STUDIES ON THE DEVELOPMENT OF INFANT FOODS FROM PLANT PROTEIN SOURCES. PART I. EFFECT OF GERMINATION OF CHICKPEA *(Cicer arietinum)* ON THE NUTRITIVE VALUE AND DIGESTIBILITY OF PROTEINS

Abdul Khaleque[1], *Luiz G. Elías*[2], *J. Edgar Braham*[2] *and Ricardo Bressani*[3]

Institute of Nutrition of Central America and Panama (INCAP), Guatemala, Guatemala, C. A.

SUMMARY

For the purpose of developing an infant food of improved dispersibility characteristics and high nutritive quality, different treatments and technologies were applied to chickpea *(Cicer arietinum)*. Samples were germinated for two and four days at room temperature (25 - 27°C). One portion of each germinated chickpea sample was boiled for 40 min and the other portion was autoclaved at 15 psi for 15 min.

These processed samples were then compared with the corresponding value of raw germinated and ungerminated samples as well as with the ungerminated processed ones for the following characteristics: chemical composition, contents of antiphysiological factors, solubility of proteins, lysine availability, net protein ratio (NPR), and digestibility of proteins. Germination caused an increase in the protein content of the seeds. No appreciable changes were observed in the trypsin inhibitor and tannin contents during germination. Availability of lysine was found slightly lower in the germinated seeds. The solubility of the nitrogenous constituents was markedly increased during germination. Along with processing, germination had no beneficial effect in improving protein quality, although digestibility of the proteins was increased. Boiling was more advantageous in the case of germinated seeds than autoclaving, whereas the reverse was true in the case of ungerminated seeds.

Manuscrito original recibido: 24—5—84.

1 United Nations University (UNU) fellow from the Bangladesh Council of Scientific and Industrial Research, Dacca, Bangladesh.

2 Professionals from the Division of Agricultural and Food Sciences, Institute of Nutrition of Central America and Panama (INCAP), P. O. Box No. 1188, Guatemala, Guatemala, C. A.

3 Head of the above-mentioned Division.

Publication INCAP/UNU-34.

INTRODUCTION

Legumes are important sources of proteins in the diets of almost all the temperate and tropical areas of the world (1). Due to the introduction of high yielding varieties of rice, wheat and maize, the production of legumes has either decreased or remained stagnant. Recently, various attempts have been made to increase protein availability in the diet by improving its nutritive value (2).

Germination has a marked effect on improving the nutritional quality of some legumes. A dramatic increase in the ascorbic acid content of legume seeds is observed during germination (3). The concentration of a number of other vitamins is also found high in germinated legume seeds (4).

The digestibility of legume proteins is relatively low due to the presence of antiphysiological factors (5) and structural characteristics (6, 7). Various attempts have been made to study the effect of germination on the reduction of antiphysiological factors and changes of the organic constituents in legumes in order to improve their nutritive value, but contradictory results are reported in the literature. The nutritional quality of proteins was found to increase during germination in soybean (8) and Mash bean (9), but decreased in navy beans (10), mung beans (11). and common beans (12), and was not affected in some pulses (13).

Chattopadhyay and Banerjee (14) showed an increment of the biological value in chickpea proteins during germination without any change in trypsin inhibitor activity, whereas no improvement of the biological value of chickpea proteins during germination was observed by Venkataraman *et al.* (15).

Chickpea is a notorious inducer of flatulence in human subjects due to the presence of raffinose and stachyose, and germination has an effect in reducing these components (16). During germination, a significant portion of starch is hydrolyzed (17). This is an added advantage of the process, because chickpea starch is known to affect the utilization of proteins to some extent (18).

Recent studies have shown that the polyphenolic compounds or tannins present in food grains interfere with protein digestion (19). Singh and Jambunathan (20) determined the tannin content in various cultivars of chickpea and their effect on the digestibility of proteins. These compounds are heat-resistant (19) but germination has a beneficial effect (21).

