EFFECT OF AUTOCLAVING ON THE PROTEIN QUALITY OF LEGUME SEEDS

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ULLAH

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AN ABSTRACT OF THE THESIS OF

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Title: Effect of autoclaving on the protein quality of legume seeds.

Legumes are good sources of protein and contain from 20 to 40 per cent protein. About 15-30 per cent of the total protein calories of average diets in this part of the world and other developing countries is derived from legume proteins. And heat processing has been shown to bring about an increase in the nutritive value of legume proteins and inactivation of trypsin inhibitor, hemagglutinins and other growth depressing factors present in them.

In the present study, the effect of autoclaving for 5 and 20 minutes at 121° C on protein quality of 19 different legume seeds was investigated. The amino acid content, antitryptic activity and hemagglutinating activity of these legumes were determined. Protein efficiency ratio (PER) and net protein utilization (NPU) were used to evaluate their protein quality.

The results, according to 1957 FAO provisional pattern, showed that all the raw legume seeds investigated were most deficient in their sulphur amino acids followed usually by tryptophan. However, according to the rat requirements, threonine and lysine appeared to be the limiting amino acids next to the sulphur amino acids. Autoclaving for 5 and 20 minutes did not cause any appreciable loss in the amino acids determined. Trypsin inhibitor activity was destroyed to the extent of 72-80 per cent by autoclaving for 5 minutes and to 89-94 per cent by autoclaving for 20 minutes. Similarly, hemagglutinating activity was almost completely destroyed in Phaseolus vulgaris and soybean, that had the highest activity, by autoclaving for 20 minutes; while in other legumes, complete destruction was observed by autoclaving for 5 minutes.

Protein efficiency ratio (PER) of the legume seeds was improved by autoclaving for 5 minutes. But less improvement was observed when legumes were autoclaved

for 20 minutes. However, PER values of local vetch and lupine did not improve on autoclaving. Estimation of protein quality of legumes determined by NPU method was in agreement to those determined by the PER assays. Both methods were found to be highly correlated (P .01) as was observed by many workers.

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I. INTRODUCTION

Edible leguminous seeds are important sources of protein in the dietaries of people living in Afro-Asian and southern American countries of the world. About 15-30 per cent of the protein calories consumed are derived from legumes (9, pp 19-36, 97, pp 14-15). Legumes constitute about eight per cent of the proteins consumed in the world as shown in the following table:

Table 1. World production[1] of certain legumes; FAO estimates (9, p 18).

Legumes	1948/49-1952/53	1958/59	1959/60	1960/61
	1000 metric tons			
1. Beans, peas, lentils, broad-beans and chick-peas	23,470	25,940	29,440	28,280
2. Pigeon peas, cowpeas, vetches, lupine, and other legumes	5,460	4,970	5,650	5,900
3. Total, all legumes	28,930	30,910	35,910	34,180

1. Excluding USSR and Mainland China.

The protein content of legumes is high and ranges from 20 to 40 per cent. However, contribution of

a dietary protein to the requirements of the animal depends not only on the quantity of the protein present in the diet, but also on its quality; mainly, on its amino acid composition (100, 101). A diet deficient in a single amino acid will cause cessation to the process of protein synthesis, although all other amino acids may be present in adequate amounts (7, pp 19-22).

Protein quality of legumes, like other foodstuffs, is affected to a great extent by processing and cooking methods. Overcooking may result in damage to protein quality by lowering digestibility (denaturation) and causing the loss of sulphur amino acids. This is particularly importnat, since the legumes are endogenously deficient in methionine (44, 87).

Apart from their protein content most legumes are known to contain some toxic factors such as hemagglutinins and trypsin inhibitors, which may show adverse effects when the legumes are fed untreated to animals. In turn then, the presence of these factors will affect the utilization of proteins. However, these factors are also known to be destroyed to a great extent by processing. Thus the application of heat treatment will affect the nutritive value of legumes by affecting both the toxic factors present and quality of the protein (102, pp 304-321).

The use of pressure cooking has become popular and the use of autoclaving for preparation of legumes for consumption is commonly practised. Therefore, the effect of autoclaving on protein quality of locally grown legumes seems essential for evaluating the protein quality of these legumes for human consumption.

The purpose of the present study was to determine the amino acid composition, the presence of hemagglutinins and trypsin inhibitor in locally grown legumes as affected by various treatments of autoclaving. The effect of the presence of these factors and autoclaving on protein quality, namely, protein efficiency ratio (PER) and net protein utilization (NPU) of the legume seeds, has also been investigated.

II. REVIEW OF LITERATURE

Legumes as a source of protein in human diets have a long history. They are mentioned in the Holy Books, and lentils cooked with garlic were eaten around 2000 B.C. The first experiment to compare the effects of pulse and water as a complete diet with a diet of meat fed to children for 10 days was recorded in 1637, and gave surprisingly good effects that were expected to be inferior (9, pp 1-12). However, legumes have been known to contain toxic factors and have low digestibility (77, pp 281-298, 63), yet no systematic studies have been reported until the beginning of the present century. With advances in chemical analysis and the analysis of essential amino acids, the whole concept of human nutrition has changed and the concept of quality and quantity of protein evolved (86, 90). Modern nutrition and food technology paved the way for better utilization of all foodstuffs by conserving the maximum amount of nutrients during processing. Thus, the approaches made in this direction will be reviewed under the following headings: (A) Evaluation of protein quality in legumes by chemical methods, (B) The assessment of protein quality of legume seeds, raw and processed, by bioassay

procedures.

A. Chemical Studies

Amino acids: The primary function of a dietary protein is to provide amino acids in the proper proportion for the synthesis of tissue proteins for growth and maintenance of cellular nitrogenous constituents, and for other metabolic needs (100, 101). The early investigations of Osborne and Mendel (94) and Rose (105) have established the concept of essentiality of some amino acids. Thus, the quality of protein has come to indicate the proportions of the essential amino acids present in meeting the requirements of the organism fed this protein.

The proportion of other essential amino acids present in the proteins have also been found to affect the utilization of the limiting amino acid and results reported indicate that even a small surplus of a certain amino acid can sometimes increase the needs of the limiting amino acid to overcome the depression when the total protein intake is low. This phenomena is referred to as amino acid imbalance (7, pp 19-22, 100-101).

Block and Weiss (20, pp 316-321) stated that legume proteins are good sources of lysine and threonine but are deficient in sulphur amino acids and tryptophan, which has been reported by many workers (92, pp 54-56,

10, 30, 46, 61, 101, 111, 112, pp 399-410). Jaffe (61) and others (30, 46, 101, 106, 108) reported that supplementing legumes with methionine improved their nutritive value to that of casein. Kakade and Evans (67) also arrived at a similar conclusion, and indicated that navy beans are limiting in methionine.

The first observation (95) that heating increased the nutritive value of soybean seeds focussed the attention of other investigators (37, 47-48) to find the optimum time and temperature of heating. Jaffe (63) autoclaved all legumes at a pressure of 10 lbs per square inch (psi) for 20 minutes, but kidney beans were soaked overnight and autoclaved for 30 minutes at 10 psi. De Muelenaere (41) also observed improvement in digestibility on autoclaving, thereby increasing the availability of amino acids, particularly the sulphur amino acids.

Block and Weiss (20, pp 32-130) have described the chemical, chromatographic and microbiological assay procedures for analysis of amino acids. Orr and Watt (92, pp 16-21) have reported the amino acid contents of various legumes. Recently Bandemer and Evans (10) and others (111, 112, pp 399-410) have also reported the amino acid content of various species of plants including legumes. All reports confirmed the inherent deficiency of legumes in sulphur amino acids, followed by tryptophan.

Evans et al. (48) found that long autoclaving resulted in some loss of cystine and methionine. Delcueto et al. (39) reported chick-peas to be a good source of lysine. They also observed a 10 per cent reduction in lysine when samples were autoclaved for 30 and 60 minutes at 121° C in both 10 and 50 per cent water. The losses were higher at lower moisture content. Stilling and Hackler (110) reported no significant loss of amino acids on autoclaving tempeh, a soybean product, for 2 hours at 100° C. Johnson et al. (66) suggested that in soybeans there is a nitrogen and sulphur containing complex which is absorbable, but not utilizable for tissue building purposes; a finding which has been confirmed by Donoso et al. (44) and recently by Miller et al. (87).

Studies of Evans et al. (47-48), Richardson (103), Sherwood et al. (108) and Russel et al. (106) performed to determine whether supplementation of the unheated legume proteins with various amino acids would achieve the same effect of improving protein quality as heating, have shown that the addition of methionine or cystine to unheated samples improves protein utilization to the same extent as proper heating. Kakade and Evans (67) remarked that raw navy beans, even if supplemented with all the deficient amino acids, did not bring about any improvement in nutritive value. However, autoclaved beans when supplemented with methionine alone or all

amino acids supported growth like casein.

It also seems that the difficulty in the interpretation of amino acid values in foods, whether determined by microbiological or chromatographic methods, is that the amino acid values obtained do not necessarily reflect their availability (7, p 56; 19, pp 249-278). Many legumes are known to contain toxic factors like trypsin inhibitor and hemagglutinins which affect the biological availability of amino acids (72, 75, 81, 23, 16). Other toxic factors have also been mentioned by Liener (77, pp 281-298) and Rao et al. (102, pp 304-321). Trypsin inhibitor: As early as 1917, Osborne and Mendel (95) reported that raw soybean did not support growth of rats, while cooked soybean promoted good growth. This finding has subsequently been confirmed by many workers (22, 23). Accordingly, it was concluded that raw soybean contained a substance that inhibits the action of the enzyme trypsin, thus interfering with the breakdown and digestion of proteins. Melnick et al. (81) remarked that methionine in raw soybean was liberated more slowly by the proteolytic enzymes of the intestines than the other essential amino acids. This concept gained strength later by the discovery of antitryptic factors in raw soybean (27-28). The purification (22-23, 28) and characterization (28, 69) of this heat labile protein in soybean that inhibited the proteolytic activity of

trypsin was established. Active antitryptic fractions from unheated soybean have been demonstrated to retard the growth of rats (23-25), mice (116) and chicks (4, 23, 54). From the observation that the protein efficiency of partially heated flour increased in proportion to the destruction of the trypsin inhibitor, Westfall and Hauge (115) concluded that the trypsin inhibitor was the major cause of the poor utilization of soybean. Trypsin counteracted the growth depression of rats (25-26) and chicks (36) fed raw soybean. However, Kakade and Evans (67) did not confirm this finding on rats fed raw navy beans with various concentrations of trypsin. They pointed out rather the growth depressing effects of such additions of enzyme.

Thus, there appears to be little doubt that the poor growth promoting quality of raw soybean and other legumes could be attributed to a large extent to a trypsin inhibitor. Although Melnick's hypothesis (81), referred to earlier, would seem to explain the mode of action of trypsin inhibitor, none of the subsequent reports could confirm his theory. In vitro studies have shown that the trypsin inhibitor does not specifically retard the enzymatic release of methionine, but appears to affect all the amino acids to the same extent (72). According to Desikachar and De (43) and Westfall et al. (116) active antitryptic preparations retard the growth

of rats and mice even when added to diets containing predigested protein, where intestinal proteolysis would not play a part with respect to the availability of essential amino acids.

