plained on a dilution basis and is not understood at this time. Possibly the dietary effect was a result of differences in ruminal metabolism which concurrently influenced pesticide absorption from the gastrointestinal tract. Sink *et al.* (1972) reported that DDT metabolism was different in the rumen of concentrate *vs.* forage-fed sheep and Rumsey *et al.* (1970) reported that the concentration of total DDT residues was different between concentrate *vs.* forage-fed steers.

The average residue concentration of fat tissue was lower in DES-implanted heifers (26.8 *vs.* 35.1 ppm, $P < 0.01$) than in heifers receiving no DES. The difference was primarily due to the low concentrations found in the implanted heifers that were fed forage (19.4 *vs.* 30.6 for forage plus or minus DES, respectively, and 34.1 *vs.* 39.7 for concentrate plus or minus DES, respectively, $P < 0.01$). These differences may reflect an apparent increase in metabolic rate of DES-implanted cattle. A consistently higher heart rate was found in the present trial for DES-implanted heifers than for heifers that received no implants (Rumsey and Bond, 1972) and also in a separate trial with steers (Rumsey *et al.*, 1973). DES may also have a direct effect on certain hepatic enzyme systems (Bitman and Cecil, 1970; Street *et al.*, 1966).

Davison (1970) and Murphy and Korschgen (1970) indicated that dieldrin accumulation plateaus after about 28 weeks of exposure, the plateau concentration being dependent on the dosage level. Assuming that a plateau was reached in the present study (approximately 16 months exposure), the data suggest that the plateau concentration is influenced by type of diet and the use of DES and that both the use of DES and urea interact with type of diet to change the plateau concentration. Apparently the effect on residue concentration operates across all fat depots, possibly related to overall changes in absorption or metabolic balance, or both, within the animal.

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Effect of Wet Heating on the Physicochemical Properties of Groundnut Proteins[1]

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Defatted groundnut meal was heated with steam at atmospheric pressure and under 1 and 2 kg/cm^2 pressure. The extractability of the proteins in aqueous solution and DEAE-cellulose chromatographic, gel filtration, gel electrophoresis, and spectral characteristics of the extracted proteins were studied and compared with those of the untreated sample. The sample heated at atmospheric pressure did not differ much from the untreated sample in the measured properties. Samples heated under pressure showed considerable differences. The changes were such as to suggest that pressure heating depolymerized the proteins. The phosphorus and carbohydrate contents of the protein increased due to wet heating.

Heat treatment of oilseed and oilseed meal is an inherent feature of their processing. Application of heat is involved at various stages such as decuticling of kernel, mechanical removal of oil, and desolventization of solvent-extracted meal. Dry roasting (Lee *et al.*, 1969) and wet heating (Coomes *et al.*, 1966) have been reported to reduce the aflatoxin content of groundnut. Several studies have been made on the nutritive value of heat-treated groundnut proteins (Mcosker, 1962; Dunn and Goddard, 1948; Fournier *et al.*, 1949; Balasundaram *et al.*, 1958; Dawson, 1968; Neucere *et al.*, 1972). In general, drastic heat treatment reduces the nutritive value. The solubility of the protein in 10% NaCl and buffer solutions decreases (Woodham and Dawson, 1966; Neucere, 1972). Differences in the amino acid composition of the conarachin fraction from heated oilseed meals have been observed (Dawson and Woodham, 1966). Moving boundary electrophoretic patterns also show differences (Cama *et al.*, 1958). Neucere (1972) has reported differences in disc electrophoretic and immunoelectrophoretic patterns.

In the processing of edible groundnut meal, where extrusion cooking is involved, the protein is subjected to heat under steam pressure. Therefore, it was of interest to study the effect of wet heating on groundnut proteins. In this investigation the solubility, DEAE-cellulose chromatographic, gel filtration, polyacrylamide gel electrophoresis, and spectral characteristics of proteins extracted from wet heated groundnut meal have been studied. The phosphorus and carbohydrate contents of the protein were also estimated. The nutritive value of such a groundnut meal

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[1] Part II in the series "Studies on Groundnut Proteins."