It appears that no systemat'c study has been carried out to establish th effect of germination on chickpea. The present work was therefore undertaken to determine any effect on the nutritive value and digestibility of chickpea proteins during germination. This study forms part of a general program of research on the preparation, processing and nutritional quality of an infant food, using chickpea as a protein source.

MATERIALS AND METHODS

Chickpea

The chickpea seeds used in the present study were grown in Guatemala

and obtained in the local market. The seed coat was light brown in color and accounted for 5.15o/o of the whole seed.

Germination of Seeds

The chickpea seeds were disinfected by treating them with a solution of 70o/o ethyl alcohol and 3o/o calcium chloride for two minutes. The treated seeds were thoroughly washed with tap water followed by distilled water, and soaked in distilled water for six hours at room temperature (25-27oC). They were then allowed to germinate for two and four days at room temperature in the dark in a plastic tray padded with wet filter paper. Distilled water was added occasionally to keep the seeds moist.

Processing of Chickpea Samples

The ungerminated chickpea was soaked in distilled water for six hours at room temperature (25-27oC) and the soaking water was discarded. The presoaked chickpea sample was divided into two portions: one portion was boiled for 40 min in fresh tap water, and the other was autoclaved at 15 psi for 15 min. The germinated chickpea, either for two or four days, was divided into three portions. The first portion was boiled, and the second one was autoclaved under identical conditions as in the case of ungerminated chickpea. The third portion was not subjected to any processing. The cooked chickpeas were separated from the broth by cloth filtration and the filtrate was discarded. All chickpea sampleas were then freeze-dried and stored in a cold room at −4oC until needed. The chickpeas were dehulled and the cotyledons ground into a powder in a Wiley mill to pass 40 mesh for experimental purposes.

Chemical Analyses

Moisture, ash, fat, crude fiber and nitrogen contents were determined by the AOAC methods (22). The crude protein content was calculated using a conversion factor of o/o N x 6.25; the carbohydrate was estimated by difference. The trypsin inhibitor activity was assayed by the method of Kakade *et al.* (23), and the tannin content was determined according to the method of Folin-Denis (24). Available lysine was established by the technique of Conkerton and Frampton (25) as modified by Carpenter (26), and nitrogen solubility index (NSI), according to the standard method of the AOCS (27).

Biological Assays

Weanling rats of the Wistar strain, from INCAP's animal colony, were distributed in groups consisting of four males and four females, each. The animals within each sex were randomly distributed by weight among the several experimental groups; the mean initial weight among the groups differed by not more than 1 g. The animals were kept in individual all-wire cages with raised screen bottoms. Diets and water were fed *ad libitum.* All chickpea samples were incorporated into a basal diet to provide 10o/o protein. The amounts varied from 45.98 to 49.76o/o,

depending on the protein content of the sample. The control diet (10%/o protein) was prepared using casein (11.31%/o) as the protein source. A group of rats was fed a protein-free diet. All diets were supplemented with cottonseed oil 5%/o, mineral mixture 4%/o (28), cod liver oil 1%/o, and sufficient cornstarch to adjust to 100. Five ml of a solution of vitamins (29) were added to each diet.

The experiment for the determination of net protein ratio (NPR) was conducted for a period of 10 days. The determination of protein digestibility was carried out at the end of the NPR experiment by keeping the rats in the same cages and offering the same experimental diets. Feces were collected daily for five days and stored in a cold room; the feces were dried, cleaned, and ground into a powder, and the nitrogen content was determined by the Kjeldahl method (22). NPR and protein digestibility were calculated using the standard formulae for protein foods (30).

RESULTS AND DISCUSSION

The cotyledon portion of the chickpea and those of various germinated and processed ones were analyzed for their proximate composition, as shown in Table 1. Fair agreement is apparent between the composition of cotyledons of ungerminated raw chickpea and those reported by Flores *et al.* (31). It can be seen that the protein content is slightly increased during germination. This may be due to the use of carbohydrate as a source of energy to support the germination process. A similar increase in protein content in other legume seeds during germination was obtained by Hsu *et al.* (32) and Fordham *et al.* (3). The results also show that tl e protein and ash contents in the processed samples are slightly lower than those in the unprocessed ones, probably due to losses in the cooking water, losses which were higher in the case of germinated seeds.

