Effect of Heat on Peanut Proteins. II. Variations in Nutritional Quality of the Meals

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Peanut meals prepared from whole seeds that were heated at temperatures varying from 110 to 155°C for 1 hr at two moisture levels were evaluated nutritionally by rat feeding tests. The protein efficiency ratio (PER) of each meal correlated with the available lysine (AVL) content. Total amino acid compositions of the total proteins were not affected by heat. Mild heat increased both AVL and PER values; temperatures above 120°C gradually impaired nutritional values and caused a reduction in AVL. The highest AVL and PER values for wet-heated seeds occurred at 110°C and for dry-heated seeds at 120°C. The increasing order of limiting amino acids for rats in all meals was cystine, lysine, threonine, isoleucine, and methionine.

Nutritional value of proteins differs because of variations in amino acid content and availability. Nutritionists have observed a reduction in the nutritional values of some protein foods after heating, higher temperatures causing the greater losses. Some investigators believe that these effects are not due to actual destruction of amino acids but rather to their reduced availability after heating (Horn, 1969; Osner and Johnson, 1968). Other characteristics which affect the nutritional quality of plant foods are digestibility, availability of trace minerals, and the presence of enzyme inhibitors. Mild heating, for example, improved both the biological value and the digestibility of soybean meals (Dimler, 1969; Evans and Bandemer, 1967; Osborne and Mendel, 1917).

The discovery that a peanut meal (Blount, 1961) was the carrier of a toxic factor (mycotoxin) led to intensive investigations for controlling mold contamination. Since processing of peanuts for food uses includes a roasting step, additional decontamination can be obtained under optimum conditions of heat and moisture. Lee *et al.* (1969) showed that dry roasting as in commercial processing caused over 75% reduction of aflatoxin in inoculated peanuts. Other investigators showed that proper storage of peanuts at a maximum of 10% moisture reduced aflatoxin contamination (Lensler and Natoli, 1969).

The effects of various roasting conditions on enzyme inhibitors have been reported. Woodham and Dawson (1968) showed that trypsin inhibitor activity in peanut meals was inversely proportional to dry heat applied, and chick growth tests on these meals showed a reduction in gross protein value with increasing temperature. Wet heat, however, readily deactivated the inhibitor at lower temperatures without notable decreases in nutritional value.

Commercial processing of peanuts varies in different parts of the world. For practical roasting, a temperature of 170°C for 36 min has been reported (McOsker, 1962). In the present study, 145°C for 1 hr seems to be an ideal roast. General information on this subject has been reported by Woodham and Dawson (1968). The object of the current work is to compare the effects of wet and dry heat on nutritional value of seeds heated within a temperature range approaching practical roasting. Whole seeds which were heated up to 155°C for 1 hr at two different moisture levels were defatted, and diets were prepared from these meals and fed to growing rats. Specific proteins were not isolated and amino acid supplementation was not employed.

EXPERIMENTAL

Seed Treatment. Dehulled intact Virginia 56-R peanuts (11 kg) including the testae (1968 crop) were divided into 1-kg lots. One lot was unheated and served as the control. Five samples were allowed to imbibe distilled water for 16 hr at 25°C to a final moisture content of 40%, placed in ventilated trays, and were heated for 1 hr in a forced draft oven at 110, 120, 130, 145, and 155°C, respectively. Five other samples were heated on an "as is" basis, 5% moisture, under identical conditions.

After equilibration to room temperature, each sample was homogenized in 1:1 hexane–acetone, using 3:1 solvent per kg of seeds in a Sorvall Omnimixer for 5 min at 5°C. The homogenate was filtered under vacuum, with each batch yielding approximately 600 g of a fine meal.

Analytical Methods. Total nitrogen contents were determined by the Kjeldahl method and residual lipid contents by Soxhlet extraction. Quantitative amino acid analyses were carried out by ion-exchange chromatography (Moore and Stein, 1963) through a Technicon Autoanalyzer. Available lysine was determined by the method of Conkerton and Frompton (1959). Fiber and ash contents were determined according to the AOAC method (1965).