Table I. Protein Content of the Samples and Protein Extractable in 1 *M* NaCl or Alkali Solution

Sample	Protein content, %	Protein extracted by 1 *M* NaCl, %	Protein extracted by alkali, %
I	53	41	45
II	57	40	45
III	56	16	21
IV	55	6	7

depends to a large extent on the degree of steam pressure during autoclaving (Rama Rao, 1974).

EXPERIMENTAL SECTION

Preparation of Sample. Edible grade expeller pressed groundnut flour was passed through a B.S.S. 44-mesh sieve and divided into four batches of 1 kg each. One batch was used as the experimental control (I) and the other three were spread in covered trays and steamed for 30 min each at (1) atmospheric pressure (98°) (II), (2) 1 kg/cm^2 pressure (121°) (III), and (3) 2 kg/cm^2 (135°) pressure (IV). Pressure was quickly released and the samples cooled and dried at room temperature. They were then powdered and passed through a 44-mesh sieve. The moisture content of the samples, determined by the hot-air oven method, was found to vary between 6 and 8%.

Protein extractability was determined both in 1 *M* NaCl and alkali (pH 10) solutions. Ten grams of the sample was placed in 100 ml of 1 *M* NaCl solution and stirred for 30 min at room temperature on a magnetic stirrer. With the alkali extraction, 10 g of the sample was stirred with 100 ml of distilled water for 15 min, the pH was adjusted to 10 by the addition of NaOH, the sample was stirred another 15 min and centrifuged at 4000 rpm for 15 min, and an aliquot of the supernatant was taken for protein estimation.

Protein Estimations. Nitrogen was measured by the micro-Kjeldahl method and a factor of 6.25 was used to cover the nitrogen value to crude protein.

DEAE-Cellulose Chromatography. DEAE-cellulose (Sigma Chemicals, NOD-8507) was regenerated by the standard procedure (Peterson and Sober, 1956). It was equilibrated with 0.01 *M* phosphate buffer (pH 7.9) and packed into a column, 2.5 × 28 cm. The protein extracted in 1 *M* NaCl was dialyzed extensively against the phosphate buffer. An aliquot of 5.0 ml of the protein solution was loaded on the column and allowed to be absorbed. It was then eluted with a linear gradient of NaCl; the gradient vessels contained 250 ml of phosphate buffer (pH 7.9; 0.01 *M*) and 250 ml of phosphate buffer (pH 7.9; 0.01 *M* containing 0.6 *M* NaCl). Four-milliliter fractions were collected on an automatic fraction collector. Absorbance at 280 nm was measured. The chromatographic experiments were at room temperature (25°).

Phosphorus Estimation. The method of Taussky and Shorr (1953) was used on aliquots of 0.2 ml of 1 *M* NaCl extracted protein solution (after dialysis). With each sample the estimation was made in triplicate and an average value was taken.

Carbohydrate Estimation. The method of Montgomery (1961) was used on aliquots of 0.1 ml of 1 *M* NaCl-extracted, dialyzed protein solution. The carbohydrate content has been expressed as per cent "glucose equivalent."

Gel Filtration. The gel (Bio-Gel agarose A 0.5m column (100–200 mesh), from Bio-Rad, Richmond, Calif., control no. 4974) was washed with 1 *M* NaCl solution, packed into a 1.2 × 66 cm column, and washed with 1 l. of 1 *M* NaCl solution.

A known volume of protein solution (1–2 ml containing 25 mg/ml), which had been extracted in 1 *M* NaCl solution and dialyzed, was carefully layered on the gel column and allowed to be absorbed. The column was then eluted with 1 *M* NaCl solution at 24 ml/hr. Fractions of 3 ml were collected on an automatic fraction collector and the absorbance of the fractions at 280 nm was measured. The recovery of the material from the column was checked by measuring the absorbance of protein solution loaded and that recovered; the recovery in all the cases was better than 90%.