The trypsin inhibitor activity and tannin contents are reported in Table 2. The data indicate that germination of chickpea has little effect in reducing the trypsin inhibitor activity. Chattopadhyay and Banerjee (14) also reported no change of the trypsin inhibitor activity during germination of chickpea. However, in studies with other legume seeds, Subbulakshmi *et al.* (33) showed appreciable decrease of the trypsin inhibitor activity during germination. The trypsin inhibitor of chickpea is found to be heat labile, since boiling for 40 min causes about 80%/o destruction of the activity. But the heat sensibility of the inhibitor varies in germinated seeds, possibly due to synthesis of more heat-stable trypsin inhibitors during germination (34).

Apparently, no change in tannin content is observed during germination of chickpea. The chickpea samples, which were boiled for 40 min, contain a low level of tannins, presumably due to leaching out of the component in the soaking and cooking waters. Singh and Jambunathan (20) have found that tannin content varies with the varieties of chickpea and the color of the seed coat; nevertheless, the present results are found similar to those notified by Kahn *et al.* (35).

TABLE 1

PROXIMATE COMPOSITION OF THE COTYLEDON FLOUR PREPARED FROM GERMINATED AND PROCESSED CHICKPEA SAMPLES
(Dry weight basis)

Samples	Crude protein o/o	Crude fat o/o	Ash o/o	Crude fiber o/o	Carbohydrate (by difference) o/o
Chickpea (raw)	21.34	7.11	2.26	1.80	67.49
Chickpea (boiled)	21.15	7.08	1.55	2.01	68.21
Chickpea (autoclaved)	21.11	7.63	1.57	1.99	67.70
2-day germinated chickpea (raw)	22.68	7.57	2.23	1.97	65.55
2-day germinated chickpea (boiled)	21.50	7.91	1.27	1.85	67.47
2-day germinated chickpea (autoclaved)	21.86	7.29	1.54	1.81	67.50
4-day germinated chickpea (raw)	22.65	7.21	2.22	1.85	66.07
4-day germinated chickpea (boiled)	21.68	7.72	1.38	1.76	67.68
4-day germinated chickpea (autoclaved)	21.90	7.46	1.46	1.93	67.25

Results on the determination of nitrogen solubility index (Table 2) (NSI) indicate that the solubility of nitrogenous constituents in water is markedly increased during germination. This is probably due to the fact that during germination the proteolytic enzymes become active and hydrolyze the proteins to some extent into peptides and amino acids (36). The results also show that 2-day germinated seeds contain more soluble nitrogen than 4-day germinated ones. It has been reported that during germination, new proteins are synthesized from the degradation product of the reserve proteins, and the amino acid pattern of the newly formed proteins is different from that of the reserve proteins (37). The difference in solubility of the proteins in the 2-day and 4-day germinated seeds is probably due to differences in synthetic protein contents and their amino acid composition. The solubility of the nitrogenous constituents is greatly reduced when subjected to heat processing, and the NSI of autoclaved samples of both ungerminated and germinated seeds are almost similar. Boiling for 40 min is shown to be more pronounced in reducing the solubility of nitrogen in the case of germinated seeds.

The data in Table 2 also show that the availability of lysine decreased as germination progressed. Geervani and Theophilus (38) reported a decrease of available lysine even at 16 hr-germination of chickpea. Similar results were also obtained by Elías *et al.* (12) in the case of common beans. Autoclaving, under the conditions used in the present study,

TABLE 2

EFFECT OF GERMINATION AND PROCESSING OF CHICKPEA ON THE TRYPSIN INHIBITOR AND TANNIN CONTENTS

Days of germination	Processing conditions	Lysine g/16 g N	NSI o/o	Trypsin inhibitors TIU/ml extract*	Tannin (as tannic acid) o/o
0	0	7.8	63.4	4.3	0.87
0	Boiling for 40 min	7.9	26.2	1.0	0.67
0	Autoclaving at 15 psi for 15 min	6.8	25.4	0.9	0.71
2	0	7.4	84.4	3.0	0.81
2	Boiling for 40 min	7.1	21.1	2.7	0.61
2	Autoclaving at 15 psi for 15 min	6.4	29.9	1.5	0.77
4	0	7.0	67.1	3.6	0.97
4	Boiling for 40 min	7.2	18.1	1.0	0.55
4	Autoclaving at 15 psi for 15 min	6.8	24.4	0.0	0.77

