PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 8 (4): 408-413, 2009
ISSN 1680-5194

Biological Evaluation of Protein Meals for Making Nutrient Dense Food Bar

Omer Mukhtar Tarar[1], Salim-ur-Rahman[1], Tahir Zahoor[1], Khalid Jamil[2] and Aamer Jamil[3]
[1]National Institute of Food Science and Technology, [3]Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad, Pakistan
[2]Food and Marine Resources Research Center, PCSIR Labs. Complex, Karachi, Pakistan

Abstract: The study was undertaken for biological evaluation of protein meals, differing in raw or processed chickpea (*Cicer arietinum* L.) and vetch (*Lathyrus sativus* L.) flour for nutritionally rich food bar making. The indigenous food processing technologies such as controlled and natural fermentation and germination were utilized to improve the protein quality of legumes. The resultant flours were thereafter blended with other protein sources to produce balanced protein meal. The protein quality of these diets was assessed by implying *in-vivo* rat assays. The values for relative protein efficiency ratio (RPER) and relative net protein ratio (RNPR) in close proximity to each other for processed meals are an indicator of good protein quality of these meals. It could be concluded that food bars with good protein quality can be produced by using meals carrying processed vetch or chickpea.

Key words: Germination, controlled fermentation, RPER, RNPR

INTRODUCTION

In Pakistani diet, chickpea (*Cicer arietinum* L.) is one of the popular legumes. Its supplementation to wheat flour at different levels has been of extensive interest (Awan *et al.,* 1996). It contains protein with high lysine and threonine contents. However similar to other legumes it has low digestibility and carries certain negative factors such as tannins, trypsin inhibitors, phytates, and hemagglutinins (Bressani, 1993).

Vetch (*Lathyrus sativus* L.*)* is one of the cheapest legumes rather least investigated source with high protein portion. It is grown in Pakistan, India, Bangladesh, Nepal, China, Middle East, France, Spain, Australia and some African countries (Hanbury *et al.,* 1999). Vetch is also rich in lysine. In addition to other antinutritional factors, the presence of the neurotoxin, β-N-oxalyl- α,β-diaminopropionic acid (β-ODAP), is a concern for public health as well as a barrier to the utility of vetch (Yigzaw *et al.*, 2004). After depleting its toxic factors, its supplementation in wheat flour would help to improve the nutritional quality of the products (Lodhi *et al.,* 2003). The use of indigenous food processing technologies such as fermentation (Sarkar *et al.*, 1997) and germination (Donangelo *et al.*, 1995) could reduce the antinutritional factors in legumes.

Increasing demand for nutritious meals and snacks, have prompted the food researchers to develop new products that combine convenience and nutrition (Izzo and Niness, 2001). Food bars are the snack foods of good sensory characteristics due to their nutrient combinations. One of the strategies to produce the food bars with good protein quality is cereal-legume complementation (Hernandez *et al.*, 1995).

Biological evaluation is the best tool for judging the quality of protein, since numerous factors decide the ultimate *in-vivo* quality of the protein. Chickpeas and field peas have shown better biological value than some popular legumes through the rat balance method (Sarwar *et al.,* 1989). Conversely studies on nutritive value of fermented or germinated chickpea and vetch flours supplemented food meals are limited. The present study was undertaken for biological evaluation of the protein qualities of food meals differing in raw or processed chickpea and vetch flours components.

MATERAIALS AND METHODS

Chickpea, vetch, wheat flour, farina and other Ingredients were purchased from local market. Casein (M/S Sigma Chemical Co., St. Louis, MO, USA) was procured from local scientific store. Mineral and vitamins mixes were prepared from analytical grade chemicals.

Germination of legume seeds: Vetch and chickpea seeds were germinated according to procedure described by Khalil *et al.* (2007).