Alumot and Nitsan (5) and Chernick *et al.* (36) noticed that chicks fed raw soybean oil meal developed marked hypertrophy of the pancreas. Contrary to what has been generally assumed, the amount of trypsin found in the intestine of rats (80) and chicks (70) fed raw soybean was actually greater than that observed in animals fed the heated soybean, as was also pointed out by Lyman (79). A crystalline preparation of the inhibitor produced the same effects as raw soybean oil meal (70). It appears, therefore, that the growth depression caused by the trypsin inhibitor has no relation to the inhibition of intestinal proteolysis, but may be the result of an endogenous loss of essential amino acids from a hyperactive pancreas which is responding in a compensatory fashion to the effects of the trypsin inhibitor. However, it needs to be established in the case of rats as in the case of chicks (70).

Trypsin inhibitor has also been found in a large number of other legumes (21, 25, 115) and its presence provides the clue to the observation that heating increases the *in vitro* digestibility of some legume

proteins (113). Jaffe (63) observed that those legumes which had the highest antitryptic activity were also those in which the digestibility, as measured *in vivo* with rats, was best improved by cooking. As in the case of soybean, it is not certain that the trypsin inhibitor exerts its deleterious effect on growth by an inhibition of intestinal proteolysis, since Klose *et al.* (68) found that fractions of lima bean protein which were high in antitryptic activity inhibited the growth of rats fed acid hydrolysed casein.

Jaffe (63) found the antitryptic activity of lentils, cowpeas, soybean and red kidney bean to be 1.78, 1.91, 4.15, 4.25 x 10^{-4} units/g, respectively. Borchers and Ackerson (21) found 16.6, 41.4, 32.3, 0, 0, 0, 21.8, 44.1, 0, 35, 0, 0, and 43.7 x 10^{-3} units/ml of extract of *Arachis hypogea*, carob bean, chick-pea, guar bean, lentils, lupine, *Phaseolus coccineus*, *Phaseolus vulgaris*, peas, soybean, *Vicia faba*, *Vicia sativa* and cowpeas, respectively. Kunitz (69) reported antitryptic activity of raw soybean to be 6.2 x 10^{-3}/ml of extract. Bielorai and Bondi (17) did observe antiproteolysis exhibited by extracts from raw peas, peanuts and soybean and reported that toasting at 120° C for 15 minutes of raw soybean, very little antitryptic activity was left that did not interfere with growth.

Ham et al. (54) also showed that soybean heated in an autoclave at 15 psi for 30 minutes had no anti-proteolytic activity. Treating extracts of trypsin inhibitor with $(NH_4)_2SO_4$ at pH 2.0, and acetone at pH 4.5 plus papain was found to have no significant effect on growth of rats. Westfall and Hauge (115) pointed out that autoclaving soybean at 108° C for 15 minutes destroyed the inhibitor potency. Kakade and Evans (67) and Honavar et al. (58) also found negligible activity left in navy beans and kidney beans autoclaved at 121° C for 15 minutes. Sohonie and Bhandarker (109) reported that in cowpeas the trypsin inhibitor was destroyed by heating for 1 hour at 100° C. Patwardhan (97, pp 12-30) stated that "the application of heat treatment will have to be done with discretion in dealing with legumes." Thus, it can be concluded that a moderate degree of heat applied in preparation and cooking is beneficial.

He has further remarked that "trypsin inhibitor in legumes need not be of major concern while considering the nutritive value of these foodstuffs."

Hemagglutinins: Borchers and Ackerson (24) and Jaffe (62) concluded that there is no obvious relationship between presence of the trypsin inhibitor and beneficial effect of heat treatment. This discrepency had served to focus attention on the possible presence of other

growth inhibitors in raw legumes that were eliminated by heat. Numerous reports (38, 40, 49-51) confirmed the early finding of Osborne and Mendel (93) that rats would not grow if the source of dietary protein was derived from the kidney bean (Phaseolus vulgaris), and that the prolonged feeding of raw beans caused death of the rats. Since then many workers arrived at similar conclusions while working with a wide variety of Phaseolus vulgaris. Besides Jaffe (64), de Muelenaere (42) also found a high degree of toxicity in rats fed Phaseolus lunatus. Liener (73, 74) observed similar growth retardation by feeding of raw soybean. In view of the lack of the ability of a casein digest to counteract this toxicity, Jaffe (65) concluded that the toxic principle involved here was not a trypsin inhibitor. This confirmed the early recognition of the presence of substances in legumes which have the ability to agglutinate the red blood cells from various species of animals (82).

These hemagglutinins are sometimes referred to as phytoagglutinins (29). Although a toxic reaction may be produced in animals by the direct injection of the purified hemagglutinin of the soybean (71-74), Liener (75) called attention to the fact that the oral ingestion of the purified hemagglutinin of soybean, i.e., 'soyin,' would inhibit the growth to the extent of 25 per cent. Li and Osgood (71) and Rigas and Osgood (104) prepared

purified hemagglutinins of Phaseolus vulgaris. Honavar et al. (58) and Jaffe (62, 64-65) showed that purified preparations of this hemagglutinin could markedly inhibit growth of rats. It is believed that the action of hemagglutinins is to combine with the cells lining the intestinal wall and thus interfere with intestinal absorption of nutrients, i.e., amino acids (65).

It may be pointed out here that there have been several instances reported in which manifestations of toxicity have been observed in subjects who have eaten insufficiently cooked scarlet runner bean (50) and broadbean (38) or inadequately heated kidney bean flour (50). Eating of raw soybean was noticed to cause symptoms of nausea, vomitting and diarrhea in Japanese prisoners of war (34). It appears logical to suspect hemagglutinins to be responsible in part for the intoxication of human subjects ingesting certain raw legumes.

Creger and Gifford (38) reported the correlation between toxicity and the presence of hemagglutinins. On the contrary to what was found by Liener and Hill (76), de Muelenaere (40) reported that only 3 out of 7 rats died by intraperitoneal injection of soybean hemagglutinin, and reported 30,240 hemagglutinating units (H.U.) activity, which was completely destroyed by 30 minutes autoclaving. Natal

bean and Umgarbi were reported by de Muelenaere (40) to contain 155,520, and 45,200 H.U.; whereas Honavar et al. (58) reported hemagglutinating activity of Phaseolus vulgaris to be 2,456-3,560 H.U. More recently de Muelenaere (42) reported that one variety of Phaseolus vulgaris did not exhibit toxicity indicating varietal variability; while soybean had moderate toxicity which is clear from the following table, taken from de Muelenaere:

Table 2. Effect of heat treatment of legumes' hemagglutinating activity and intraperitoneal toxicity.

	Hemagg. units/g		Toxicity[1]	
	Raw	Heated	Raw	Heated
Phaseolus vulgaris	155,000	50	7/7	0/7
" "	45,000	0	6/11	0/7
" "	40,000	20	0/11	0/7
" "	8,000	4	3/10	0/5
Phaseolus acutipolias	6,000	0	1/5	0/5
Phaseolus multifloris	4,000	14	5/5	0/5
Glycine max	8,000	4	4/7	0/7
Vicia sativa	120	10	0/5	0/5
Vicia faba	90	11	0/5	0/5
Pisum sativum	80	4	0/5	0/5
Phaseolus aureus	78	0	0/5	0/5
Vigna sinenses	6	2	0/5	0/5

1. Given as number of animal deaths 72 hours after injection.

Thus it seems evident that even hemagglutinins in properly cooked legumes have little effect in interfering with growth except for few legumes in which hemagglutinins are not destroyed as confirmed by Dreyer et al. (45).

B. Evaluation of Proteins by Bioassay Methods

Hegsted and Chang (55) pointed out that the metabolic utilization of dietary proteins in man and the rat have close resemblence, thus pointing the applicability of results of rat growth assays for protein evaluation of human dietaries. The primary objective of animal assays is to explore the quality of protein in relation to its amino acid composition and the effect of processing on the nutritive value of the protein. Thus, growth will be directly related with essential amino acid composition and their proportion to each other in the test diet (7, p 23).

Protein efficiency ratio: Protein efficiency ratio (PER) is a procedure developed by Osborne et al. (96) based on the growth promoting value of a protein in a diet containing adequate amounts of other nutrients. The nutritive value (PER) is expressed as the ratio of the gain in body weight (g) to the amount of protein (g) consumed in a specified period of time. Because of its simplicity, this method has become a very widely used method of protein evaluation. Originally, the method

involved the determination of PER at different levels of protein intake, the maximum value being taken as the PER of the test diet. Maximum value is obtained at 10 per cent as reported by Middleton et al. (83). However, Chapman et al. (35) and Campbell (32, pp 11-22) have recommended 10 per cent protein level and ad libitum feeding to obtain maximum PER. Numerous factors affect the PER determinations as have been shown in recent reviews (32, pp 11-22; 7, pp 22-24). The most important of these factors are: the level of protein in diet; age; sex and strain of rats; variation in food intake; and duration of the experimental period.

Protein level of diet: The effect of increasing levels of protein on PER values is dependent on protein quality (32, pp 11-22). PER values fall markedly when good quality protein is fed at a level higher than 10 per cent. While in case of a poor quality protein, there may be some increase followed by a decrease. The presence of toxins, as in legumes and rape seeds, may affect food intake and utilization. Allison (2-3) and Kakade and Evans (67) have used two levels of proteins, 10 and 20 per cent, to study the effect of toxic factors, and when there is more than one amino acid limiting in the diet. However, most investigators use a single level.

Age and sex of rat: Chapman et al. (35) reported higher PER values when 22 days old rats rather than 29 days old rats were used. Weanling rats, 3 weeks old, are more sensitive to differences in protein quality. The sex of rats may also affect the PER ditermination. However, Block and Mitchel (19, pp 249-278) pointed out that there is no advantage of such distinction. Whereas Morrison and Campbell (89) showed that female rats tend to give maximum PER values at lower levels of dietary protein than males. The two sexes did not show the same difference between different proteins. Further, the females did not gain weight so rapidly as males and the variation within the groups was greater than that observed with males. Marked variation in PER values was obtained using different strains of rats. This shows that the same strain of rat has to be used for comparing PER values of different foods.

Variation in food intake: Sherwood and Weldon (107) introduced the technique of feeding amounts of protein based on the weight of the animal at any time. More recently Carpentor (33) pointed out the justifiability of ad libitum feeding in case the diets under comparison were equally palatable and the animals ate them in quantities proportional to their maintenance requirements over the experimental period.

Length of assay period: Several workers (14-15) have reported that shorter experimental periods gave higher PER values than longer periods. Morrison and Campbell (89) showed that differences in the PER of different proteins tend to decrease as the experiment progresses. Most workers, therefore, have used a four-week experimental period. Bender and Doell (15) and Bender (14) determined PER on as short a period as 10 days and recommended 60 days. Chapman et al. (35) also showed less variation in four weeks than in two weeks. They corrected PER values of different proteins taking a constant PER value of 2.5 for casein. Since PER values may vary significantly with different batches of rats, the use of standard reference as casein in PER determination has been stressed. However, Campbell (32, pp 11-22) has standardized these conditions, the use of which for performing PER assays will give reproducible results.