* Trypsin units (TIU) as defined for the BAPA method.

exerted an effect in reducing the availability of lysine, probably due to an increased amount of free sugars in the case of the germinated seeds, which leads to an increased Maillard reaction, as compared to the ungerminated ones. A similar effect of autoclaving of chickpea was reported by Datta and Datta (39).

The values of net protein ratio (NPR) of ungerminated and germinated chickpea, with or without processing and of casein are compared in Table 3. Findings demonstrate that no significant increment of nutritive value of proteins occurred during germination or processing of chickpea. A slight increase in NPR value, however, was obtained in autoclaved ungerminated chickpea, boiled and autoclaved 2-day germinated chickpea, boiled 4-day germinated chickpea, and approached that of casein. These results are in agreement with those reported by Chandrasekhar and Jayalakshmi (13); they showed no significant improvement of PER during autoclaving or germination of chickpea. Jaya *et al.* (40) found that the PER value of 24-hr germinated both raw and cooked chickpea, and of 72-hr germinated cooked chickpea, were equal to the PER of casein, whereas 48-hr germinated both raw and cooked chickpea gave a significantly lower PER value. They also indicated that an ungerminated cooked sample gave equal PER to that of the best germinated cooked sample. Geervani and Theophilus (38) likewise, showed that there are no

TABLE 3

EFFECT OF GERMINATION ON THE NUTRITIVE VALUE OF CHICKPEA PROTEINS

Chickpea samples	Protein in diets o/o	Average diet intake g	Average weight gain g/10 days *	Net protein ratio NPR
Raw	9.51	109.4	26.7	$3.52^{b} \pm 0.40$
Boiled	10.96	111.6	34.9	$3.53^{b} \pm 0.16$
Autoclaved	10.25	115.5	35.4	$3.67^{ab} \pm 0.23$
2-day germinated (raw)	10.25	102.9	29.6	$3.58^{b} \pm 0.41$
2-day germinated (boiled)	10.25	102.9	29.6	$3.86^{ab} \pm 0.37$
2-day germinated (autoclaved)	10.38	115.9	37.0	$3.75^{ab} \pm 0.24$
4-day germinated (raw)	10.28	96.7	27.7	$3.60^{b} \pm 0.32$
4-day germinated (boiled)	10.26	113.5	34.5	$3.67^{ab} \pm 0.26$
4-day germinated (autoclaved)	10.50	109.0	32.8	$3.58^{b} \pm 0.22$
Casein (control)	10.13	114.1	39.1	$4.08^{a} \pm 0.22$

* Initial weight = 45.0 g.
Means carrying the same superscript are not significantly ($p > 0.05$) different. (Mean ± SD).

s'gnificant differences between the PER of raw ungerminated and germina ed chickpea, whereas boiling or autoclaving improves PER significantly.

The apparent a d true d gestibilities of proteins in raw and processed chick ea for the ungerminated and germinated samples are given in Table

The pro e' s' d gest bility in the raw samples showed a progressive i cr ase with g rmin t'on time. Heat processing (boiling or autoclaving) 'ncre sed it s'gnificantly in both ungerminated and germinated chickpea, as com ared to t e corresponding values of the unprocessed samples. The o'l ge m'na e.l samples gave better results in protein digestibility as compared to autocla ed samples.

The results of the present study correlate well with those reported by Geerv.ni nd Theophilus (38). On the contrary, Venkataraman *et al.* (15) found that cooking of ungerminated and germinated chickpea caused a i ificant reduction of digestibility coefficients as compared to that of the ungerminated uncooked chickpea.