Controlled fermentation of legume flours: Fermentation of legume flours by thermophilic lactic culture (a mixed strain culture containing *Lactobacillus helveticus* and *Streptococcus thermophilus*) was carried out as suggested by Doblado *et al.* (2007).

Natural fermentation of legume flours: Legume flours were fermented naturally following the method proposed by Martin-Cabrejas *et al.* (2004).

Preparation of meals: The protein meal formulation on the basis of cereal-legume complementation is shown in Table 1. The standard (casein) and test protein diets were adjusted to 10% protein, whereas non protein diet was devoid of protein. The fat level of test diet was analysed to be 9%, to attain the same level for standard and control (protein free) diets, refined corn oil (Rafhan Maize Industries, Faisalabad) was added. Basic meal, legume meal and whey protein concentrate were blended to give a uniform mixture. Thereafter these were baked at 149°C for 20 min (PCSIR Drying Oven, DO-1-30/02, PCSIR Labs., Lahore, Pakistan) to simulate the processing steps to which the protein blends were about to be subjected during food bar preparation (Brisske *et al.*, 2004). Then other ingredients were added and pellets prepared by adding little water.

Biological assay: Biological evaluation was done by measuring the protein quality of diets containing protein blends as per treatments (Table 1). Albino rats of the *Sprague-Dawley* strain were used. Weaning was done at 21 days of age. The rats were then put on stock diet for 7 days prior to the experiment. They were arbitrarily divided into experimental units of 3 rats each in such a way that the initial weight of the rats in each cage was 135g; 2 experimental units were randomly allotted to each diet. The rats were fed the allotted diet *ad libitum* for a period of 14 days. During this period fresh, clean water was made available at all times and room temperature was maintained at 24-27°C. The weight of each replicate was recorded weekly. The faecal matter from each cage was collected daily, dried to a constant weight and stored in glass bottles for nitrogen determination (Miller and Bender, 1955).

Nutritional indices: The amount of the feed given on dry weight basis was computed, based on moisture content while moisture free refusal was obtained by drying overnight at 100°C. The difference between diet given and refusals was taken as feed intake, which was further used to calculate protein intake. Feed intake, protein intake and body weight gain were used to compute the following nutritional indices:

Feed efficiency (FE) = Gain in body wt (g)/Feed intake (g)

Feed utilization (FU) = Feed intake (g)/Gain in body wt (g)

Protein efficiency ratio (PER) = Gain in body wt (g)/Protein intake (g)

Corrected protein efficiency ratio (C-PER) = PER of Casein (2.5) x PER of Test Protein/Exp. PER of casein

Protein utilisation (PU) = Protein intake (g)/Gain in body wt (g)

Net protein ratio (NPR) = (weight gain of test rat/weight loss of non protein rat)/protein consumed by test rat

Relative PER (RPER) and relative NPR (RNPR) values calculated using the following equations (Sarwar and Peace, 1994):

RPER = (PER of test diet/PER of control diet) x 100

RNPR = (NPR of test diet/NPR of control diet) x 100

RESULTS AND DISCUSSION

Feed/protein intake: The mean feed intake was 165.0 g for casein group followed by G-CBM (152.5g) and G-VBM (141.9g), on a dry weight basis, respectively during test period (Table 2). It was observed that the casein diet was consumed maximally, followed by the germinated vetch and chickpea flour blended meals while the non-protein diet was consumed the least (99.6g). Statistical analysis revealed that there was no significant difference between feed intake of germinated and controlled fermented flour blended meals. Among test proteins, the feed intake of raw vetch and chickpea flour blended meals groups of rats was the minimum and did not differ significantly while that of N-VBM closely followed these. Protein intake of casein and G-CBM diet groups was maximum (16.42 and 15.19g, respectively) and did not differed significantly among them followed by G-VBM, C-CBM and N-CBM diet groups. There are certain factors which affect the feed intake by the rats. These factors include the amino acid balance of diet (Peters and Harper, 1985), fat contents (LeMagnen, 1983), saccharose contents and antinutritional factors (Nestares *et al.*, 1996). The higher intake of casein diet could thus be attributed to the saccharose content and absence of any nutritional factor.