The validity of PER assay method has been criticized by Bender and Doell (15), Bender (14) and Hegsted and Chang (55), besides other reviewers (32, pp 11-22; 7, 23-24). The chief objections pointed out are the following: i) The assumption that the gain in body weight is constant in composition is not always valid. ii) The results will vary with dietary protein intake and protein level. iii) No allowance is made for

protein requirement for maintenance and full utilization of dietary protein is assumed. iv) The method does not permit evaluation of proteins that do not support growth.

Hegsted and Chang (55) reported that PER is correlated with weight gain. Therefore, PER is not characteristic of protein, but of rate of gain of animals consuming the diet. Contrary to this, Middleton et al. (83) stated that growth was due to the addition of nitrogenous constituents. PER values cannot be used as a basis for working out relative protein requirements of different proteins (55). They further stated that some 1,500 investigations have been made on PER, and remarked that "it is not unfair to say that PER has none of the characteristics of good bioassay."

Bender (14) also criticized that the variation in PER was due in part to the variation in food intake, which is influenced by the quality of the protein. Errors were high due to inadequate intake and he pointed out that 60 days' period is essential for accuracy. However, a highly significant correlation coefficient $r = 0.803$ (p .001) between PER and net protein utilization (NPU) was obtained and the following regression equation was obtained: $Y = 40.0 + 12.6X$; while Block and Mitchel (19, pp 249-278) obtained $r = 0.838$ and $Y = 37.2 + 14.05X$. PER could not be determined in protein which gave a NPU value of less

than 40. He remarked that "with all drawbacks it is surprising that the average PER values correlate so well with NPU." Evidence gathered only partly supported the criticisms of PER.

However, Campbell (32, pp 11-22) pointed out the simplicity and accuracy of this method provided standard conditions are carefully followed. Thus many investigations find that PER gives an over all idea of nutritive value of the protein.

Osborne and Mendel (95) were the first to find out that soybeans, unless cooked for several hours, would not support normal growth in rats. This has been subsequently confirmed (22-25). Evans et al. (47, 48, 115) showed that autoclaving at 120° C for 30 minutes is essential for increasing digestibility over raw and 130° C autoclaved samples. While Clandidin et al. (37) showed that a good quality protein food was obtained by autoclaving at 140° C for four minutes. Whereas Jaffe (63) preferred autoclaving for 10-15 minutes at 120° C. Liener (77, pp 281-298), however, summarized that for a positive improvement in protein quality, it made no difference whether the legume was germinated, parched, heated in water or autoclaved. However, many workers arrived at the conclusion that the application of heat treatment has to be done with great care when dealing with legumes. Bressani et al. (30) pointed out that

10-30 minutes' autoclaving at 121° C was optimum for Phaseolus vulgaris and 20 minutes' autoclaving at 121° C has been recommended by Liener (76) for soybean. In contrast, Kakade and Evans (67) reported that 5 minutes' autoclaving is optimum for Phaseolus vulgaris and Honavar et al. (58) recommended autoclaving in water to improve protein quality. Peanuts were reported to exhibit a decrease in protein quality on autoclaving for more than 15 minutes (16, 31).

Feeding lentils, both raw and autoclaved for 15 minutes at 15 psi to rats at 12 per cent protein level, PER values of 0.9 and 0.8 were obtained by Hirwe and Magar (57). Similar observations were indicated by Blaizot (18). Borchers and Ackerson (24) did observe an improvement by autoclaving. Adolph et al. (1) found that cooking enhanced the growth promoting value of lentils and PER of 1.15 was reported; Patwardhan (97, p 21) stated that PER of lentils ranged from 0.1 to 0.9.

Hirwe and Magar (57) obtained PER values of 1.65 and 2.01 for raw and autoclaved samples of chick-peas respectively. Adolph et al. (1) reported PER values of 1.47 and 2.05 for raw and cooked chick-peas. Russel et al. (106) found a PER value of 1.8 for cooked chick-peas. Blaizot (18) found little improvement in chick-peas autoclaved at 15 psi for 15 minutes. Contrary to this, Borchers and Ackerson (24) and Honavar et al. (58)

did obtain an improvement in PER of chick-peas by autoclaving, while ordinary cooking resulted in little improvement in nutritive value. Patwardhan (97, p 21) quoted a range in PER of 1.3-2.1 for optimally cooked or autoclaved chick-peas.

Elias et al. (46) reported PER values ranging from 1.42 to 2.30 for eight cooked varieties of cowpeas. A PER value of 1.41 for cowpeas was reported by Aykroyd and Doughty (9, p 74). Borchers and Ackerson (24) reported gain in weight of 9.84 g and 15.4 g for raw and autoclaved cowpeas (30 minutes at 15 psi). Jaffe (61) also observed an improvement in growth promoting value of cowpeas cooked for two hours. Sherwood et al. (108) stated that a very slight improvement resulted from cooking, while different varieties and localities showed different effects. Supplementation of cowpeas with 0.3 per cent methionine caused a significant improvement. A similar conclusion was arrived at by Richardson (103). Patwardhan (97, p 21) assigned an average PER value of 1.3 to cowpeas.

Inamdar and Sohonie (60) reported that raw broadbeans, when supplemented with methionine, tryptophan, leucine and valine and fed to rats, resulted in retardation in growth, while a similar diet of broadbeans autoclaved for 30 minutes at 15 psi showed an improvement in growth. Adolph et al. (1) reported a PER value

of 1.13 for broadbeans cooked for two hours, and a PER value of 1.2 has been mentioned by Patwardhan (97, p 21).

Russel et al. (106) reported a PER value of 0.2 for cooked Phaseolus vulgaris whereas Adolph et al. (1) found a value of 1.51. Bressani et al. (30) showed that fours hours' cooking had a beneficial effect similar to 10-30 minutes autoclaving at 16 psi with regard to growth promoting value of Phaseolus vulgaris. However, at various protein levels, black bean gave PER values of 0.43 to 1.20 and, on supplementation with sulphur amino acids, an improvement in growth was observed. Lysine and tryptophan supplementation did not result in an increase in PER, but affected an improvement in feed efficiency. These results indicated the low biological availability of these amino acids even though kidney bean is a good source of both. All rats in the study died within 2 weeks.

Everson and Heckert (49) reported PER of 0.8-0.9 for Phaseolus vulgaris autoclaved for 45 minutes at 15 psi; Honavar et al. (58) obtained similar results. They stated that presoaking is essential and, if performed, followed by autoclaving the nutritive value (PER) was equal to that of casein. Jaffe (65) also noticed a similar observation. In contrast to these findings, Kakade and Evans (67) reported PER values of 1.84

and 1.62 for autoclaved navy beans (5 minutes at 15 psi) fed at 10 and 20 per cent protein level. They remarked that the leaching or presoaking recommended by Honavar et al. (58) was not essential. They also confirmed that sulphur amino acid supplementation was as effective as supplementing with all essential amino acids to a diet of navy beans autoclaved for 5 minutes. These results were in agreement with Bressani's observation (30).

Borchers and Ackerson (24) did not find any improvement in heated or autoclaved common vetch. Patwardhan (97, p 21) also had no mention of vetch and lupine. However, negative PER values have been reported when lupine extract (alkaloid) was added to a diet of casein and was fed to rats (8, p 7).

Little work on guar bean and jantar seed has been reported except for that of Borchers and Ackerson (24); they showed autoclaving did not improve the nutritive value of guar beans.

Similarly, all reports to date mention little improvement in PER of heated or autoclaved peas. Beeson et al. (11) and others (49, 103) reported lower PER values for peas due to cooking or autoclaving. However, Russel et al. (106) reported PER of 0.4 for cooked peas, while Murry (91) found PER values of 1.4 for raw peas and 1.1 for cooked peas.

Net protein utilization: As early as 1924, Mitchel (88) introduced the term 'net utilization of dietary protein' which is the product of digestibility coefficient and biological value divided by 100. Later it was introduced by Bender and Miller as net protein utilization (NPU) (12). It is a measure of the amount of dietary protein incorporated in the body. The method of assay includes a group of rats fed a non-protein diet, and the experimental groups are fed the test proteins at a level of 10 per cent for 10 days. The food intake of the animals is recorded. The animals are killed at the end of the 10 days' period and carcass nitrogen is determined by Kjeldahl. The NPU value is calculated from the following formula:

$$NPU = \frac{\text{Body N of test group - body N of non-protein group} + \text{N consumed by non-protein group}}{\text{N consumed by test group}}$$

Bender and Miller (13) later reported that body nitrogen need not be determined in each experiment but can be calculated from the water content of the body using the formula: $Y = 2.92 + 0.024X$, where Y is the ratio of nitrogen to water and X is the age of animal in days. They concluded that the ratio of nitrogen to water is constant for any age group. However, it will be necessary to establish the formula for each colony of animals (84).

The NPU method is subject to considerable variation and many drawbacks as have been pointed out. Block and Mitchel (19, pp 249-278) and Chapman et al. (35) reported that NPU determination is as valuable as PER determinations. On the other hand, Campbell (32, pp 28-35) pointed out the lack of sensitivity of the method to lysine deficiency. However, Miller and Naismith (85) demonstrated the use of total sulphur analysis in evaluating nutritive value of diets. They argue that lysine deficiency will not affect NPU which may be more correlated with sulphur amino acids.

Morrison et al. (90) found a decrease in protein utilization when high protein levels were used, and also observed direct relationship between NPU values and lysine content. They observed lower NPU values when vitamins and minerals were not included.

A high positive correlation between NPU and PER were reported by Bender (14) and it was concluded that both methods give the same order of classifying proteins according to their nutritive quality. However, it has been emphasized that, "there is need for more study of the validity of the measurement of endogenous nitrogen which is an integral part of many of these procedures" (7, p 57). Hegsted and Chang (56) did not confirm the results reported by Miller and Payne (86) that NPU values decrease if more than 27 per cent calories are derived from

proteins; probably, because of differences of experimental period which was 21 days in their case. They also remarked that the nutritive value of low quality proteins has been overassessed.

In general, it appears that optimum cooking or autoclaving brings about improvement in protein quality, which may be rated in the same order by PER and NPU.

III. MATERIALS AND METHODS

Collection and preparation of samples: Samples of air-dry legume seeds; namely, peeled lentils, brown and yellow whole lentils, chick-peas, cowpeas, kidney bean, common bean, broadbean, and fenugreek were obtained from the local market in Beirut. Local vetch and cyprus vetch, Clark, Perry and Ford soybean seeds were obtained from the Agricultural Research and Education Center of the American University of Beirut. Guar bean and jantar seeds were obtained from a local market in West Pakistan.

All samples were dusted, cleaned by removing extraneous materials including damaged seeds, and were stored at room temperature.

Each sample of seeds was divided into 3 parts. Two parts were separately spread on aluminium foil and autoclaved at a temperature of 121° C, for 5 and 20 minutes respectively. The autoclaved samples were dried overnight in a forced air-draft oven at 75° C. All samples, including the untreated ones, were then ground to a fine powder for the preparation of the animal diets and for analytical determinations.