In conclusion, it can be stated that the 2-day germinated and boiled product can be considered the best one from the nutritional point of

TABLE 4

EFFECT OF GERMINATION ON THE DIGESTIBILITY OF CHICKPEA PROTEINS

Chickpea samples	Apparent digestibility (AD)	True digestibility (TD)
Raw	$69.65^{g} \pm 2.32$	$72.61^{e} \pm 2.34$
Boiled	$73.35^{ef} \pm 1.59$	$75.69^{de} \pm 1.91$
Autoclaved	$73.81^{ef} \pm 2.86$	$76.06^{d} \pm 2.90$
2-day germinated (raw)	$72.58^{fg} \pm 1.20$	$75.07^{de} \pm 1.50$
2-day germinated (boiled)	$77.53^{bcd} \pm 2.53$	$80.01^{bc} \pm 2.55$
2-day germinated (autoclaved)	$76.25^{cde} \pm 1.50$	$78.35^{cd} \pm 1.65$
4-day germinated (raw)	$75.47^{def} \pm 1.63$	$78.01^{cd} \pm 1.55$
4-day germinated (boiled)	$80.26^{b} \pm 1.99$	$82.38^{b} \pm 1.99$
4-day germinated (autoclaved)	$78.86^{bc} \pm 2.24$	$81.13^{bc} \pm 2.15$
Casein (control)	$87.71^{a} \pm 1.18$	$89.98^{a} \pm 1.00$

In each vertical column, means carrying the same superscript are not significantly ($p > 0.05$) different. (Mean ± SD).

view. Furthermore, this heating process is advantageous from the technological and economical points of view.

RESUMEN

ESTUDIOS SOBRE EL DESARROLLO DE ALIMENTOS INFANTILES A BASE DE FUENTES DE PROTEINA VEGETAL. I. PARTE. EFECTO DE LA GERMINACION DEL GARBANZO *(Cicer arietinum)* EN EL VALOR NUTRITIVO Y LA DIGESTIBILIDAD DE LAS PROTEINAS

Con el propósito de desarrollar un alimento para niños con características superiores de dispersibilidad y de alto valor nutritivo, se aplicaron al garbanzo *(Cicer arietinum)* diferentes tratamientos y tecnologías de procesamiento.

Lotes de garbanzo fueron germinados durante dos y cuatro días a temperatura ambiente (25-27°C). Una porción correspondiente a cada período de germinación se hirvió durante 40 min y otra porción se sometió a cocción en el autoclave a 15 lb/pulgada cuadrada durante 15 min.

Luego, estas muestras procesadas se compararon con los valores correspondientes de muestras crudas germinadas y no germinadas, así como también con los valores de muestras no germinadas, procesadas, con relación a las siguientes características: composición química proximal, contenido de factores antifisiológicos, solubilidad del nitrógeno, disponibilidad de lisina, razón proteínica neta (NPR) y digestibilidad de la proteína. La germinación indujo un aumento en el contenido de proteína de las semillas. No se observaron cambios apreciables en el contenido de inhibidores de tripsina y de taninos debido a la germinación. El contenido de lisina disponible fue

ligeramente más bajo en las semillas germinadas. La solubilidad de los compuestos nitrogenados aumentó significativamente durante la germinación. El proceso de germinación con el procesamiento térmico no indujo cambios favorables, que mejorasen la calidad proteínica; sin embargo, la digestibilidad de las proteínas aumentó. El proceso de ebullición fue más favorable para las semillas germinadas que la cocción en el autoclave, mientras que lo inverso ocurrió en el caso de las semillas no germinadas.