Weight gain: The average body weight gain in 14 days was found maximum for standard diet (14.42g). It was followed by G-CBM and G-VBM (42.04 and 37.91g, respectively) (Table 2). In contrast average loss of weight was 16.50g in the non-protein diet. Statistical analysis revealed that weight gain for the casein diet was significantly higher than for all the test proteins ($P < 0.05$). The weight gain was significantly lower for both raw flour groups and N-VBM. It could be attributed to the antinutritional factors and toxic constituents present in the legumes (Mortuza *et al.*, 2000). The other factors responsible for low weight gain in diets with raw legume flours are the low sulfur amino acid content and low digestibility of the protein (Huyghebaert *et al.*, 1979) and carbohydrate content (Mercier, 1979).

Feed efficiency: Efficiency could be defined as the gain in body weight per unit feed intake. Results showed that the feed efficiency was 0.304 for casein closely followed by G-CBM (0.276) (Table 3). Statistical analysis showed that test diets had significantly lower feed efficiencies than casein. Whereas minimum feed efficiency (0.213) was observed for R-VBM diet. Among the test diets, R-CBM and N-VBM also showed significantly lower feed efficiencies ($P < 0.05$).

Feed utilization: The ratio of feed intake to gain in body weight is called feed utilization. Results revealed good feed utilization in casein (3.29) followed by G-CBM (3.63), G-VBM (3.74), C-CBM (3.89). C-VBM and N-CBM also possessed reasonable feed utilization values, not differing from each other (3.98 and 3.99, respectively). Whereas minimum feed utilization, was studied for R-VBM, followed by R-CBM.

Protein utilization (PU): Protein utilization is ratio of protein intake to gain in body weight. The results revealed that the PU value for G-CBM (0.361) was in close proximity to casein (0.327) having maximum value. The statistical difference for PU values in the test protein diets and casein was observed and the minimum protein utilization was studied for R-VBM.

Protein efficiency ratio (PER): Protein efficiency ratio is gain in body weight per unit protein intake. The results revealed that the PER value for casein was 3.06 followed by G-CBM (2.77), G-VBM (2.69) and C-CBM (2.58) (Fig. 1). The close PER values for test diets to casein indicates adequate combination of essential amino acids in test diets. Plant proteins are categorized into three groups for PER, high PER (< 2.3 to > 1.6), medium PER (< 1.6 to >1.0) and low PER (< 1.0). The present study revealed that all the test and standard protein diets had high PER (Hsu *et al.,* 1978).

Net protein retention (NPR): Net protein retention is defined as the ratio of sum of weight gain of test protein group and weight loss of non protein group to that of protein intake of test protein group. The results revealed that the NPR value for both germinated bar meals (3.85) was in close proximity to casein (4.06) having maximum net protein retention value (Fig. 1). The statistical difference for PU values in the test protein diets and casein was established from data. However controlled and natural fermentation of both legume flour bar meal diets showed non significant difference among them. The enzymatic (proteases) breakdown of legume proteins during germination leads to the formation of polypeptides, oligopeptides and free amino acids. It may contribute towards improvement of nutritional indices of diets and meals carrying germinated legumes (Nielsen, 1991). Moreover germination lowers the levels of antinutritional factors e.g. trypsin inhibitors, tannins and phytates and reduces the phyto-haemagglutinating activity (Gupta and Sehgal, 1991).

Corrected protein efficiency ratio (C-PER): Corrected protein efficiency ratio is defined as ratio of PER of test protein to that of standard protein multiplied by standard value of reference proteins. The standard PER of casein is suggested as 2.5 (Chapman and Mitchell, 1959). The corrected PER for meal diets with germinated legume flours was in good correlation with standard diet (Table 4). The C-PER values of G-VBM and G-CBM indicated that these bar meals contained high quality proteins. The meal diet with raw vetch flour ranked at the bottom of the C-PER ranking order with value of 1.75. Statistical analysis revealed that standard protein had significant higher C-PER than test protein diets. The test proteins also differed highly significantly among themselves.