Chemical analysis: Moisture content was determined in all legumes according to A.O.A.C. methods (59, p 12).

Protein content was determined by macro Kjeldahl method (59, p 158). The amino acids lysine, threonine, methionine and cystine were determined by microbiological assays according to Block and Weiss (19, pp 50-70), with minor modifications made. The organisms employed were Streptococcus faecalis ATCC 8043 (American type culture collection) for threonine, and Leuconostic mesenteroides P-60 ATCC 8042 for lysine, methionine and cystine. The lyophilized cultures of the organisms were revived according to standard procedures. Stock cultures of the organisms were maintained by monthly transfers, in a medium of the following composition:

Peptonized milk	10 g
Tryptone	10 g
Agar	10 g
Tomato juice	200 ml
Distilled water	800 ml

The basal assay media were purchased in a dry form from Difco Laboratories, Michigan (U.S.A.). Manufacturer's specifications were followed for preparation and sterilization.

Culture tubes containing the sterilized broth, the above mentioned medium without agar, were inoculated and incubated at 37° C. The cells were removed by centrifugation and were suspended in 10 ml of 0.9 per cent saline solution under sterile conditions.

Standard solutions of 20 mcg of L-lysine, 20 mcg of DL-threonine (equivalent to 10 mcg of L-threonine),

5 mcg of L-methionine and L-cystine were prepared according to the methods of the Association of Official Agricultural Chemists. All four amino acids were hydrolized by refluxing a sample of 0.5 g of protein with 60 ml of 2 N HCl for eight hours except cystine, which was refluxed for two hours. More than 5 ml of HCl were evaporated on a hot plate. After cooling, 60 ml of distilled water were added to the residue and the pH was adjusted to 4.0 to precipitate the humins. The hydrolysate was then taken out of the refrigerator where it was stored for two hours and filtered through Whatman No. 1 filter paper. The pH of the filterate was adjusted to 6.8 by the addition of 1N NaOH and the volume was made to 250 ml. A series of test tubes of suitable dilutions (0.3, 0.6 and 1 ml) of the test hydrolysate and the standard ranging from 0-1 ml were prepared. The volume in all tubes was made up to 1 ml with distilled water and sterilized in the autoclave at 15 psi for 15 minutes. The corresponding basal medium was inoculated with 1 drop of the saline suspension of the proper organism for each 5 ml of the medium. One ml of this inoculum was added to each of the assays and the standard tubes under asepctic conditions and incubated at 37° C for 72 hours. The lactic acid produced in each tube was titrated with 1N NaOH, using 0.004 per cent bromothymol blue as indicator. Thus a standard curve was obtained for each amino acid

from which the concentration of the unknown could be determined.

Tryptophan was determined chemically, according to Lombard and Delange (78), by weighing about 600 mg of protein sample in triplicate, in glass-stoppered Erlenmeyer flasks, marking 2 flasks as unknown and the other as recovery. Ten ml of 100 mcg tryptophan solution were added to the recovery flask followed by adding 25 ml of 0.05 N NaOH, 10 ml of freshly prepared 2 per cent papain solution, and 10 drops of 5 per cent NaCN solution. Twenty-five ml of 0.05 N NaOH solution and 10 drops of sodium cyanide solution were added to 30 ml of the enzyme solution in another flask to determine its tryptophan content.

The flasks were stoppered and allowed to stand overnight in an oven at 70° C. After cooling, the contents of each flask were transfered and brought to a volume of 100 ml with distilled water. Five ml aliquots of these hydrolysates were then transfered to 30 ml centrifuge tubes, and to each tube 3 ml of carbon tetrachloride and 5 ml of 0.1 N KOH were added. After thorough shaking for 10 minutes, the tubes were centrifuged at 3000 rpm for 10-15 minutes.

One ml of the clear supernatant was pipetted into each of 3 test tubes, 2 of which were marked T (test)

and 1 B (blank); 1 ml of 5 per cent P-dimethylamino-benzaldehyde solution was added to the 2 test tubes and 1 ml of distilled water was added to the blank. Five ml of concentrated HCl was then added to all 3 tubes. Ten minutes after thorough shaking of the tubes two drops of 0.2 per cent sodium nitrite solution were added to each unknown and the tubes were shaken again. Intensity of the color was measured at 590 mu in a "Spectronic 20" spectrophotometer and the results were calculated as follows:

Tryptophan content of sample:

$$\frac{200 \times \text{OD "unknown"}}{\text{OD standard} \times \text{sample standard in g}} = X \text{ mg tryptophan/100 g sample}$$

Tryptophan content of enzyme solution:

$$\frac{1000 \times \text{OD "unknown"}}{3 \times \text{OD standard}} = Y \text{ mg tryptophan/100 g sample}$$

$$\text{mg tryptophan/100 g sample} = X - \frac{Y}{20}$$

Determination of trypsin inhibitor: Antitryptic activity was determined by the method of Kunitz (69) with some modifications in the extraction of the inhibitor, for which the procedures described by Ham et al. (54) and Borchers et al. (22, 23) were followed. An appropriate amount of the sample was taken in test tubes and was treated with 10 volumes of 0.05 N HCl at pH 4.2. The tubes were shaken and placed in a freezer overnight.

Then the tubes were removed from the freezer, thawed and were shaken for 10 minutes. The tubes were then centrifuged at 9,000 rpm for 30 minutes, and the supernatant was collected in a flask and kept in a refrigerator. This procedure was repeated three times, each time the supernatant was collected and treated with a fresh batch of HCl at pH 4.2 to obtain maximum extraction of the inhibitor. In later extractions, however, freezing time was decreased to three hours, so as to avoid long periods of time whereby the inhibitor may have been destroyed.

The HCl extract thus collected was treated with a sufficient volume of acetone to attain a concentration of 70 per cent acetone to precipitate the inhibitor. The contents were again centrifuged at 4,000 rpm for 30 minutes. Then the supernatant was discarded and the precipitate was dissolved in 0.0025 M HCl and the volume adjusted to 100 ml with 0.0025 M HCl. Samples of 1 ml containing 50 mcg of trypsin[1] dissolved in 0.0025 M HCl, were mixed with 1 ml of the inhibitor; 1 ml of each mixture was added to 1 ml of 1 per cent casein solution at pH 7.6 and was kept for 20 minutes at 37° C. Then this was mixed with 3 ml of 5 per cent trichloracetic acid. The separated products were filtered and the

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1. Obtained from Mann Research Laboratories, Incorporated. New York (1:300).

concentration of the filterate was determined by measuring the optical density of the solutions at 280 mu. Readings were corrected for the blank solution, prepared in a similar way except for the digestion at 37° C. A standard curve was plotted with known concentration of trypsin. This curve was used to calculate the inhibitor activity which was expressed as units of trypsin inhibited.

Determination of hemagglutinating activity: The procedure described by Liener and Hill (76) was followed for determining hemagglutinins with some modifications. Fifty ml of 0.9 per cent sodium chloride were added to 5 gm of the legume sample in a stoppered bottle and shaken. The samples were allowed to stand in a refrigerator overnight, then filtered to obtain a clean yellow solution.

A suspension of red blood cells from chicken whole blood (collected by heart puncture in an oxalated tube) was prepared in the following manner:

The hematocrit value (H) was determined on a portion of the blood, using a hematocrit tube. One volume of whole blood (vb) was diluted with 4 volumes of cold 0.9 per cent saline solution, and centrifuged at 2,000 rpm for 10 minutes. The supernatant fluid was discarded. This was repeated three times. The precipitated cells were finally suspended in a sufficient

volume of saline to give a 4 per cent concentration of red blood cells on the basis of the hematocrit value. To a series of 10 tubes was added a 0.5 ml of decreasing concentrations of the original extract, to which 0.2 ml of the 4 per cent red blood cells suspension was poured. The tubes were incubated at 37° C for 1 hour. Agglutination was read by tapping the tube and noting the clumping of the cells. Those which had not been agglutinated, dispersed readily to form a uniform suspension. A tube containing 0.5 ml of saline plus 0.2 ml of the cells suspension served as a negative control. The degree of agglutination was measured by the size of the cell aggregate by arbitrarily grading from zero to 4^+. The least amount of hemagglutinin which produced positive evidence of agglutination (1^+) was defined as 1 hemagglutinating unit (HU), under these specified conditions, and accordingly hemagglutinin activity was calculated from the formula:

$$HU/g = \frac{Da \times Db \times S}{V}$$

where

V = volume of extract in tube 1 (=0.5 ml)

Da = dilution factor of extract in tube 1 (=1 unless original extract was diluted)

Db = dilution factor of tube containing 1 HU and

S = ml of original extract/gm sample (=10)

Animal experiments: The bioassay methods of protein evaluation used in this study were protein efficiency ratio (PER) and net protein utilization (NPU).

For PER determinations, groups of 10 weanling rats were used. A reference group was also included, which was maintained on a casein diet to serve as a standard. Weanling rats of the Sprague-Dawley[1] strain were used and were fed a stock diet[2] for 2-3 days to recover from shipment. The animals were housed indivudually in mesh-bottom cages in an air conditioned room, maintained at 70 ± 2° C and at a relative humidity of about 55 ± 5 per cent. They were assigned to the experimental diets according to a randomized block design. Food and water were provided ad libitum; weekly record of food consumption and body weight were kept for obtaining PER values.

The basal diet consisted of the following proportions of the various components:

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1. Obtained from Animal Suppliers (London), Ltd., London.
2. Obtained from VITASNI Company, Beirut.

Ingredients	Percentage
Corn starch	80
Corn oil	10
Non-nutritive cellulose (alphacel)	5
Mineral mixture - USP XIV[1]	4
Vitamin mixture[2]	1

The experimental diets were prepared by mixing appropriate amounts of the test materials to provide 10 per cent protein (N x 6.25) in the diet. A 10 per cent casein diet was also prepared. All test diets were made iso-nitrogenous and iso-caloric with each other. Test proteins were added at the expense of corn starch.

Protein efficiency ratio was determined according to the standardized method suggested by Campbell (32, pp 11-22); the assay period was four weeks. PER

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1. Mineral mixture obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio; and contained: NH_4-alum, 90 mcg; $Ca(PO_4)_2$, 100 mg; $CaCO_3$, 70 mg; Ca-citrate, 300 mg; $CuSO_4$, 80 mg; $FeNH_4(SO_4)$, 15 mg; $MgCO_3$, 35 mg; $MgSO_4$, 240 mcg; KCl, 125 mg; KI, 40 mcg; KH_2PO_4, 200 mg; NaCl, 80 mg; and NaF, 500 mcg/g, respectively.

2. Obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio; and contained the following vitamins triturated in dextrose per gram of mixture: 900 IU vit A; 100 IU vit D; alpha-tocopherol, 5 mg; vit C, 45 mg; inositol, 5 mg; choline chloride, 75 mg; riboflavin, 1 mg; menadione, 2.25 mg; para-amino benzoic acid, 5 mg; niacin, 4.5 mg; pyridoxine-HCl, 1 mg; thiamine-HCl, 1 mg; calcium pantothenate, 3 mg; biotin, 20 mcg; folic acid, 90 mcg and vit B_{12}, 1.35 mcg.

was calculated from the following formula:

$$PER = \frac{\text{Weight gain of the test group (g)}}{\text{Protein consumed by the test group (g)}}$$

Corrected PER values were calculated by using the following formula:

$$\frac{2.5}{\text{Determined PER of casein}} \times \text{PER of test diet}$$

NPU determinations were performed according to the method of Miller and Bender (12, 84). A group of eight rats, housed in two cages, was assigned to each diet. A negative control group was fed a protein-free diet throughout the experimental period. Daily records of food intake and body weight were kept during the 10 days' period of the experiment. Spilled food was collected daily and was weighed at the end of the experiment. After the 10 days' period, the animals were sacrificed with chloroform after recording the body weights. Incisions were made in the cranium, thorax and abdomen of each animal, and the carcasses were dried in a forced-draft oven at 95-100° C for 2-3 days. Each group of carcasses was then ground separately in a meat-mincer twice or three times to obtain a homogenious mixture. Nitrogen was determined on a sample of the ground material. NPU was then calculated from the following equation:

$$NPU = \frac{B - BK + IK}{I}$$

where B and BK are the total body nitrogen of animals on test and protein-free diets respectively, and I and IK are their respective nitrogen intakes.

The results of the animal experiments were analyzed statistically and all possible comparisons were made by the use of the method of single degree of freedom according to Leclerg *et al*.[1]

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1. Leclerg, E. L., W. H. Leonard, and A. G. Clark. *Field Plot Technique*. (pp 146, 167). 2nd ed. 1962.

IV. RESULTS AND DISCUSSION

In the evaluation of the effects of autoclaving on protein quality of local varieties of legume seeds, both chemical methods including determinations of total protein, amino acids, trypsin inhibitor and hemagglutinating activity, as well as bioassay procedures, namely, protein efficiency ratio and net protein utilization were used in this study.

A. Chemical Studies

Moisture and protein content: The data in Table 3 show that moisture content varied from 8.11 to 14.00 per cent, in the different legume seeds investigated. This is within the normal range usually found in most legume seeds (9, pp 38, 116).

Also, Table 3 shows that chick-peas contain the lowest protein content (19.98 per cent) while lupine contains the highest protein content (39.17 per cent). The three varieties of soybean were found to have almost the same amount of protein ranging from 34.19 to 35.16 per cent. Brown whole lentils have a little higher percentage of protein than peeled brown and yellow whole lentils. All the other legumes had a protein

Table 3. Protein and moisture content of legume seeds. (Expressed per 100 gm edible portion)

Legume		Moisture	Nitrogen	Protein (N x 6.25)
Common name	Scientific name	g	g	g
1. Lentils, brown, peeled[1]	Lens esculenta	9.25	3.86	24.13
2. Lentils, brown, whole[1]	"	8.81	4.06	25.38
3. Lentils, yellow, whole[1]	"	8.11	3.83	23.93
4. Common bean[1]	Phaseolus vulgaris	10.34	3.39	21.19
5. Kidney bean[1]	"	13.30	3.59	22.43
6. Broadbean[1]	Vicia faba	8.95	4.21	26.33
7. Lupine[1]	Lupinus spp.	7.80	6.27	39.17
8. Peanuts[1]	Arachis hypogaea	5.76	4.30	26.89
9. Cowpeas[1]	Vigna sinensis	9.82	3.80	23.77
10. Peas[1]	Pisum sativum	14.00	3.52	21.98
11. Chick-peas[1]	Cicer arietinum	8.92	3.20	19.98
12. Soybean, Clark[2]	Glycine max.	8.00	5.56	34.75
13. Soybean, Ford[2]	"	8.10	5.47	34.19
14. Soybean, Perry[2]	"	7.80	5.63	35.16
15. Cyprus vetch[2]	Vicia sativa	12.00	3.70	23.13
16. Local vetch[2]	"	9.26	4.18	26.13
17. Jantar seed[3]	Sesbania aegyptiaca/ aculeata	9.63	4.73	29.53
18. Guar bean[3]	Cyamopsis psoralioides	9.11	4.81	30.06
19. Fenugreek[1]	Trigonella foenumgraecum	9.33	4.81	30.06

1. Obtained from market in Beirut.

2. Obtained from experimental station of American University of Beirut.

3. Obtained from market in West Pakistan.

content from 21.19 to 30.06 per cent. These results are in agreement with the findings of other workers (9, pp 38, 116, 92, p 54).

However, it has been pointed out by VanEtten et al. (111) that in crown vetch, hemp sesbania, guar and fenugreek, there are other naturally occurring amino acids such as L-canavanine. Thus using the factor (N x 6.25) for total protein in such legumes would give a higher value. Therefore, the need for working out the exact factor for calculation of total protein for each legume could be emphasized.

Amino acids: Tryptophan content of the various legume seeds is shown in Table 4. The data show that the three varieties of soybean had the highest tryptophan content (133-136 mg per g N) followed by kidney bean, guar bean and common bean. These were the only legume seeds which were adequate in tryptophan content according to the 1957 FAO provisional pattern (6). Cowpeas and chick-peas were found to have identical amounts of tryptophan (84 mg per g N), and lupine had the lowest amount (43 mg per g N). The results are in agreement with those of other workers (9, p 117, 20, pp 316-321, 7, 46, 92, pp 16-21, 100, 111). The amount supplied by a diet containing 10 per cent protein from most of the legumes meets the requirement of the rat. However, broadbean, peanuts, and yellow lentils provided 77-90 per cent of the requirements,

Table 4. Tryptophan content of legume seeds.

Legume	Raw	Auto-claved 5 min. at 121° C	Auto-claved 20 min. at 121° C	Amount supplied by 10g protein from raw seeds	Per cent of rat requirement at 10% protein[1]	Score based on 1957 FAO provisional pattern[2]
	mg/g N	mg/g N	mg/g N	mg	%	
1. Lentils, brown, peeled	72	72	73	115	105	80
2. Lentils, brown, whole	69	70	70	110	100	76
3. Lentils, yellow, whole	63	64	64	100	90	70
4. Common bean	123	123	123	196	178	136
5. Kidney bean	129	129	129	206	187	113
6. Broadbean	53	53	53	85	77	59
7. Lupine	43	43	43	69	63	49
8. Peanuts	54	54	54	86	78	60
9. Cowpeas	84	84	84	134	122	93
10. Peas	73	73	73	117	106	81
11. Chick-peas	84	84	84	134	122	93
12. Soybean, Clark	135	135	135	216	196	150
13. Soybean, Ford	133	133	132	212	192	148
14. Soybean, Perry	136	136	136	218	198	151
15. Cyprus vetch	74	74	75	118	107	82
16. Local vetch	61	61	61	97	88	68
17. Jantar seed	72	72	72	115	105	80
18. Guar bean	126	126	126	202	183	140
19. Fenugreek	72	72	72	115	105	80

1. Calculated according to amino acid requirements of the rat reported by Rao *et al.* (99). J. Nutrition 69, 387, 1959.

2. Calculated for the raw seeds as follows: $\text{Score} = \frac{\text{Tryptophan, mg/g N} \times 100}{\text{FAO provisional pattern (90 mg/g N)}}$

while lupine supplied only 63 per cent of the rat needs for tryptophan.

The results presented in Table 4 indicate that there was no loss of tryptophan on autoclaving for 5 or 20 minutes at 121° C. Similar conclusions have been reported by many investigators (10, 30, 31, 46, 67, 110).

The lysine content of legume seeds is shown in Table 5. It can be seen that all the legumes studied are rich in lysine according to the 1957 FAO provisional pattern (6), except for guar bean. The range of lysine content in the raw seeds was 258 to 535 mg per gram nitrogen. The requirements of the rat for lysine according to Rao et al. (99) indicate that on the average not more than 90 per cent of the needs could be met arbitrarily from those legumes containing the highest amount of lysine; guar bean supplied only 46 per cent of the requirement.

As also shown in Table 5, there was little loss of lysine due to autoclaving for 5 and 20 minutes at 121° C. Other workers (30, 31, 44, 47, 48) reported similar results. However, there may be a loss in the availability of lysine due to its binding to carbohydrates as was reported in the case of peanuts (16). Also, the loss of lysine availability may become greater when autoclaving is carried out for 20 minutes. This may be due to 'browning reactions' which involve amino acids and

Table 5. Lysine content of legume seeds.

Legume	Raw	Autoclaved 5 min. at 121° C	Autoclaved 20 min. at 121° C	Amount supplied by 10g protein from raw seeds	Per cent of rat requirement at 10% protein[1]	Score based on 1957 FAO provisional pattern[2]
	mg/g N	mg/g N	mg/g N	mg	%	
1. Lentils, brown, peeled	500			800	89	185
2. Lentils, brown, whole	496	482	466	794	88	184
3. Lentils, yellow, whole	378	—	—	605	67	140
4. Common bean	424			678	75	157
5. Kidney bean	535	542	468	856	95	198
6. Broadbean	442	—	—	707	78	164
7. Lupine	303	—	—	485	54	113
8. Peanuts	370			592	64	137
9. Cowpeas	466	458	452	746	83	173
10. Peas	503			805	90	186
11. Chick-peas	424	386	408	678	75	157
12. Soybean, Clark	516	—	—	826	91	191
13. Soybean, Ford	498	—	—	797	88	184
14. Soybean, Perry	525	—	—	840	93	194
15. Cyprus vetch	488	—	—	781	86	180
16. Local vetch	470	—	—	752	83	174
17. Jantar seed	274	—	—	438	48	101
18. Guar bean	258	—	—	413	46	96
19. Fenugreek	438	—	—	701	78	162

1. Calculated according to amino acid requirements of the rat reported by Rao et al. (99). J. Nutrition 69, 387, 1959.

2. Calculated for the raw seeds as follows: Score = $\frac{\text{Lysine, mg/g N} \times 100}{\text{FAO provisional pattern (270 mg/g N)}}$

carbohydrates that are present in large amounts in all legume seeds. These results are in agreement with those of Bressani et al. (30) and of other investigators (10, 46, 67, 110) in that they also observed no major loss when they determined total lysine. Delcueto et al. (39) reported only six per cent loss of lysine in chick-pea when autoclaved for 30 and 60 minutes at 121° C.

As shown in Table 6, the threonine content of various legume seeds varied from 162 to 294 mg per gram nitrogen. Jantar seed and guar bean had marginal values according to the 1957 FAO provisional pattern (6); all other legumes were found to be good sources of threonine. Similar ranges had been reported by other workers (20, pp 316-321, 46, 92, pp 16-21, 111). However, most legumes at 10 per cent protein level supplied only 67-91 per cent of the requirements of the rat (99). Jantar seed, guar bean and broadbean had the lowest threonine content and supplied only 51, 55 and 60 per cent of the rat requirement, respectively. Elias et al. (46) have shown that there are small differences among varieties.