BIBLIOGRAPHY

1. Aykroyd, W. R. & J. Doghty. **Legumes in Human Nutrition.** Rome, FAO, 1964. (FAO Nutritional Studies No. 19).
2. Parpia, H. A. B. Utilization problems in food legumes. In: **Nutritional Improvement of Food Legumes by Breeding. Proceedings of a Symposium sponsored by PAG, held at the Food and Agriculture Organization, Rome, Italy, 3-5 July, 1972.** M. Milner (Ed.). New York, N.Y., The Protein Advisory Group, 1973, p. 281-295.
3. Fordham, J. R., C. E. Wells & L. H. Chen. Sprouting of seeds and nutrient composition of seeds and sprouts. **J. Food Sci., 40:552-556, 1975.**
4. Chen, L. H., C. E. Wells & J. R. Fordham. Germinated seeds for human consumption. **J. Food Sci., 40:1290-1294, 1975.**
5. Bressani, R. & L.G. Elías. The problem of legume protein digestibility: In: **Nutritional Standards and Methods of Evaluation for Food Legume Breeders.** L. W. Billingsley (Ed.). Ottawa, Canada, International Development Research Centre, 1977, p. 61-72 (IDRC-TSTe).
6. Liener, I. W. Legume toxins in relation to protein digestibility. A review. **J. Food Sci., 41:1076-1081, 1976.**
7. Wallace, G. M., W. R. Bannatyne & A. Khaleque. Studies on the processing and properties of soymilk. II. Effect of processing conditions on the trypsin inhibitor activity and the digestibility *in vitro* of proteins in various soymilk preparations. **J. Sci. Food Agric., 22:526-531, 1971.**
8. Everson, G. J., H. Steenbock, D. C. Cederquist & H. T. Parsons. The effect of germination, the stage of maturity, and the variety upon the nutritive value of soybean protein. **J. Nutr., 27:225-229, 1944.**
9. Khan, M. A. & A. Ghaffor. The effect of soaking, germination and cooking on the protein quality of Mash beans *(Phaseolus mungo).* **J. Food Sci. Food Agric., 29:** 461-464, 1978.
10. Kakade, M. L. & R. J. Evans. Effect of soaking and germinating on the nutritive value of navy beans. **J. Food Sci., 31:781-783, 1966.**
11. Noor, I., R. Bressani & L. G. Elías. Changes in chemical and selected biochemical components, protein quality and digestibility of mung bean *(Vigna radiata)* during germination and cooking. **Qual. Plant. Plant Foods Hum. Nutr., 30:135-144, 1980.**
12. Elías, L. G., A. Conde, A. Muñoz & R. Bressani. Effect of germination and maturation on the nutritive value of common beans *(Phaseolus vulgaris).* In: **Nutritional Aspects of Common Beans and Other Legume Seeds as Animal and Human Foods.** Proceedings of a Meeting held in Riberão Preto, November, 1973. W. G. Jaffé (Ed.). Caracas, Archivos Latinoamericanos de Nutrición, 1975, p. 139-152.
13. Chandrasekhar, U. & K. Jayalakshmi. Evaluation of protein quality of sprouted, roasted and autoclaved legumes on albino rats. **Ind. J. Nutr. Dietet., 15:414-421, 1978.**