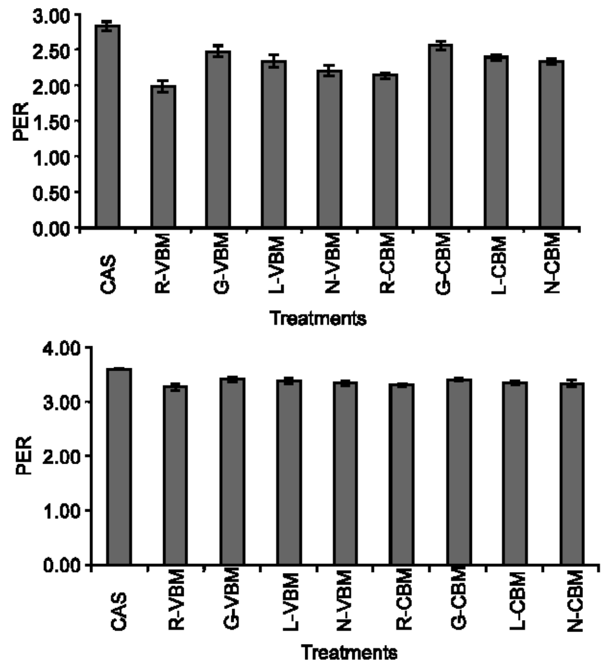

Fig. 1: Protein efficiency ratio (PER) and net protein retention (NPR) in rats for given meals.

CAS = Casein, R-VBM = Raw vetch flour bar meal, C-VBM = Controlled fermented vetch flour bar meal, N-VBM = Naturally fermented vetch flour bar meal, G-VBM = Germinated vetch flour bar meal, R-CBM = Raw chickpea flour bar meal, C-CBM = Controlled fermented chickpea flour bar meal, N-CBM = Naturally fermented chickpea flour bar meal, G-CBM = Germinated chickpea flour bar meal.

Relative protein efficiency ratio (RPER) and Relative net protein ratio (RNPR): Net protein ratio (NPR) and relative NPR (RNPR) methods were considered to be improvements over the protein efficiency ratio (PER) method for measuring the protein quality of foods (Sarwar and McDonough, 1990). These methods do not always rank the proteins in the same order as the PER method. RNPR values for poor quality proteins were much higher than the RPER values (Mitchell *et al.*, 1989). However in this study high quality protein sources i.e. processed flour blends, either carrying germinated or fermented legume flours agreed closely. RPER and RNPR values for meal diets with raw vetch flour (R-VBM) were 70 and 91 respectively with difference of 21 whereas for meal diets with raw chickpea flour (R-CBM) difference little reduced (about 16). On the basis of these

Table 1: Experimental treatments for protein meal formulation

	(%) Treatments							
Components	R-VBM	G-VBM	C-VBM	N-VBM	R-CBM	G-CBM	C-CBM	N-CBM
Raw vetch flour	7.7	-	-	-	-	-	-	-
Controlled fermented vetch flour	-	7.7	-	-	-	-	-	-
Natural fermented vetch flour	-	-	7.8	-	-	-	-	-
Germinated vetch flour	-	-	-	7.8	-	-	-	-
Raw chickpea flour	-	-	-	-	8.2	-	-	-
Controlled fermented chickpea flour	-	-	-	-	-	8.2	-	-
Natural Fermented chickpea flour	-	-	-	-	-	-	8.2	-
Germinated chickpea flour	-	-	-	-	-	-	-	8.2
Basic meal	24.4	24.4	24.4	24.4	25.7	25.7	25.7	25.7
Whey protein concentrate	5.2	5.2	5.2	5.2	5.5	5.5	5.5	5.5
Cellulose	5	5	5	5	5	5	5	5
Salt mixture	5	5	5	5	5	5	5	5
Vitamin mixture	2	2	2	2	2	2	2	2
Corn starch	sufficient for 100	sufficient for 100	sufficient for 100	sufficient for 100	sufficient for 100	sufficient for 100	sufficient for 100	sufficient for 100