In the legume samples tested there appeared to be little loss of threonine due to autoclaving at 121° C for either 5 or 20 minutes. Similar results have been reported by other investigators (10, 30, 44, 46, 67, 110). It can be concluded that legumes are generally good sources of lysine and threonine and that autoclaving

Table 6. Threonine content of legume seeds.

Legume	Raw	Autoclaved 5 min. at 121° C	Autoclaved 20 min. at 121° C	Amount supplied by 10g protein from raw seeds	Per cent of rat requirement at 10% protein[1]	Score based on 1957 FAO provisional pattern[2]
	mg/g N	mg/g N	mg/g N	mg	%	
1. Lentils, brown, peeled	214	—	—	342	67	119
2. Lentils, brown, whole	228	208	217	365	72	127
3. Lentils, yellow, whole	198	—	—	317	62	110
4. Common bean	277	—	—	443	87	154
5. Kidney bean	294	286	272	470	92	163
6. Broadbean	192	—	—	307	60	107
7. Lupine	221	—	—	354	69	123
8. Peanuts	178	—	—	285	56	99
9. Cowpeas	239	241	223	382	75	133
10. Peas	236	—	—	378	74	132
11. Chick-peas	212	203	196	339	66	118
12. Soybean, Clark	291	—	—	466	91	162
13. Soybean, Ford	273	—	—	437	86	152
14. Soybean, Perry	282	—	—	451	88	157
15. Cyprus vetch	266	—	—	426	84	148
16. Local vetch	248	—	—	397	78	138
17. Jantar seed	162	—	—	259	51	90
18. Guar bean	176	—	—	282	55	98
19. Fenugreek	226	—	—	362	71	126

1. Calculated according to amino acid requirements of the rat reported by Rao et al. (99). J. Nutrition 69, 387, 1959.

2. Calculated for the raw seeds as follows: $\text{Score} = \frac{\text{Threonine, mg/g N} \times 100}{\text{FAO provisional pattern (180 mg/g N)}}$

for 5 and 20 minutes did not cause any appreciable loss of these amino acids.

Methionine and cystine and the total sulphur amino acid contents of the legume seeds are shown in Tables 7 and 8 respectively. All legumes investigated were found to be deficient in total sulphur amino acids according to the 1957 FAO provisional pattern (6). Clark soybean had the highest amount of total sulphur amino acids (196 mg per g N), while lupine had the lowest amount (56 mg per g N). According to the rat requirements specified by Rao (99), similar results are observed. Ten per cent protein from Clark soybean supplied 64 per cent of the rat requirement, while lupine supplied only 18 per cent of the requirement. These results, however, indicate that the sulphur amino acids in all the legumes investigated are limiting to the growth of the rat. The variability due to variety was observed to be small, which is consistent with earlier findings (46).

As to the effects of autoclaving, there was greater loss of cystine than of other amino acids in legumes autoclaved for 5 and 20 minutes at 121° C. Optimum heating has been reported to be beneficial in increasing cystine and methionine availability (30, 31, 44, 47, 48, 63, 66). However, long autoclaving has been reported to render methionine unabsorbable and to cause losses of cystine up to 75 per cent (44, 87). Many

Table 7. Methionine and cystine content of legume seeds.

Legume	Methionine			Cystine		
	Raw	Autoclaved 5 min. at 121° C	Autoclaved 20 min. at 121° C	Raw	Autoclaved 5 min. at 121° C	Autoclaved 20 min. at 121° C
	mg/g N	mg/g N	mg/g N	mg/g N	mg/g N	mg/g N
1. Lentils, brown, peeled	47	-	-	52	-	-
2. Lentils, brown, whole	41	44	39	49	41	39
3. Lentils, yellow, whole	39	-	-	44	-	-
4. Common bean	73	-	-	91	-	-
5. Kidney bean	68	72	67	88	72	61
6. Broadbean	41	-	-	57	-	-
7. Lupine	28	-	-	57	-	-
8. Peanuts	48	-	-	93	-	-
9. Cowpeas	79	75	82	72	75	82
10. Peas	64	-	-	78	-	-
11. Chick-peas	85	69	78	68	89	79
12. Soybean, Clark	98	-	-	132	-	-
13. Soybean, Ford	87	-	-	128	-	-
14. Soybean, Perry	96	-	-	136	-	-
15. Cyprus vetch	36	-	-	58	-	-
16. Local vetch	35	-	-	53	-	-
17. Jantar seed	49	-	-	23	-	-
18. Guar bean	63	-	-	35	-	-
19. Fenugreek	72	-	-	106	-	-

Table 8. Total sulphur amino acids (methionine + cystine) content of legume seeds.

Legume	Total sulphur amino acids	Amount supplied by 10g protein from raw seeds	Per cent of rat requirement at 10% protein[1]	Score based on 1957 FAO provisional pattern[2]
	mg/g N	mg/g N	%	
1. Lentils, brown, peeled	94	150	31	35
2. Lentils, brown, whole	82	131	27	30
3. Lentils, yellow, whole	78	125	25	29
4. Common bean	146	233	47	54
5. Kidney bean	136	218	44	50
6. Broadbean	82	131	27	30
7. Lupine	56	90	18	21
8. Peanuts	96	154	31	36
9. Cowpeas	151	242	49	56
10. Peas	128	205	42	47
11. Chick-peas	153	245	50	57
12. Soybean, Clark	196	314	64	73
13. Soybean, Ford	174	278	57	64
14. Soybean, Perry	192	307	62	71
15. Cyprus vetch	72	115	24	27
16. Local vetch	70	112	23	26
17. Jantar seed	72	115	24	27
18. Guar bean	98	157	32	36
19. Fenugreek	144	230	47	53

1. Calculated according to amino acid requirements of the rat reported by Rao et al. (99). J. Nutrition 69, 387, 1959.

2. Calculated for the raw seeds as follows: Score = $\frac{\text{Total sulphur amino acids, mg/g N} \times 100}{\text{FAO provisional pattern (270 mg/g N)}}$

observations (10, 30, 46, 67, 110), however, on short time autoclaving have shown negligible losses of sulphur amino acids, which is in support of the results obtained in the present investigation.

It appears from the above results that the sulphur amino acids are the most limiting amino acids in all the legumes tested to the growth of the rat and also so according to the 1957 FAO provisional pattern. Among all the legumes, sulphur amino acids were most limiting in lupine, and least limiting in Clark soybean.

According to the 1957 FAO provisional pattern, tryptophan appears to be the second limiting amino acid in all the legumes; while mostly threonine or lysine in some legumes appear to be the second limiting amino acid to the growth of the rat according to Rao et al. (99). However, it is interesting to note that tryptophan was more limiting in lupine than in all other legumes; and, according to both criteria, kidney bean had the highest scores for both lysine and threonine.

Antitryptic activity: The values for antitryptic activity exhibited by the legumes, presented in Table 9, show that soybean, kidney bean, common bean, peanuts, chick-peas, jantar seed, peas and yellow lentils had a range of 7.60 to 11.60 x 10^{-4} tryptic inhibiting units/ml of extract; in cowpeas there were 2.80 x 10^{-4} tryptic inhibiting units/ml. However, lentils, brown, peeled and

Table 9. Antitryptic activity of raw and autoclaved legume seeds[1]

Legume	Raw	Autoclaved 5 min. at 121° C	Autoclaved 20 min. at 121° C
	$X10^{-4}$ T.I.U./ml	$X10^{-4}$ T.I.U./ml	$X10^{-4}$ T.I.U./ml
1. Lentils, brown, peeled	nil	nil	nil
2. Lentils, brown, whole	nil	nil	nil
3. Lentils, yellow, whole	7.60	1.70	0.48
4. Common bean	9.60	2.65	0.86
5. Kidney bean	11.60	3.00	1.02
6. Broadbean	nil	nil	nil
7. Lupine	nil	nil	nil
8. Peanuts	8.40	1.90	0.75
9. Cowpeas	2.80	0.83	0.32
10. Peas	8.40	2.25	0.86
11. Chick-peas	8.40	1.90	0.62
12. Soybean, Clark	11.00	2.20	0.75
13. Soybean, Ford	9.00	1.85	0.58
14. Soybean, Perry	10.00	2.05	0.67
15. Cyprus vetch	nil	nil	nil
16. Local vetch	nil	nil	nil
17. Jantar seed	8.40	2.34	0.84
18. Guar bean	nil	nil	nil

1. Expressed in terms of tryptic units inhibited. Tryptic unit is defined as the increase of one unit of extinction at 280 mμ per minute of digestion under the experimental conditions.

whole, broadbean, lupine, cyprus vetch and local vetch, and guar bean did not show any antitryptic activity. The results are in agreement with those reported by Borchers and Ackerson (21), except for yellow lentils and peas which, according to them, had no anti-tryptic activity. However, in the present investigation yellow lentils and peas gave positive tests for inhibiting intestinal proteolysis; these results are in agreement with those of Jaffe (63) and Bielorai and Bondi (17).

Antitryptic activity was found to be destroyed to the extent of 72-80 per cent in legumes autoclaved for 5 minutes and to the extent of 89-94 per cent in samples autoclaved for 20 minutes. Kakade and Evans (67) and other investigators (22-24, 30, 58) have reported similar observations. Bielorai and Bondi (17) mentioned that autoclaving at 120° C for 15 minutes destroyed the anti-tryptic activity of soybean, and that the moderate degree of inhibitor potency left did not interfere with the breakdown and release of amino acids.

Antitryptic activity was not determined in fenugreek because it was difficult to extract this factor due to the high content of mucilage in the sample.

Hemagglutinating activity: The hemagglutinating activity of the legume seeds investigated is shown in Table 10. High activity was observed in raw kidney bean, common bean, soybean, brown lentils, whole and peeled and yellow

Table 10. Hemagglutinating activity of raw and autoclaved legume seeds[1].

Legume	Raw	Autoclaved 5 min. at 121° C	Autoclaved 20 min. at 121° C
	H. units/g	H. units/g	H. units/g
1. Lentils, brown, peeled	640	40	0
2. Lentils, brown, whole	640	40	0
3. Lentils, yellow, whole	480	20	0
4. Common bean, white	8,200	160	20
5. Kidney bean	10,560	320	40
6. Broadbean	80	0	0
7. Lupine	0	0	0
8. Peanuts	20	0	0
9. Cowpeas	20	0	0
10. Peas	80	0	0
11. Chick-peas	0	0	0
12. Soybean, Clark	2,560	160	20
13. Soybean, Ford	2,560	160	20
14. Soybean, Perry	2,560	160	20
15. Cyprus vetch	160	0	0
16. Local vetch	160	0	0
17. Jantar seed	0	0	0
18. Guar bean	20	0	0

1. Expressed as hemagglutinin unit which is the least amount of hemagglutinin that will produce positive evidence of agglutination (1^+) of 0.2 ml 4 per cent suspension of washed chicken red blood cells after 1 hr. of inoculation at 37° C.

lentils. The values ranged from 480 to 10,560 hemagglutinin units per gram. Broadbean, local vetch, cyprus vetch and peas exhibited a moderate degree of hemagglutinating activity, while peanuts, cowpeas and guar bean had the least activities. Lupine, chick-peas and jantar seed exhibited no hemagglutinating activity. Other investigators (30, 36, 40, 42, 53, 58, 76) have reported similar results. However, the presence of hemagglutinating activity in guar bean, peanuts and cowpeas has been questioned by Liener (77, pp 281-298) and Rao et al. (102, pp 304-321) perhaps because of the presence of glucose and other sugars in legumes which may interfere with the agglutination test (98).