Immunoelectrophoresis, according to Grabar and Williams (1953), was performed in 1.5% Ionagar #2 gel (Oxoid Limited, London) in 0.25 *M* veronal buffer, pH 8.2, employing the LKB immunoelectrophoretic kit. A voltage gradient of 4 V/cm was applied for 2 hr at room temperature. Each well was filled with 1.5 mg of protein prior to electrophoresis. After separation, each trough was filled twice with antiserum against the total proteins of the peanut. Slides were stained with 1.0% amido black and destained with 7.0% acetic acid.

Preparation of Diets. Peanut protein diets were adjusted to correspond to a casein diet of the following percent composition: protein, 10.0; lipid (corn oil), 8.0; USP salt mixture 14 fortified with zinc and cobalt, 5.0; cellulose, 1.0; vitamin mixture, 2.0; cornstarch, 20.0; dextrose, 49.0; and water, 5.0.

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Table I. Composition of Peanut Meals[a]

No.	Heat treatment, °C	Total solids, %	H_2O, %	Nitrogen, %	Fat, %	Fiber, %	Ash, %
1	None	91.7	8.26	8.80	21.3	3.78	3.85
2	110 Wet heat	91.7	8.34	8.16	17.1	4.29	4.01
3	120	90.1	9.94	7.71	18.7	4.02	3.81
4	130	90.1	8.93	8.13	23.8	4.10	3.86
5	145	92.4	7.59	8.53	15.2	3.91	3.91
6	155	93.5	6.54	8.66	20.5	4.46	4.17
7	110 Dry heat	93.4	6.65	8.12	19.6	4.03	3.87
8	120	94.0	6.04	7.78	22.2	3.20	3.77
9	130	93.9	6.13	7.90	19.6	4.14	3.78
10	145	93.5	6.53	8.03	18.2	4.40	3.86
11	155	93.6	6.38	8.11	20.4	4.26	3.81

[a] Dry matter basis.

Feeding Experiments. For each diet five male weanling rats (Sprague–Dawley Strain), 22 days old, weighing about 56 g each were employed. Food and water were given *ad libitum* for 28 days and the final mean weights of the rats ranged from 84 to 140 g. Fecal waste for digestibility analyses was collected during the second trial week.

RESULTS

Composition of Meals. The chemical composition of the peanut meals is listed in Table I.

Animal Response to Diets. Comparisons of each diet relative to casein are given in Table II. The highest weight gains and PER values were obtained with diets from seeds wet-heated at 110°C (2) and those dry-heated at 120°C (8). As noted with soybeans (Osborne and Mendel, 1917), the diets prepared from unheated seeds (1) gave a lower PER value than those prepared from seeds heated below 130°C. Though digestibility is constant for each diet, the availability of digested products and, consequently, nitrogen retention are decreased with increased heat as evidenced by the lower PER values. There was an inverse linear relationship between applied heat and PER for both sets of samples; the most severe nutritional impairment occurred above 130°C.

Changes in AVL closely paralleled those in PER. The initial rise in AVL to a maximum of 3.08% after wet heating at 110°C was followed by a progressive decrease as heating increased. Dry-heating at 110°C increased the AVL but the maximum of 3.08% was not achieved until the seeds had been heated at 120°C; AVL decreased thereafter as temperature increased.

Table II. Comparison of Nutritional Characteristics with AVL of Peanut Meals

Number	Final mean body weight,[a] g	Digestibility[b]	PER[c]	AVL[d]
1 (control)	99.0 ± 7.3[e]	93.7	1.41	1.95
2 Wet heat	112.6 ± 16.8	94.8	1.81	3.08
3 Wet heat	103.6 ± 12.6	94.9	1.55	2.78
4 Wet heat	107.0 ± 19.1	94.6	1.54	2.40
5 Wet heat	91.0 ± 5.1	94.7	1.25	2.03
6 Wet heat	86.4 ± 8.2	95.0	1.09	2.03
7 Dry heat	100.6 ± 13.3	94.4	1.50	2.48
8 Dry heat	128.4 ± 16.1	93.6	1.94	3.08
9 Dry heat	106.0 ± 10.5	94.9	1.64	2.63
10 Dry heat	88.2 ± 7.7	94.8	1.23	2.33
11 Dry heat	84.2 ± 7.9	94.3	1.00	2.18
Casein	140.6 ± 21.9	96.1	2.50	

[a] Average of five male rats; initial age 22 days; initial weight 56.5 g. [b] Feed intake minus moisture-free fecal weight divided by feed intake times 100 recorded during 1 week. [c] Body weight gain in g per g protein eaten, average of five rats. [d] Determined as E-DNP-L-Lysine, percent in protein. [e] Standard deviations.