*Basic meal comprises of rusk crumb, roasted patent wheat flour and farina in the ratios of 24:4.5:4.5. R-VBM = Raw vetch flour bar meal, C-VBM = Controlled fermented vetch flour bar meal, N-VBM = Naturally fermented vetch flour bar meal, G-VBM = Germinated vetch flour bar meal, R-CBM = Raw chickpea flour bar meal, C-CBM = Controlled fermented chickpea flour bar meal, N-CBM = Naturally fermented chickpea flour bar meal, G-CBM = Germinated chickpea flour bar meal.

Table 2: Feed intake, protein intake and weight gain values in rats for given meals

Treatments	Feed intake g/rat/14days	Protein intake g/rat/14days	Weight gain g/rat/14days
CAS	165.0 (±5.29)a	16.42 (±0.53)a	50.16 (±0.51)a
R-VBM	106.7 (±6.35)f	10.63 (±0.65)f	22.76 (±0.37)g
G-VBM	141.9 (±7.81)bc	14.13 (±0.80)bc	37.91 (±1.12)c
C-VBM	128.8 (±7.08)cde	12.82 (±0.72)cde	32.34 (±0.65)e
N-VBM	119.8 (±6.65)def	11.91 (±0.63)def	28.41 (±0.56)f
R-CBM	116.6(±7.58)ef	11.59 (±0.79)ef	26.78 (±1.38)f
G-CBM	152.5 (±8.25)ab	15.19 (±0.78)ab	42.04 (±1.18)b
C-CBM	139.1 (±9.96)bcd	13.82 (±0.99)bcd	35.72 (±2.10)cd
N-CBM	133.5 (±12.4)bcde	13.28 (±1.29)bcde	33.48 (±2.72)de
CNT	99.6(±7.95)fg	0.74(±0.09)g	-16.503(±1.29)h

Means followed by different letters, within a column, are significantly different ($P < 0.05$). CAS = Casein, R-VBM = Raw vetch flour bar meal, C-VBM = Controlled fermented vetch flour bar meal, N-VBM = Naturally fermented vetch flour bar meal, G-VBM = Germinated vetch flour bar meal, R-CBM = Raw chickpea flour bar meal, C-CBM = Controlled fermented chickpea flour bar meal, N-CBM = Naturally fermented chickpea flour bar meal, G-CBM = Germinated chickpea flour bar meal, CNT = No protein

Table 3: Feed efficiency, feed utilization and protein utilization values in rats for given meals

Treatments	Feed efficiency	Feed utilization	Protein utilization
CAS	0.304(±0.007)a	3.29(±0.07)f	0.327(±0.007)f
R-VBM	0.213(±0.009)g	4.69(±0.20)a	0.467(±0.021)a
G-VBM	0.267(±0.006)c	3.74(±0.10)de	0.373(±0.011)de
C-VBM	0.251(±0.009)e	3.98(±0.14)cd	0.396(±0.015)cd
N-VBM	0.237(±0.008)f	4.22(±0.15)bc	0.419(±0.014)bc
R-CBM	0.230(±0.003)f	4.35(±0.06)b	0.433(±0.007)b
G-CBM	0.276(±0.007)b	3.63(±0.09)e	0.361(±0.008)e
C-CBM	0.257(±0.003)cd	3.89(±0.05)d	0.387(±0.005)de
N-CBM	0.251(±0.003)de	3.99(±0.05)cd	0.397(±0.006)cd

Means followed by different letters, within a column, are significantly different ($P < 0.05$).