Autoclaving for 5 minutes destroyed all hemagglutinating activity present in raw legume seeds having initially low activity, whereas in those exhibiting higher activity, it was reduced to trace amounts. Hemagglutinating activity was almost completely destroyed in kidney bean (Phaseolus vulgaris) and soybean when autoclaved for 20 minutes. Similar conclusions have been reported by many investigators (40, 42, 45, 49, 58, 76).

It appears that autoclaving whole legume seeds for 20 minutes caused complete destruction of hemagglutinins; whereas autoclaving for 5 minutes lowered the activity to amounts that do not interfere with

protein utilization (67).

Hemagglutinating activity of fenugreek was also not determined due to the difficulty met in extracting the factor from the sample which had a high content of mucilage.

B. Animal Studies

Animal studies were performed to evaluate the effect of autoclaving on the protein quality of the legume seeds by determining the protein efficiency ratio (PER) and net protein utilization (NPU) of these legumes.

Protein efficiency ratio determinations: The effect of autoclaving on the protein efficiency ratio (PER) of 11 legume seeds was determined in four different experiments. Different experiments were performed because of the large number of legume samples investigated. In each experiment a casein diet was included in addition to the legume diet to serve as a standard. Two treatments to each legume seed were given; namely, autoclaving for 5 minutes at 121° C and autoclaving for 20 minutes at 121° C. It was also the purpose to determine which of the two treatments gave better nutritive results. Untreated or raw seeds were also included in each experiment. The fourth experiment, however, did not include any samples autoclaved for 20 minutes.

Results of the four experiments are shown in Table 11 and Figure 1. Statistical comparisons of PER values obtained are also shown in the same table. The results demonstrate that autoclaving for 5 and 20 minutes increased the PER values of raw and brown lentils from -0.03 to 0.12 and 0.06 respectively, and from 1.42 to 2.07 and 1.67 for chick-peas. Adolph et al. (1) found higher values of PER (1.15) for lentils, which may be due to a difference in variety, or treatment of samples. Other workers (24, 102, pp 304-321) reported an improvement in PER on autoclaving; and a PER range of 0.1-0.9 was mentioned by Patwardhan (97, p 21). In contrast, no improvement in PER values of lentils on autoclaving was obtained by other investigators (18, 57).

Adolph et al. (1) and others (57, 58, 106) reported an increase in PER values of chick-peas on cooking or autoclaving. However, Blaizot (18) found no improvement in PER by autoclaving chick-peas for 15 minutes at 15 psi.

Results of experiment 2 (Table 11) show PER values of 0.42, 0.50 and 0.22; 1.08, 1.81 and 1.37 for raw, 5 and 20 minutes autoclaved broadbean and cowpeas, respectively. Kidney bean (Phaseolus vulgaris) autoclaved for 5 and 20 minutes had PER values of 1.03 and 0.50, respectively.

Table 11. Effect of autoclaving on protein efficiency ratio and growth of rats fed raw and autoclaved legume seeds.

Experiment 1.

Legumes / Parameters	Raw brown whole lentils	Brown whole lentils auto. 20 min. at 121° C	Brown whole lentils auto. 5 min. at 121° C	Raw chick-peas	Chick-peas auto. 20 min. at 121° C	Chick-peas auto. 5 min. at 121° C	Casein
Weight gain/g 4 weeks	-0.5	1.1	1.9	39.9	45.7	64.2	86.2
Feed intake/g 4 weeks	153.0	165.6	158.1	257.6	248.1	295.6	311.5
PER Mean±	-0.03	0.06	0.12	1.42	1.67	2.07	2.50
PER SE	±0.08	±0.13	±0.05	±0.11	±0.10	±0.14	±0.08
Significant at 5%							
Significant at 1%							

Table 11. (Continued)

Experiment 2.

Parameters	Broad-bean auto. 20 min. at 121° C	Raw broad-bean	Broad-bean auto. 5 min. at 121° C	Kidney bean auto. 20 min. at 121° C	Kidney bean auto. 5 min. at 121° C	Raw cow-peas	Cow-peas auto. 20 min. at 121° C	Cow-peas auto. 5 min. at 121° C	Casein
Weight gain g/ 4 weeks	3.4	7.9	8.7	9.4	19.6	21.6	26.3	51.4	89.50
Feed intake g/ 4 weeks	158.7	177.9	173.1	170.5	191.0	191.01	85.0	256.4	327.40
PER Mean±	0.22	0.42	0.50	0.50	1.03	1.08	1.37	1.81	2.50
PER SE	±0.08	±0.05	±0.05	±0.13	±0.11	±0.13	±0.03	±0.09	±0.07
Significant at 5%									
Significant at 1%									

Table 11. (Continued)

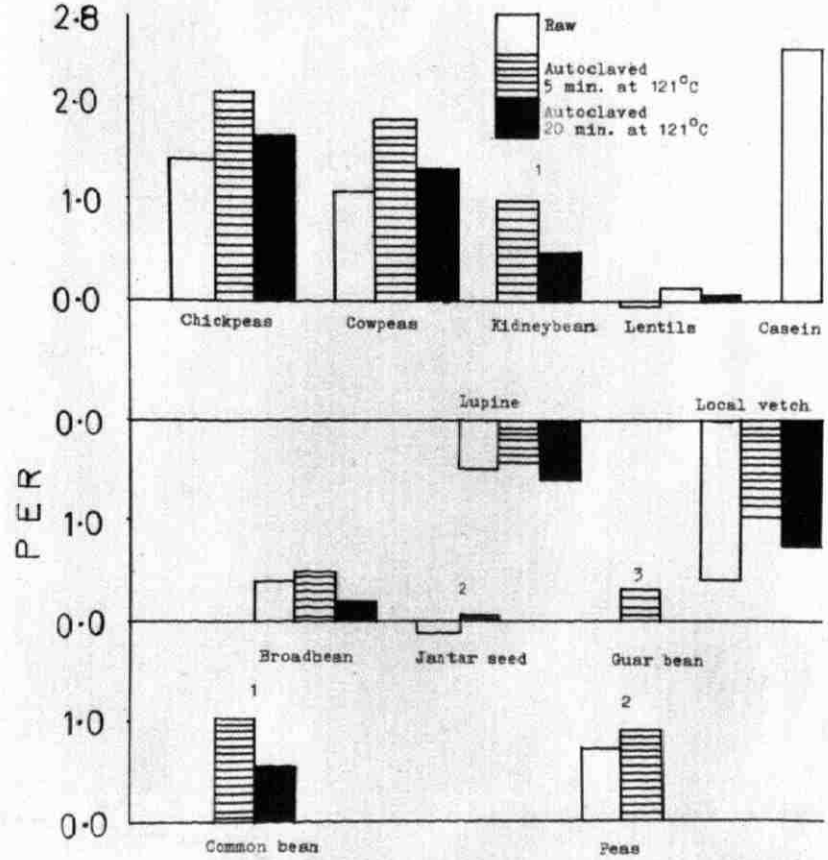
Experiment 3.

Parameters	Local vetch auto. 20 min. at 121° C	Raw local vetch	Local vetch auto. 5 min. at 121° C	Lupine auto. 20 min. at 121° C	Raw lupine	Lupine auto. 5 min. at 121° C	Common bean auto. 20 min. at 121° C	Common bean auto. 5 min. at 121° C	Casein
Weight gain g/4 weeks	-27.5	-22.5	-16.1	-10.4	-8.0	-7.5	12.7	26.2	84.5
Feed consumption/ g/4 weeks	159.5	165.0	164.0	172.1	165.4	162.1	203.7	251.3	328.0
PER Mean±	-1.63	-1.28	-0.94	-0.58	-0.46	-0.43	0.54	1.03	2.50
PER SE	±0.21	±0.09	±0.09	±0.18	±0.09	±0.10	±0.08	±0.06	±0.14
Significant at 5% and 1% levels									

Table 11. (Continued)

Experiment 4.

Legumes / Parameters	Raw jantar seed	Jantar seed auto. 5 min. at 121° C	Guar bean auto. 5 min. at 121° C	Raw peas	Peas auto. 5 min. at 121° C	Casein
Weight gain g/ 4 weeks	-1.7	1.2	5.9	14.0	19.2	78.6
Feed intake g/ 4 weeks	135.3	163.0	158.3	163.0	188.2	287.0
PER Mean±	-0.11	0.06	0.34	0.72	0.91	2.50
PER SE	±0.10	±0.13	±0.16	±0.11	±0.05	±0.08
Significant at 5%						
Significant at 1%						



1. All rats of groups not shown in figure died before the end of the experimental period.
2. PER was not determined on groups not shown in figure.
3. All rats fed raw guar beans died before the end of the experimental period, and PER was not determined on 20 min. autoclaved guar beans.

Figure 1. Protein efficiency ratio of raw and autoclaved legume seeds.

Inamdar and Sohonie (60) observed depressed growth of rats when fed raw broadbean, even when supplemented with the deficient amino acids, while improvement in growth was observed on a similar diet of broadbean autoclaved for 30 minutes at 15 psi. Adolph et al. (1) and Patwardhan (97, p 21) reported, respectively, PER values of 1.13 and 1.20 for broadbean cooked for 2 hours.

Elias et al. (46) reported PER values ranging from 1.42 to 2.3 for eight cooked varieties of cowpeas. A PER of 1.41 as stated by Aykroyd and Doughty (9, p 74) and similar values were reported by many other workers (24, 97, p 21). Sherwood et al. (108) did not report any significant improvement in PER values of cowpeas due to cooking. Varieties and locations were observed by them to have pronounced effects.

Adolph et al. (1) found a PER value of 1.51 for cooked Phaseolus vulgaris and Russel et al. (106) obtained a value of 0.2 Bressani et al. (30) found that cooking Phaseolus vulgaris in water for 4 hours increased PER values as much as autoclaving at 121° C for 10-30 minutes. Other investigators (49, 67, 97, pp 12-30) found that rats fed raw kidney bean died within a period of two weeks; and that cooking and autoclaving improved PER values.

PER values for local vetch, lupine and common bean are also presented in Table 11. The results show that negative PER values were obtained for raw, 5 and 20 minutes autoclaved local vetch and lupine.

All rats fed raw common bean died within two weeks. However, common bean autoclaved for 5 minutes gave a PER value of 1.03, while a value of 0.54 was obtained for the sample autoclaved for 20 minutes. Thus, it can be seen that lupine and local vetch did not improve on autoclaving, while common bean improved considerably.

The results of experiment 4 (Table 11) show that autoclaving for 5 minutes at 121° C increased the PER values of raw jantar seed, guar bean and peas. However, all rats fed raw guar bean died within one week, thus PER values could not be determined.