Amino Acid Analysis. Amino acid contents determined on five selected samples, the control and those heated at the lowest and highest temperatures, are given in Table III. One acid hydrolysis period, 20 hr, was used.

Table III. Total Amino Acid Composition (g/100 g meal) and Percent of Essential Amino Acids Required by the Growing Rat in Peanut Meals

Amino acid	Control	Wet heat		Dry heat			Percent MEAR				
	1	2	6	7	11	MEAR[a]	1	2	6	7	11
Asp	5.3	6.6	5.8	6.0	6.4						
Ser	2.1	2.3	2.4	2.4	2.6						
Glu	9.4	10.0	10.3	8.8	12.0						
Pro	1.5	2.3	2.3	2.1	2.4						
Gly	2.7	3.0	3.0	2.9	3.3						
Ala	1.7	2.0	2.0	2.0	2.2						
Thr	1.1	1.4	1.4	1.3	1.5	0.5	50.0	63.5	68.5	67.0	67.0
Cys	0.3	0.5	0.4	0.6	0.6	0.34	21.5	43.0	45.0	48.0	32.0
Met	0.5	0.5	0.5	0.5	0.6	0.16	71.0	76.0	84.0	75.0	75.0
Val	2.1	2.0	2.0	1.9	2.3	0.55	85.5	78.0	94.0	82.0	78.0
Iso	1.6	1.7	1.7	1.5	2.0	0.55	66.5	67.0	82.0	74.5	74.5
Leu	3.0	3.1	3.3	3.2	3.6	0.70	97.5	112.0	115.0	107.0	113.0
Tyr	1.7	2.1	1.8	1.9	2.0	0.30	147.0	154.0	150.0	168.0	144.0
Phe	1.9	2.6	2.6	2.5	2.4	0.42	103.0	145.0	138.0	150.0	150.0
Lys	1.4	1.8	2.0	1.9	2.2	0.90	28.6	51.5	55.5	48.0	54.0
His	1.1	1.3	1.8	1.3	1.1	0.25	100.6	127.0	99.0	125.0	173.0
Arg[b]	5.1	5.8	6.5	5.8	6.0	0.20	580.0	635.0	675.0	705.0	790.0

[a] Minimal essential amino acid requirement (percent of diet) in a 10.0% protein diet for the growing rat with nonessential amino acids same as in casein (Rao *et al.*, 1964). [b] MEAR for arginine reported by Albanese (1959) for a 20.0% protein diet.

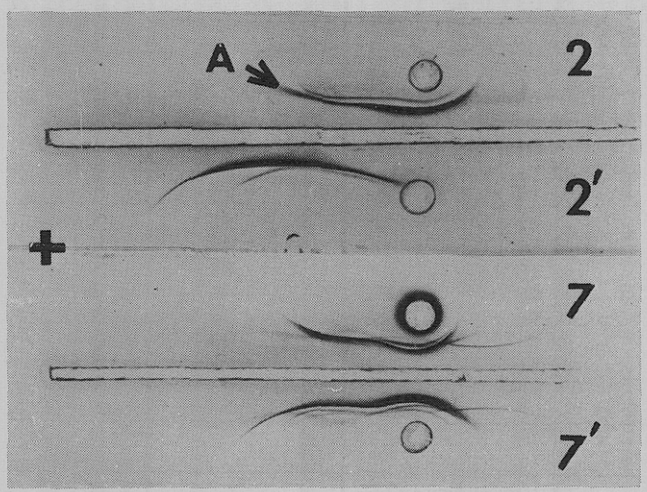

Figure 1. Detection of trypsin inhibition by immunoelectrophoresis. Numbers refer to meals 2 and 7 in Table I; primes indicate the same samples incubated with 0.1% trypsin for 20 hr. A refers to α-arachin

Tryptophan was not determined on these samples, but it has been shown that, like threonine, it is deficient in the peanut and poorly utilized by the rat (Evans and Bandemer, 1967). Waldroup and Harms (1965) also found peanut meal deficient in tryptophan for growing chicks. Another study showed that free tryptophan is slightly decreased after roasting, suggesting a possible side reaction involving flavor components (Newell *et al.*, 1967).