Table 4: Corrected protein efficiency ratio (C-PER), Relative protein efficiency ratio (RPER) and Relative net protein retention (RNPR) in rats for given meals

Treatments	C-PER	RPER	RNPR
CAS	2.50(±0.00)a	100(±0.14)a	100(±0.14)a
R-VBM	1.75(±0.04)h	70(±1.70)h	91(±1.14)d
G-VBM	2.20(±0.01)c	88(±0.57)c	95(±0.89)b
C-VBM	2.07(±0.04)e	83(±1.27)e	94(±1.27)bc
N-VBM	1.95(±0.02)f	78(±0.99)f	93(±0.78)bcd
R-CBM	1.89(±0.01)g	76(±0.28)g	92(±0.28)cd
G-CBM	2.27(±0.01)b	91(±0.21)b	95(±0.57)b
C-CBM	2.12(±0.02)d	85(±0.71)d	93(±0.35)bcd
N-CBM	2.06(±0.01)e	83(±0.35)e	93(±1.13)bcd

Means followed by different letters, within a column, are significantly different ($P < 0.05$).

criteria, G-CBM ranked as best quality meal with difference of 4 followed by G-VBM, having difference of 7 between RPER and RNPR values.

Conclusion: The biological evaluation of protein meals showed that indigenous processing technologies improved the protein quality of legumes. The processed flours blended with other protein sources produced nutritionally balanced protein meals. On the basis of nutritional indices, germinated legume flours (germinated vetch flour bar meal and germinated chickpea flour bar meal) ranked as the best quality meals closely followed by controlled fermented legume flours. It could be thus concluded that food bars with good protein quality can be produced by incorporating these processed vetch or chickpea meals.

REFERANCES

Awan, J.A., M.I. Siddique and A.H. Gilani, 1996. Fortification of wheat flour. Pak. J. Food Sci., 6: 39-50.

Bressani, R., 1993. Grain quality of common beans. Foods Rev. Int., 9: 237-297.

Brisske, L.K., S.Y. Lee, B.P. Klein and K.R. Cadwallader, 2004. Development of a prototype high-energy, nutrient-dense food product for emergency relief. J. Food Sci., 69: S361-S367.

Chapman, G. and H.H. Mitchell, 1959. Evaluation of protein in foods: 1-A Method for the determination of protein efficiency ratio. Canad J. Biochem. Phys., 37: 679.

Doblado, R., J. Frias, R. Munoz and C. Vidal-Valverde, 2003. Fermentation of *Vigna sinensis* var. *Carilla* flours by natural microflora and *lactobacillus* species. J. Food Prot., 12: 2313-2320.

Donangelo, C.M., L.C. Trugo, M.F. Trugo and B.O. Eggum, 1995. Effect of germination of legume seeds on chemical composition and on protein and energy utilization in rats. Food Chem., 53: 23-27.

Gupta, C. and S. Sehgal, 1991. Development, acceptability and nutritional value of weaning mixtures. Plant Foods Hum. Nutr., 41: 107-116.

Hanbury, C.D., K.H.M. Siddique, N.W. Galwey and P.S. Cocks, 1999. Genotype -environment interaction for seed yield and ODAP concentration for *Lathyrus sativus L.* and *L. cicera L.* in Mediterranean-type environments. Euphytica, 110: 45-60.

Hernandez, M., I. Montalvo, V. Sousa and A. Sotelo, 1995. The protein efficiency ratios of 30:70 mixtures of animal: vegetable protein are similar or higher than those of the animal foods alone. J. Nutr., 126: 574-581.

Hsu, H.W., N.E. Sutton, M.O. Banjo, L.D. Satterlee and J.G. Kendrick, 1978. The C-PER and T-PER assay of protein quality. Food Tech., 32: 69-73.