These results are in contrast to the finding of Borchers and Ackerson (24) who did not find any beneficial effect on autoclaving guar bean. The results with peas, which show a slight increase in PER, were not different from those of other workers (11, 49, 91, 103, 106) who observed a slight decrease in PER values on autoclaving or cooking.

Jantar seed and guar bean, which are less commonly used, gave PER values equal to or better than lentils which are more commonly consumed. Thus, it

should be pointed out that the use of these legumes, which can be grown under saline and drought conditions, could help overcome the protein shortage in certain areas. The need for further work on increasing their palatability by removing their pungency is obvious.

In general, foods with PER values below 1.0 are considered to contain poor quality protein; PER values for lentils, jantar seed, guar bean, broadbean, and peas were all below 1.0, indicating their low protein quality. However, when these legumes are consumed with cereals (1, 9, p 74, 100-102, pp 304-321) PER values of greater than 1.8 have been reported, indicating their important role as a supplement. Phaseolus vulgaris (kidney bean and common bean), cowpeas and chick-peas had a range of PER values from 1.03 to 2.07, which show that they contain better quality proteins. Russel et al. (106) pointed out that methionine is probably more available in chick-peas and soybean than in other legumes he investigated, which is perhaps the reason for their higher nutritive value.

The effect of autoclaving on PER values of each legume seed was statistically analyzed. The analysis is shown also in Table 11. The same bar drawn under different treatments indicates no statistically significant difference.

Statistically significant improvement in PER values was observed on autoclaving for 5 minutes the raw seeds of chick-peas and cowpeas at both 1 per cent and 5 per cent levels of significance. PER values obtained from samples autoclaved for 20 minutes were statistically higher than PER values for raw samples only in the case of cowpeas. Where applied, other than in the above mentioned comparisons, no statistically significant improvement in PER was obtained by autoclaving the raw legume seeds.

PER values for casein in all experiments were statistically higher than all other PER values except for that of chick-peas autoclaved for 5 minutes when compared at the 1 per cent level of significance. This indicates that chick-peas, autoclaved for 5 minutes, had the highest protein quality of all the legume samples tested.

It should be mentioned here that in the case of kidney bean, common bean and guar bean, all the rats fed the raw seeds of these legumes died; and, therefore, positive PER values obtained from the other autoclaved samples when compared to none obtained from the raw samples show that autoclaving has a significant effect.

In the present investigation, the results of feeding experiments on rats shown in Table 11 indicate that raw lupine, local vetch, and brown lentils, which were observed to have no antitryptic activity, supported

no growth of rats fed on them, thus had negative PER values. This may be due to the presence of some other toxic factors. However, chick-peas and cowpeas, which showed positive evidence of the inhibitor, supported good growth of rats and had the highest PER values for the raw seeds. It has been reported (77, 97) that trypsin inhibitor does not affect growth to a large extent by intestinal inhibition, but that poor growth is due to the endogenous loss of amino acids, which is due to hypertrophy of the pancreas (5, 36, 70). The results of the present study are in agreement with those stated by Patwardhan (97, pp 12-30) and others (77, pp 281-298, 73, 102, pp 304-321) who remarked that many reports about the toxic potential of trypsin inhibitor are exaggerated.

Net protein utilization: A fifth experiment was performed on lentils, kidney beans and common beans to assess their protein quality by net protein utilization (NPU) method. It was also intended to compare NPU with PER values which are affected by reduced food intake due to the presence of endogenous toxic factors. The results, shown in Table 12, indicate that NPU values were highly correlated to PER, growth and feed consumption values. A value of 40 or below for NPU was obtained when rats failed to grow; in such a case, negative PER's were obtained. With positive growth of rats on a test diet, a positive PER value and an NPU

Table 12. Effect of autoclaving on the net protein utilization and growth of rats fed raw and autoclaved legume seeds.

Legume	Treatment[1]	Wt. gain g/10 days	Food intake g/10 days	NPU
Kidney beans	Raw	-56	90	0
" "	Autoclaved for 5 minutes	+10	174	48
" "	Autoclaved for 20 minutes	+12	178	43
Common beans	Raw	-55	99	0
" "	Autoclaved for 5 minutes	+22	188	49
" "	Autoclaved for 20 minutes	+10	170	44
Lentils, yellow, whole	Raw	-4	151	40
" " "	Autoclaved for 5 minutes	+2	162	43
" " "	Autoclaved for 20 minutes	+1	172	42
Non-protein	—	-34	122	1.5

1. All treated samples were autoclaved at 121° C.

value of more than 40 was obtained. A correlation coefficient with the value of r = 0.898 (p .01) was obtained for PER and NPU and a regression equation of Y = 31.05 + 6.85X is obtained. Other workers (14, 19) reported similar correlations.

Hegsted and Chang (56) did not confirm the finding of other reports (86, 90) that lowering of protein utilization occurs with higher protein intake. The highest protein level they tested was 45.26 per cent. They also remarked that legumes and other poor quality proteins have been overassessed. However, it was observed that proteins according to their quality ranked in the same order when evaluated by PER or NPU method.

Toxicity of legume seeds: The mortality rate of rats fed the experimental diets (Table 13) show that feeding of raw kidney bean and common bean resulted in the death of all rats within two weeks. These results indicate that these legumes contain toxic factors; namely, trypsin inhibitor and hemagglutinins or others. Guar bean was found to be highly lethal, since the animals started dying 36-48 hours after ingestion of the raw diet and all rats died within one week. When raw local vetch and raw lentils were fed, four animals died during the 4-week experimental period. Raw jantar seed was found to be as toxic as raw peas. However, jantar seed and guar bean contained some substances that had a pungent smell which

Table 13. Mortality of rats used in experiments 1 through 4 shown in Table 11[1].

Legume	Raw					Autoclaved for 5 minutes at 121° C					Autoclaved for 20 minutes at 121° C				
	1 wk	2 wk	3 wk	4 wk	Total No. of dead rats per 4 wk	1 wk	2 wk	3 wk	4 wk	Total No. of dead rats per 4 wk	1 wk	2 wk	3 wk	4 wk	Total No. of dead rats per 4 wk
Lentils	-	2	1	1	4	-	1	1	-	2	-	-	-	-	0/10
Chick-peas	-	-	-	-	0	-	-	-	-	0[2]	-	-	-	-	0/10
Broadbeans	-	-	-	1	1	-	-	-	-	0	-	-	-	-	0/10
Rat kidney beans	6	4	-	-	10	-	-	-	1	1	-	-	-	2	2/10
Cowpeas	-	-	-	1	1	-	1	-	-	1	-	-	-	-	0/10
Jantar seed	2	1	-	-	3	-	1	2	-	3	Not determined				
Guar bean	10	-	-	-	10	-	-	-	2	2	Not determined				
Peas	1	1	1	-	3	-	-	-	-	0[2]	Not determined				
Local vetch	-	1	3	-	4	-	-	1	2	3	-	1	-	2	3/10
Lupine	-	-	-	-	0	-	-	-	1	1	-	-	-	-	0/9[2]
Common beans	1	9	-	-	10	-	1	-	-	1	-	-	-	1	1/9[2]

1. Initial number of rats in all groups was ten, except where indicated otherwise.

2. Initial number of rats per group was nine.

were not eliminated during autoclaving. Various reports (97, pp 12-30, 102, pp 304-321, 114, pp 565-669) indicated the presence of the highly toxic cyanogenic glucosides in these legumes, which may perhaps be present in higher amounts in guar bean.

Autoclaving for 5 minutes could not bring about any improvement in mortality rates of rats fed raw vetch and jantar seed, perhaps because of some other heat-stable toxic factors which were not affected by autoclaving. The effect of autoclaving for 20 minutes on the toxicity of jantar seed, guar bean and peas was not determined because of the shortage of facilities in the animal room.

V. SUMMARY AND CONCLUSIONS

The effect of two different autoclaving treatments on the protein quality of 19 legume seeds was investigated. Autoclaving was performed at 121° C for 5 and 20 minutes. Chemical determinations before and after autoclaving included: 1) content of tryptophan, lysine, methionine, cystine and threonine; and 2) level of trypsin inhibitor and hemagglutinating activity. In addition, rat studies were performed to determine PER on 11 of the legume seeds before and after treatment; three of them were evaluated biologically by the NPU method.

According to the 1957 FAO provisional pattern (6), the most limiting amino acids were the sulphur amino acids, followed in most cases by tryptophan. However, according to the rat requirements specified by Rao *et al.* (99), threonine, and in some cases lysine appeared to be the second limiting amino acids when the diets contained 10 per cent protein from the raw legume seeds. Autoclaving of the legume seeds for 5 and 20 minutes at 121° C had no significant effect on the content of tryptophan, lysine, threonine, methionine and cystine.

The raw seeds of brown lentils, broadbean, lupine, cyprus vetch, local vetch and guar bean exhibited

no antitryptic activity. However, the activity of the trypsin inhibitor present in 11 of the raw legume seeds was greatly reduced by autoclaving. After autoclaving at 121° C for 5 minutes, common bean showed the smallest decrease of 72 per cent in its antitryptic activity, while soybean showed the largest decrease of 80 per cent. However, a greater decrease in antitryptic activity was observed when the seeds were autoclaved for 20 minutes at 121° C; the decrease ranged from 89 per cent in cowpeas to 94 per cent in lentils and soybean.

Hemagglutinating activity was found absent in raw lupine, chick-peas and jantar seed, while peanuts, cowpeas and guar bean showed very small activity. Hemagglutinating activity was completely destroyed when raw broadbean, peas, cyprus vetch, and local vetch were autoclaved for 5 minutes at 121° C; however, less destruction was observed in lentils, common bean, kidney bean and soybean. Autoclaving for 20 minutes at 121° C destroyed completely the hemagglutinating activity present in the raw seeds.

Chick-peas, cowpeas, common beans, kidney beans, and peas gave higher values for PER than broadbean, guar bean, lentils and jantar seed thus, indicating higher protein quality. Lupine and local vetch did not support growth even after autoclaving, probably because of some heat-stable, growth depressing factor. There was a

statistically significant improvement in PER values for chick-peas and cowpeas after autoclaving the raw seeds for 5 minutes. After autoclaving for 20 minutes, significant improvement was observed only in cowpeas. With the three legumes tested by both NPU and PER methods of protein evaluation, a good correlation between the results was obtained.

The following conclusions can be drawn from the present study:

1) Autoclaving of legume seeds at 121° C for 5 and 20 minutes did not cause any appreciable loss of the amino acids: tryptophan, lysine, threonine, methionine and cystine.

2) Hemagglutinins and trypsin inhibitor in various legumes were destroyed almost completely by autoclaving at 121° C for 20 minutes; whereas the activity of these heat labile, toxic factors was reduced to a level that did not affect protein utilization when the seeds were autoclaved for 5 minutes at 121° C.

3) The protein quality of legumes as measured by PER and NPU methods was improved on autoclaving for 5 minutes at 121° C. Less improvement was obtained by autoclaving for 20 minutes.

4) Guar bean and jantar seed, which are less commonly consumed than other legumes, contain about 30 percent protein, and possibly could be more extensively consumed if the inherent pungency in those seeds could be removed.

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