Polyacrylamide Gel Electrophoresis. The protein extracted in 1 *M* NaCl solution was dialyzed against several changes of glycine–NaOH buffer of pH 9.5 and 0.05 *M*. Polyacrylamide gels (7.5%) were prepared in this buffer by standard procedure. Protein (50–200 μg) in 100 μl of buffer solution containing 40% sucrose and indicator dye (Bromophenol Blue) was loaded on the top of the gel. Electrophoresis was carried out for 120 min at a constant current of 4 mA/tube. Then the gels were stained for 2 hr in 0.5% Amido Black in 7.5% acetic acid solution. They were then destained and stored in 7.5% acetic acid solution.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis. The protein solution, dialyzed against glycine–NaOH buffer, was made 1% with respect to both sodium dodecyl sulfate and β-mercaptoethanol. This solution was incubated at 37° for 2 hr, dialyzed 24 hr against the buffer containing 0.1% sodium dodecyl sulfate and 0.1% β-mercaptoethanol, and then subjected to polyacrylamide gel electrophoresis. Both the running buffer and gel buffer contained 0.1% sodium dodecyl sulfate; the running buffer in addition contained 0.1% β-mercaptoethanol. Electrophoresis was carried out for 60 min at a constant current of 4 mA/tube. Electrophoretic mobility was calculated by the method of Weber and Osborn (1969). Molecular weight markers of bovine serum albumin, egg albumin, pepsin, trypsin, and lysozyme were used.

Absorption Spectrum. The absorption spectrum of the protein extracted in 1 *M* NaCl solution, after dialysis and appropriate dilution, was recorded in the range 230–300 nm with a Beckman recording spectrophotometer DK-2.

Other Measurements. Absorption measurements were made with a Carl-Zeiss uv spectrophotometer. A Radiometer pH meter TTT2 was used for pH measurements.

RESULTS AND DISCUSSION

The protein content of the sample and the per cent protein extracted in 1 *M* NaCl (and alkali) are given in Table I. All the values have been expressed on the moisture-free basis of the samples. The extractability in alkali in each case was slightly higher than in 1 *M* NaCl solution. The extractability in samples I and II was about 70–75% of the protein content but it decreased sharply with samples III and IV. The protein content of the carbohydrate residue, after protein extraction, should quantitatively account for the unextracted protein. However, a precise estimate of the protein content of the residue was not possible for the following reason. During the separation of the insoluble carbohydrate residue from the protein solution by centrifugation, a part of the protein liquor was held by the residue. Repeated washings to remove this did not lead to a constant value; possibly some of the protein associated with the residue also leached out. However, a trend was observed that the protein content of the residue increased with increasing heat treatment.

The solubility results would suggest that heating at atmospheric pressure (II) did not affect the solubility of the proteins whereas heating under pressure did; working with the whole seed Neucere (1972) has reported that in the case of wet heating the solubility *vs.* temperature of heating profile shows a complex behavior. The observation that heating under pressure reduced protein extractability might be due to either denaturation of the protein or complex formation between protein and carbohydrate; the

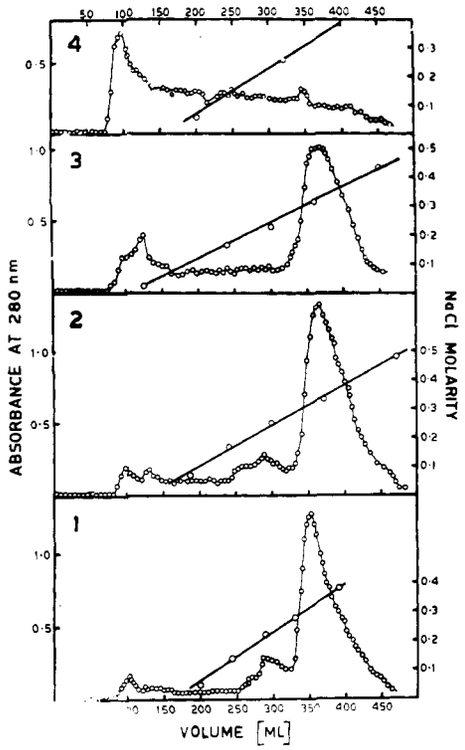

Figure 1. DEAE-cellulose chromatographic pattern of the samples: (1) sample I; (2) sample II; (3) sample III; (4) sample IV.

complex may not be quantitatively extractable in aqueous solution.

In Figure 1 the DEAE-cellulose chromatographic pattern of the samples is shown. With sample I, three peaks were obtained; the first immediately after the void volume of the column, the second at 0.2 *M* NaCl, and the third at 0.30–0.32 *M* NaCl. The pattern was similar to that obtained by Dechary *et al.* (1961). The first two fractions may be attributed to the conarachin fractions and the third to arachin. The chromatographic pattern of sample II was almost identical with that of sample I, in terms of peak position and proportion; the only discernible difference was that the arachin peak was broader.