Huyghebaert, G., G. Fontaine and G. de Groote, 1979. Broad bean (*Vicia faba*) as an alternative protein source in feed for broiler chicks. 1. The effect of different thermomechanical treatment. Rev. Agric., 32: 1243-1255.

Izzo, M. and K. Niness, 2001. Formulating nutrition bars with inulin and oligofructose. Cereal Foods World, 46: 102-106.

Khalil, A.W., A. Zeb, F. Mahmood, S. Tariq, A.B. Khattak and H. Shah, 2007. Comparison of sprout quality characteristics of desi and kabuli type chickpea cultivars (*Cicer arietinum* L.). LWT-Food Sci. Tech., 40: 937-945

Le Magnen, G., 1983. Body energy balance and food intake: a neuroendocrine regulatory mechanism. Physiol. Rev., 63: 314-386.

Lodhi, S.A., S. Rehman and N. Huma, 2003. Effect of supplementation of detoxified matri flour on the quality of pan bread. Pak. J. Sci. Ind. Res., 46: 207-210.

Martin-Cabrejas, M.A., B. Sanfiz, A.Vidal, E. Molla, R. Esteban and F.J. Lopez-Andreu, 2004. Effect of fermentation and autoclaving on dietary fiber fractions and antinutritional factors of beans (*Phaseolus vulgaris* L.). J. Agric. Food Chem., 52: 261-266.

Mercier, C., 1979. The á-galactosides of legume seeds. In “Matieres Premieres et Alimentation des Volailles”. Institut National de la Recherche Agronomique, Versailles, France, pp: 79-90.

Miller, D.S. and A.E. Bender, 1955. The determination of the net utilization of proteins by a shortened method. Br. J. Nutr., 9: 382-388.

Mitchell, G.V., M.Y. Jenkins and E. Grundel, 1989. Protein efficiency ratios and net protein ratios of selected protein foods. Plant Foods Hum. Nutr., 39: 55-58.

Mortuza, M.G., N. Newaz, M.A. Hossain and M.H. Rahman, 2000. Nutritional response of rats to faba beans (*Vicia faba* L. minor black 'Kalimattar') and its fractions. Int. J. Food Sci. Nutr., 51: 5-9.

Nestares, T., M.L. Frias, M. Barrionuevo and G. Urbano, 1996. Nutritional assessment of raw and processed chickpea (*Cicer arietinum* L.) protein in growing rats. J. Agric. Food Chem., 44: 2760-65.

Nielsen, S.S., 1991. Digestibility of legume proteins. Food Technol., 9: 112-118.

Peters, J.C. and A.E. Harper, 1985. Adaptation of rats to diets containing different levels of protein: Effects on food intake, plasma and brain amino acid concentrations and brain neurotransmitter metabolism. J. Nutr., 115: 382-398.

Sarkar, P.K., L.J. Jones, G.S. Craven, S.M. Somerset and C. Palmer, 1997. Amino acid profiles of kinema, a soybean fermented food. Food Chem., 59: 69-75.

Sarwar, G., R.W. Peace, H.G. Botting and D. Brulé. 1989. Digestibility of protein and amino acids in selected foods as determined by a rat balance method. Plant Foods Hum. Nutr., 39: 23-32.

Sarwar, G. and F.E. McDonough, 1990. Evaluation of protein digestibility-corrected amino acid score method for assessing protein quality of foods. J. Assoc. Off. Anal. Chem., 73: 347-356.

Sarwar, G. and R.W. Peace, 1994. The protein quality of some enteral products in inferior to that of casein as assessed by rat growth methods and digestibility - corrected amino acid scores. J. Nutr., 124: 2223-2232.

Yigzaw, Y., L. Gorton, T. Solomon and G. Akalu, 2004. Fermentation of seeds of teff (*Eragrostis teff*), grass-pea (*Lathyrus sativus*) and their mixtures: aspects of nutrition and food safety. J. Agric. Food Chem., 52: 1163-1169.