The minimal essential amino acid requirements (MEAR) for the growing rat are given in Table III. Percent MEAR in each meal showed that the order of limiting amino acids was cystine, lysine, threonine, isoleucine, and methionine, respectively. Arginine, phenylalanine, and histidine occurred significantly above marginal requirements. These results were in general agreement with previous experiments employing varied heat applications (McOsker, 1962).

Detection of Trypsin Inhibition. Trypsin inhibitors are known to occur in certain seeds, including the soybean and the peanut. Figure 1 shows the results of measuring trypsin inhibitor activity based on the work of Daussant *et al.* (1969). They showed that 0.1% trypsin induced a shift in the electrophoretic mobility of α-arachin, the major peanut globulin. This shift was not observed when soybean trypsin inhibitor is used on the control. In the present study, the lack of trypsin inhibition after wet heat at 110°C (2,2′) is evident, whereas the same experiment after dry heat (7,7′) showed no electrophoretic shift of arachin. Other experiments on the dry-heated samples showed the trypsin effect only after heating at 130°C or above. Although the inhibitor is still active at 120°C, the highest PER value for the dry-heated seeds occurred at 120°C. Hence no correlations exist between trypsin inhibitor and protein quality. For wet heat, loss of nutritional quality is directly related to heat, since the inhibitor is readily deactivated at 110°C.

DISCUSSION

Because intact heated seeds rather than heated defatted meals were used in this study, some discrepancies with previous workers were noted. Although different varieties of peanuts were reported to vary chemically and nutritionally (Woodham and Dawson, 1968), a comparable study on whole Spanish peanut seeds showed results similar to those reported in the present study (McOsker, 1962). Seeds dry-heated (blanched) at 95°C for 14 min gave an average PER value of 1.81 with rats, compared to 2.44 for casein. Fortification of a diet prepared from the latter meal with lysine, methionine, and threonine showed better growth support for rats than did casein. Other experiments in this study showed these three amino acids were equally limiting; fortifications with other essential amino acids in the absence of any one did not significantly improve nutrition.

Anatharaman and Carpenter (1969) reported that wet and dry heat treatments at 107°C and 121°C for 0.5 hr did not affect the AVL of peanut meals. However, exposure of the meal to dry heat at 121°C for 4 hr reduced AVL. In the present experiments, dry and wet heat treatments at 120°C for 1 hr had no affect on AVL, but significant reductions were noted under both conditions by heat treatments for 1 hr above 130°C. Apparently, time as well as temperature of heating affects lysine availability.

The extent of enzymatic digestion can also offset nutritional value of proteins. Model systems, for example, comparing acylated lysine with heat-damaged protein in protein quality studies showed that both heat-damaged proteins and diets supplemented with propionyl–lysine inhibited rat growth (Bjarnason and Carpenter, 1969). Most of the lysine in the heat-damaged protein was found in fecal and urinary wastes, suggesting the formation of indigestible peptides. For the diets with acylated lysine, a specific kidney enzyme was found to hydrolyze the derivative, liberating free lysine into the urine. Since threonine in roasted peanuts has been reported to be 30% unavailable to the rat (McOsker, 1962), perhaps heat induces similar reactions.

In the peanut, trypsin inhibitor activity is reduced by low levels of wet heat but not by dry heat (Woodham and Dawson, 1968). This has been confirmed in the present study. A study of trypsin inhibitor contents of several legumes, however, showed no correlation with rat growth after autoclaving seeds at 120°C for 30 min (Borchers and Ackerson, 1950). Dechary (1970) has reported a wide disagreement on nutritional aspects of protease inhibitors. It appears that limiting amounts and availability of essential amino acids are more significant in affecting nutrition than trypsin inhibitors, as evidenced in the present work.