In the pattern of sample III, the proportion of the first two peaks had increased and the arachin peak was even more diffuse. Sample IV, however, gave a very different pattern. There was a prominent peak immediately after the void volume and no peak corresponding to the arachin fraction. The ability of a protein to be absorbed by DEAE-cellulose cannot be generally correlated with its molecular weight. However, in the light of the discussion to follow, the observation that the proportion of the first two peaks had increased in samples III and IV may be interpreted as due to dissociation of the high molecular weight fractions of the protein.

The gel filtration pattern of the samples is given in Figure 2. Sample I gave three peaks in the proportion 20:30:40%. Arachin, which forms about 67% of the total (Johnson and Shooter, 1950), has a mol wt of 350,000. Conarachin has a lower molecular weight (Naismith and MacDavid, 1958). Separation in gel filtration is due to differences in molecular size (Ackers, 1970). Since the proportion of the first peak did not correspond to that of the arachin fraction it was difficult to assign the peaks to arachin and conarachin fractions. This may be due to the fact that both arachin and conarachin undergo association–dissociation reactions which are influenced by pH and salt concentration (Johnson and Shooter, 1950; Johnson and Naismith, 1953).

Sample II gave a pattern almost identical with that given by sample I, in regard to peak proportion and position suggesting that proteins of samples I and II were similar. Samples III and IV showed differences. The proportion of the first two components decreased and that of the third component (low molecular weight) increased. Furthermore, the presence of a new low molecular weight component in the sample was suggested by the appearance of a peak around 60 ml. In sample IV, the intermediate peak observed with samples I, II, and III was absent. These results suggested that heating under pressure would lead to the depolymerization of the groundnut proteins.

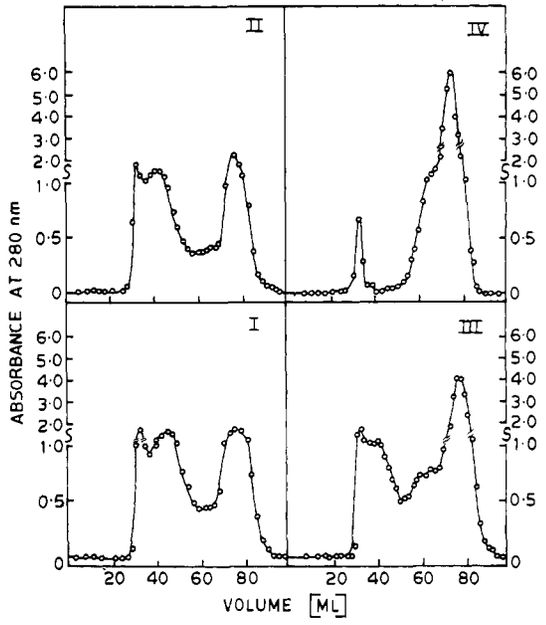

Figure 2. The gel filtration pattern of the samples. Inset numbers indicate the sample number.

It was observed that a less complex gel filtration pattern was obtained when the experiments were done in glycine–sodium hydroxide buffer (pH 9.5 and 0.05 *M*); this is, perhaps, due to the effect of salt on association–dissociation reactions. In these experiments the protein extracted in 1 *M* NaCl solution was dialyzed against glycine–NaOH buffer and run on an agarose column equilibrated with the buffer. The patterns obtained with samples I and IV are given in Figure 3. Sample I gave only two peaks in the proportion 80:20, the high molecular weight fraction being the major one. With sample IV only two peaks were obtained; however, the proportion of the low molecular weight component was higher. These results would lend further support to the conclusion that

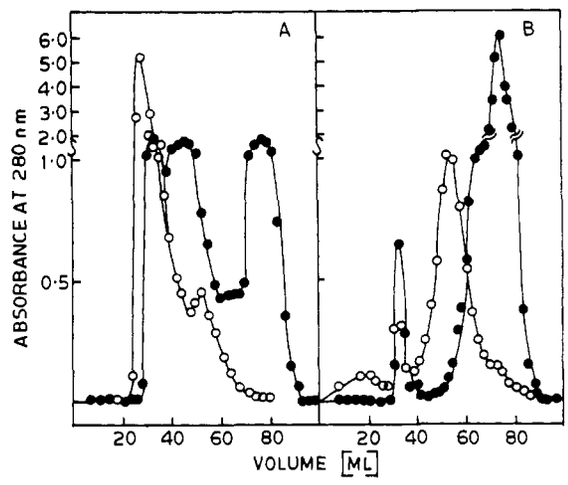

Figure 3. The gel filtration pattern of samples in NaCl solution and buffer: (A) sample I; (B) sample IV; (○) glycine–NaOH buffer; (●) 1 *M* NaCl solution.