14. Chattopadhyay, H. & S. Banerjee. Effect of germination on the biological value of proteins and the trypsin-inhibitor activity of some common Indian pulses. **Ind. J. Med. Res.**, 41:185-189, 1953.
15. Venkataraman, L. V., T. V. Jaya & K. S. Krishnamurthy. Effect of germination on the biological value, digestibility coefficient and net protein utilization of some legume proteins. **Nutr. Reps. Internat.**, 13:197-205, 1975.
16. Aman, P. Carbohydrates in raw and germinated seeds from mung bean and chick pea. **J. Sci. Food Agric.**, 30:869-875, 1979.
17. Jaya, T. V. & L. V. Venkataraman. Changes in the carbohydrate constituents of chickpea and greengram during germination. **Food Chem.**, 7:95-104, 1981.
18. Geervani, P. & F. Theophilus. Influence of legume starches on protein utilization and availability of lysine and methionine to albino rats. **J. Food Sci.**, 46:817-818, 1981.
19. Elías, L. G., D. G. de Fernández & R. Bressani. Possible effects of seed coat polyphenolics on the nutritional quality of bean proteins. **J. Food Sci.**, 44:524-527, 1979.
20. Singh, U. & R. Jambunathan. Studies on Desi and Kabuli chickpea *(Cicer arietinum* L.) cultivars: levels of protease inhibitors, levels of polyphenolic compounds and **in vitro** protein digestibility. **J. Food Sci.**, 46:1364-1367, 1981.
21. Chavan, J. K., S. S. Kadam & D. K. Salunkhe. Changes in tannin, free amino acids, reducing sugars, and starch during seed germination of low and high tannin cultivars of sorghum. **J. Food Sci.**, 46:638-639, 1981.
22. Association of Official Agricultural Chemists. **Official Methods of Analysis of the AOAC.** 12th ed. Washington, D. C., The Association, 1975.
23. Kakade, M. L., N. Simons & I. E. Liener. An evaluation of natural vs. synthetic substrates for measuring the antitryptic activity of soybean samples. **Cereal Chem.**, 46:518-526, 1969.
24. Joslyn, M. A. **Methods of Food Analysis.** 2nd. ed. New York, N. Y., Academic Press Inc., 1970.
25. Conkerton, E. J. & V. L. Frampton. Reaction of gossypol with free-amino groups of lysine in protein. **Arch. Biochem. Biophys.**, 81:130-134, 1959.
26. Carpenter, K. J. The estimation of available lysine in animal-protein foods. **Biochem.**, 77:604-610, 1960.
27. American Oil Chemists Society. **Official and Tentative Methods of the AOCS.** Rev. 1969, corrected 1979. (Nitrogen solubility index - Ba. 11-65). Chicago, Illinois, The Society.
28. Hegsted, D. M., R. C. Mills, C. A. Elvehjem & E. B. Hart. Choline in the nutrition of chicks. **J. Biol. Chem.**, 138:459-466, 1941.
29. Manna, L. & S. M. Hauge. A possible relationship of vitamin B_{13} to orotic acid. **J. Biol. Chem.**, 202:91-96, 1953.
30. Pellett, P. L. & V. R. Young (Eds.). **Nutritional Evaluation of Protein Foods.** Tokyo, Japan, The United Nations University World Hunger Programme, 1980. (Food and Nutrition Bulletin Supplement 4).
31. Flores, M., Z. Flores, B. García & Y. Gularte. **Tabla de Composición de Alimentos de Centro América y Panamá.** 4th ed. Guatemala, Guatemala, Instituto de Nutrición de Centro América y Panamá (INCAP), 1960.
32. Hsu, D., H. K. Leung, P. L. Finney & M. M. Morad. Effect of germination on nutritive value and baking properties of dry peas, lentils, and faba beans. **J. Food Sci.**, 45:87-92, 1980.
33. Subbulakshmi, G., K. GaneshKumar & L. V. Venkataraman. Effect of germination on the carbohydrates, proteins, trypsin inhibitor, amylase inhibitor and hemag-

glutinin in horsegram and mothbean. **Nutr. Reps. Internat., 13:19-31, 1976.**
34. Pusztai, A. Metabolism of trypsin inhibitory proteins in the germinating seeds of kidney beans *(Phaseolus vulgaris).* **Planta (Berl.)., 107:121, 1972.**
35. Khan, M. A., I. Jacobsen & B. O. Eggum. Nutritive value of some improved varieties of legumes. **J. Sci. Food Agric., 30:395-400, 1979.**
36. Beevers, L. Protein degradation and proteolytic activity in the cotyledons of germinating pea seeds *(Pisum sativum).* **Phytochemistry, 7:1837, 1968.**
37. Chen, L. H. & R. Thacker. Germination and nitrogenous constituents of pea seeds *(Pisum sativum).* **J. Food Sci., 43:1884-1885, 1978.**
38. Geervani, P. & F. Theophilus. Effect of home processing on the protein quality of selected legumes. **J. Food Sci., 45:707-710, 1980.**
39. Datta, S. & S. C. Datta. Available lysine in cooked pulses. **Ind. J. Nutr. Dietet., 15:128-130, 1978.**
40. Jaya, T. V., K. S. Krishnamurthy & L. V. Venkataraman. Effect of germination and cooking on the protein efficiency ratio of some legumes. **Nutr. Reps. Internat.. 12:175-183, 1975.**