography. The effects of various salt concentrations on protein profiles of boiled seeds are shown in Figure 1. HPLC resolved protein from unboiled peanut seed into 10 peaks. The observed protein profile is similar to the one reported for this cultivar by Basha and Pancholy (1981). Peak I represents the void volume and contained polymers of arachin (the major storage protein of peanut) and other high molecular weight ($>10^6$ Da) components. Peak III contained the arachin monomer, while peak VI contained the methionine-rich protein (Basha and Pancholy, 1981). Total protein analysis and HPLC data indicated that boiling caused no major changes in the seed protein content but it altered the seed protein composition. As seen in the figure, boiling caused great reduction in the size of peaks I–III, VI, and X. Thus, within 10 min of boiling the

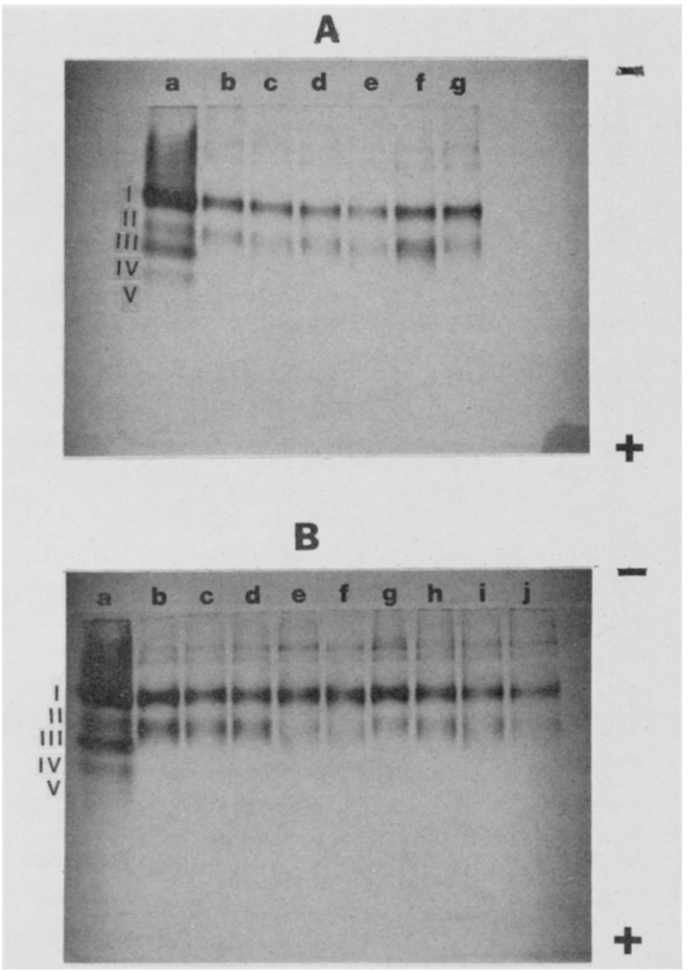

Figure 3. Gel electrophoretic profiles of seed proteins from boiled peanuts. A: unboiled control (a); boiled in presence of 0% (b), 1% (c), 2% (d), 3% (e), 4% (f), and 5% (g) salt. B: boiled for 0 min (a), 10 min (b), 20 min (c), 30 min (d), 40 min (e), 50 min (f), 60 min (g), 75 min (h), 90 min (i), and 105 min (j). Twenty micrograms of protein was applied per gel.