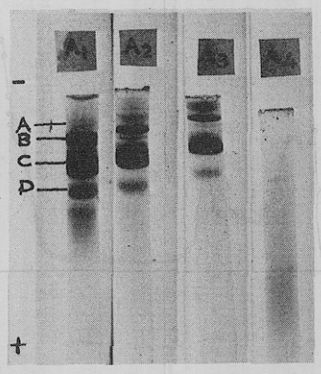

Figure 4. Polyacrylamide gel electrophoresis patterns: (A_1) sample I; (A_2) sample II; (A_3) sample III; (A_4) sample IV.

heating of groundnut protein under pressure caused depolymerization of the proteins.

The samples were also analyzed by polyacrylamide gel electrophoresis where resolution is based on both electrophoretic mobility and molecular sieving. The patterns are given in Figure 4. With samples I and II seven bands were obtained. The major bands can be assigned to the various fractions (Neucere, 1972; Tombs, 1965; Dawson, 1971): (A) α-conarachin; (B) "dimer" of arachin; (C) "monomer" of arachin; (D) β-conarachins. Samples I and II gave nearly identical patterns suggesting that dry heating had not caused any major changes in the composition of the groundnut proteins. In sample III the following was observed: (1) the proportion of arachin "dimer" and of β-conarachin decreased; (2) the proportion of α-conarachin increased; and (3) the proportion of arachin "monomer" was practically the same. Dawson (1968) has reported that wet heating (at 108° for 15 and 45 min) of groundnut meal increased the intensity of α-conarachin and β_2-conarachin components whereas that of other β-conarachins decreased. Our results are in qualitative agreement with this observation. Sample IV gave a totally different pattern. There were no bands corresponding to α- or β-conarachins or arachins (monomer and dimer). Only a single diffuse band with high mobility was obtained. The position of this band would suggest that it was a highly depolymerized protein. Thus the results from gel filtration and gel electrophoresis were mutually compatible.

It was observed that although identical amounts of protein were loaded for gel electrophoresis, sample IV took up less stain and gave a faint band. This protein had a high carbohydrate content and probably could not be stained properly (Tombs, 1965).

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis has been used to determine the molecular weight of subunits of proteins (Weber and Osborn, 1969; Shapiro *et al.*, 1967; Dunker and Rueckert, 1969). The patterns obtained from sodium dodecyl sulfate–polyacrylamide gel electrophoresis are given in Figure 5. Samples I and II each gave three major bands and three–four minor bands; the relative mobilities (and hence the molecular weight) were the same. Sample III gave two major bands with higher mobilities (and lower molecular weight). Sample IV gave a major (diffuse) band with a high mobility.

The molecular weights of the major components are given in Table II. Samples III and IV contain a high proportion of low molecular weight components, possibly due to the depolymerizing effect of heating under steam pressure.

Neucere (1972) has reported that during wet heating of the *whole* groundnut, arachin is relatively stable and is not depolymerized. Our results show that heating under pressure depolymerizes arachin (and conarachin). Neucere (1972) has pointed out that the stability of arachin may

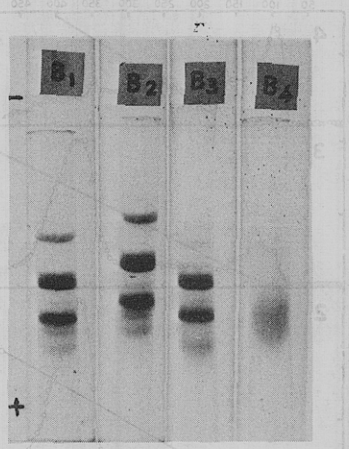

Figure 5. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis patterns: (B_1) sample I; (B_2) sample II; (B_3) sample III; (B_4) sample IV.