Mycotoxin contamination is a major problem with improperly stored peanuts and other foodstuffs. Aflatoxins are known to occur in several oilseeds; Mann *et al.* (1967) showed that both aflatoxin-infested cottonseed and peanut meals are detoxified by heat. About 80% reduction in levels of aflatoxin B_1 and B_2 was achieved by heating meals at 100°C for 2 hr at 20% moisture. Because these conditions are comparable to those of Sample 2 (110°C, 1 hr, 40% moisture) in the present work, wet heat might be advantageous in producing high-quality meals with decreased mycotoxin contamination.

The results of investigations so far do not present a clear picture on interactions of proteins with lipids and carbohydrates which may impair nutrition. For carbohydrates, Maillard-type reactions occur with lysine, arginine, and the phenolic groups of other amino acids (Ellis, 1959). Many investigators reported protein–lipid interactions in both meat and plant foods. Venolia and Tappel (1958) showed that excess heat induced protein reactions with oxidized unsaturated fats. In the absence of carbohydrates and lipids, amino acid unavailability was also observed. This was reported for reconstituted casein by McCollum and Davis (1915). In a review article of Osner and Johnson (1968), it was suggested that temperatures above 100°C for more than 1 hr can diminish the availability of lysine, arginine, methionine, cystine, leucine, tryptophan, and histidine in most foods.

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Physical Chemical Characterization of Grain Sorghum Prolamine Fractions and Components

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The prolamines of grain sorghum have the unique property of forming gels at low protein concentrations in a variety of solvents. Differences in the solubility properties and amino acid composition of prolamine fractions are described, and evidence is presented to show that protein from sorghum prolamines undergoes noncovalent interaction even in such solvents as 6 *M* guanidine hydrochloride.

The proximate composition and proteins of three American grain sorghum hybrids were previously described (Jones and Beckwith, 1970). We noted that the prolamine class of protein from these hybrids readily formed gels in alcohol–water systems, as well as in such solvents as dimethyl sulfoxide and 8 *M* urea solutions. In the alcohol–water system the addition of 1.5 *M* guanidine hydrochloride (GHCl) prevented gel formation, whereas the addition of sulfhydryl blocking agents or disulfide bond-breaking compounds did not influence the gelling phenomena.

As a continuing study of the properties of these proteins, we wish to report observed differences in solubility properties and amino acid composition between two fractions from the prolamines of the three hybrids used earlier. In addition, the sedimentation equilibrium behavior of these isolates was examined as a function of protein concentration and pH in solutions of 6 *M* GHCl which is considered to be strong noncovalent bond-disrupting agent.

MATERIALS AND METHODS

For quantitative measurements, the GHCl used was an ultra-pure grade obtained from Mann Research Laboratories. All other standard chemicals used in preparing solutions or solvents were reagent grade.

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PROTEIN ISOLATION

The method used to extract the prolamines from grain sorghum flour has been presented earlier (Jones and Beckwith, 1970). The prolamine fraction is dispersed in 95% ethanol and then cooled to 9–10°C. After centrifugation at this temperature, the solution is treated with decolorizing carbon to remove red pigments. Evaporation of the alcohol yields a white protein preparation used as one fraction in these studies.

The fastest migrating gel electrophoretic component of the prolamines (Jones and Beckwith, 1970) was isolated from a 0.5% w/v solution of decolorized prolamine in 60% v/v *tert*-butanol–water containing 1.5 *M* GHCl to prevent gelling. Water (1.8 vol) is slowly added to the solution at room temperature. After centrifuging at about 10,000 × *g*, the centrifugate is dialyzed against water and then freeze-dried. The crude product is taken up in 6 *M* GHCl (8–10% w/w total protein concentration) and passed over a 96 × 5 cm Sephadex G-150 column at 25°C with a flow rate of 10 ml per hour using 6 *M* GHCl as eluent. The first 1020 ml of effluent are discarded and the next 200 ml are collected, exhaustively dialyzed against water, and then freeze-dried.

Amino Acid Analysis and Electrophoresis. The methodology was described in our earlier report (Jones and Beckwith, 1970).

Ultracentrifugation. A Spinco Model E ultracentrifuge equipped with RTIC control, photoelectric scanner attachment, multiplexing accessory, and spherical mirror optical system was used to examine sedimentation equilibrium be-