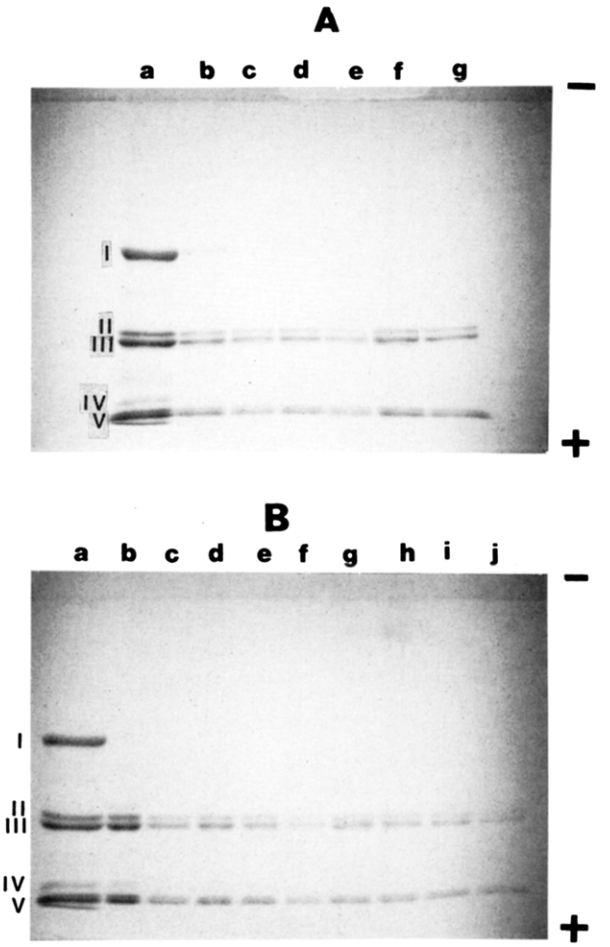

Figure 4. Sodium dodecyl sulfate gel electrophoretic profiles of boiled peanuts. A: unboiled control (a); boiled in the presence of 0% (b), 1% (c), 2% (d), 3% (e), 4% (f), and 5% (g) salt. B: peanuts boiled for 0 min (a), 10 min (b), 20 min (c), 30 min (d), 40 min (e), 50 min (f), 60 min (g), 75 min (h), 90 min (i), and 105 min (j). Forty micrograms of protein was applied per gel.

ratios of peaks I/VIII and III/VIII changed from 0.89 and 0.77 to 0.11 and 0.15, respectively. Boiling in the presence of various salt concentrations did not have any major effect on protein profiles, indicating that heating alone affected protein composition.

Peaks I, II, and VI decreased rapidly within 10 min of boiling while peak III decreased gradually with increasing periods of boiling (Figure 2). Before boiling, the ratios of peaks I/VIII and III/VIII were 0.69 and 0.65; however, within 10 min of boiling these ratios changed to 0.11 and 0.42 and afterward decreased gradually to ca. 0.07. In contrast, peak VIII, a low molecular weight (<75 000 Da) protein, remained high throughout the 2-h boiling period. After 10 min of boiling, a new peak (arrow) appeared adjacent to peak III. The data suggest that low molecular weight polypeptide components resulting from the breakdown of high molecular weight proteins are the dominant protein species of the boiled seeds.

One-Dimensional Gel Electrophoresis. Changes in the seed protein and polypeptide composition were also monitored by gel electrophoresis under nondenaturing conditions. Major protein bands evident after electrophoresis were identified as I–V (Figure 3). Compared to the control, boiled peanuts showed a decreased protein band density and contained fewer protein bands. Three fast-moving acidic components (III–V) disappeared within 10 min of boiling. Addition of different amounts of salt had no effect (Figure 3A) on seed protein composition. Likewise, boiling periods between 10 and 75 min showed no major changes in protein profiles, but heating over 75 min caused a decrease in the density of bands I and II. The decrease in protein band density may be due to either failure of the proteins to enter the gel as a result of heat coagulation or breakdown of high molecular weight proteins into fast-migrating low molecular weight polypeptides.

Differences in seed polypeptide profiles were monitored after the seed proteins were dissociated and the polypeptides resolved by SDS gel electrophoresis (SDS-PAGE). The major polypeptide bands were identified as I–V. Molecular weights of these components ranged between 14 000 and 70 000 Da. The SDS-PAGE showed (Figure 4) a reduction in the seed polypeptide content as well as disappearance of polypeptides I (MW 70 000) and IV (MW 26 000) due to boiling. In addition, the amount of protein in bands II, III, and V decreased in the boiled peanuts compared to that in the control. Addition of different amounts of salt had no effect on seed polypeptide composition (Figure 4A). Polypeptides I and IV disappeared within 10 min of boiling (Figure 4B), indicating their susceptibility to boiling. Additional boiling caused no major changes in the other polypeptide components.

Overall, the data suggest that while boiling did not greatly affect the amount of major seed components such as protein, oil, and carbohydrates, it did cause loss of free sugars and free amino acids and altered the seed protein

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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