Table II. Molecular Weight of the Major Components of the Samples after Dissociation with Sodium Dodecyl Sulfate

Sample	Molecular weight		
I	58,000	40,000	31,000
II	60,000	44,000	33,000
III	53,000	35,000	
IV			22,000

be due to the fact that in groundnut cells arachin is located in aleurone grains which are surrounded by oil droplets; these and the surrounding membrane may act as a heat shield. When the cells are disrupted, as during the preparation of the groundnut meal, this protection is no longer available and the arachin fraction would also be susceptible to the action of heat.

The absorption spectrum of the samples is given in Figure 6. Sample I gave a typical protein spectrum with a maximum at 277 nm and a minimum at 250 nm. The spectrum of sample II was similar to that of sample I. However, sample III gave a spectrum with a broad peak which had shifted to lower wavelengths (260–270 nm) and

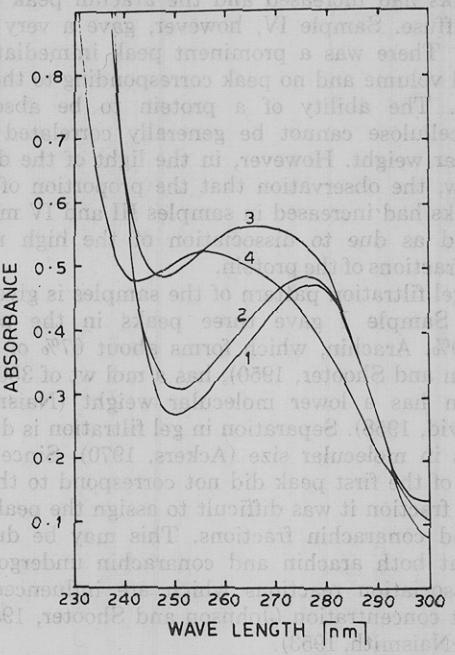

Figure 6. Absorption spectrum of the samples: (1) sample I; (2) sample II; (3) sample III; (4) sample IV.

Table III. $R_{280/260}$, Nucleic Acid Content, Nucleic Acid Phosphorus, Total Phosphorus, and Carbohydrate Content of the Protein Samples

Sample	$R_{280/260}$	Nucleic acid, %	Nucleic acid phosphorus, %	Total phosphorus, %	Carbohydrate, %
I	1.38	0.75	0.06	0.12 ± 0.02	2.0 ± 0.3
II	1.25	1.50	0.12	0.14 ± 0.02	2.1 ± 0.4
III	0.91	4.00	0.34	0.62 ± 0.10	7.9 ± 1.3
IV	0.71	10.00	0.85	1.71 ± 0.40	42 ± 10

the minimum occurred at 247 nm. In the case of sample IV, the maximum shifted to even lower wavelength (255–260 nm) and the minimum to 242 nm.

The ratio of absorption at 280 and 260 nm ($R_{280/260}$) was observed to decrease from 1.38 (sample I) to 0.71 (sample IV) (Table III). From the values of $R_{280/260}$ the nucleic acid content of the proteins was estimated (Layne, 1957). It increased from 0.75 to 10%. On the basis that a nucleotide (nucleic acid) contains 8.5% phosphorus, the phosphorus content of the samples due to nucleic acid impurity was calculated. Both the total phosphorus and nucleic acid phosphorus increased due to wet heating. Possibly the spectral changes were due to this increased phosphorus content. The value of 0.12% for the total phosphorus content of sample I was lower than the value of 0.65% reported by Pons and Guthrie (1946) for the groundnut (peanut) protein. This could be due to varietal differences.

The carbohydrate content was estimated and has been expressed as per cent "glucose equivalent" (Table III) and was found to increase due to wet heating. Sample IV was found to have a high carbohydrate content and was examined further. The NaCl extract (1 *M*) of sample IV was dialyzed extensively against distilled water. The dialysate was evaporated to dryness and dried at 110° for 2 hr. A known weight of the dried material was used for nitrogen analysis by the micro-Kjeldahl method. The protein content was found to be 45 ± 5%, checking well with the carbohydrate content of 42 ± 10%.

The results suggested that groundnut proteins depolymerized during wet heating. Also they combined with the other constituents of groundnut meal; the extent of this reaction depended on pressure (temperature).

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