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EVALUATION OF NUTRITIONAL QUALITY OF VARIOUS TEMPEH PREPARATIONS WITH *RHIZOPUS OLIGOSPORUS* IN RATS

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Abstract: Tempehs were prepared from soybean, bengal gram, cowpea and field beans with pure spore culture of Rhizopus oligosporus MTCC 556. Prepared soybean tempeh (ST), bengal gram tempeh (BT), cowpea tempeh (CT), field bean tempeh (FT) and basal diets were evaluated through diet intake, body weight gain, protein efficiency ratio (PER), feed efficiency ratio (FER), fecal loss, blood haemoglobin, liver and serum proteins as well as enzymes profile and organ weights to see any correlation between different proteins in these cultivars and their nutritive value as a source of protein for weanling albino rats (Charles foster strain). Significant differences were observed in the diet intakes, body weight gain, PER, FER, fecal loss, blood haemoglobin, liver protein, alkaline phosphatase (ALP), lactate dehydrogenase (LDH) enzyme, serum protein, albumin, globulin and organ weight, while, A:G ratio, alanine transaminase (ALT) and aspartate transaminase (AST) enzymes did not differ significantly when unfermented wheat:bengal gram (WB) (80:20) and various tempeh powders were fed. The enzymes ALP, ALT, AST and LDH did not differ significantly within the tempeh groups. BT and ST had better protein quality than that of other tempeh, and the values of most traits for FT group were lowest. BT and ST appeared palatable, digestible, nutritive, safe and quite efficient for growth in rat bioassay.

Key words: Tempeh, *Rhizopus oligosporus*

INTRODUCTION

Fermented foods are among the oldest processed foodstuff and have formed a traditional part of the diet in almost all countries for millennia. Today they continue to form major sectors of the food processing industries, including baked products, yoghurt, cheese and soy products among many others. Soybeans are often described as wonder seeds. With its 40 percent good quality protein, 20 percent oil and other nutrients content and ability to grow in diverse ecological conditions, it can become a miracle seed to serve the human need of protein in India.

Cereals are major dietary protein source, especially for those living in developing countries, where protein calorie malnutrition is prevalent. Soybean has a great potential to combat protein calorie malnutrition and provides many health benefits at an affordable cost. However, due to the presence of some anti-nutritional factors and characteristic beany flavour, it requires careful processing to make it fit for human consumption. These defects are overcome by the mold fermentation process involved in the preparation of soybean foods like Tempeh [1,2].

Tempeh is a traditional fermented soybean food in Indonesia. It is obtained by cooking, dehulling of soybean and inoculation with different strains of *Rhizopus* (R. oligosporus, R. oryzae and R. stolonifer) leading to a solid-state fermentation [3]. Fermentation influences the content of desirable constituents such as vitamin, protein and fatty acids [4,5].

Utilization of tempeh is likely to increase because of the interest of youth towards new foods. Also there is a need for more foods in the face of a growing world population. Therefore, this study was

undertaken in an attempt to develop tempeh from soybean and some of the under utilized or neglected leguminous pulses and beans and to observe the growth promoting ability and the food safety aspects of the products on weaning albino rats (Charles foster strain) as potential low cost foods.

MATERIALS AND METHODS

Various beans purchased at a retail outlet in Anand were used in the study. The soybeans (*Glycine max Merr*) were cultivars of Madhya Pradesh, while bengal gram (*Cicer arietinum*), cowpea (*Vigna cotjang*) and field bean (*Dolichos lablab*) were cultivars of Gujarat, but the history of production and storage of these beans was not known.

Dietary ingredients and their chemical analysis: The wheat grain and bengal gram as well as various tempeh prepared from soybean, bengal gram, cowpea and field bean were analyzed for moisture, protein and fat contents. Each analysis was done in triplicate on two replicates. The moisture and ash contents were determined by drying using an air oven [7], the crude protein content by micro-kjeldahl (% protein= % N × 6.25) [8], crude fat content by soxhlet extraction [9], carbohydrate by difference and kilocalories by calculation.

Preparation of culture: The culture used for the processing of tempeh was pure spores of *R. oligosporus* MTCC 556, obtained from culture collection center, Chandigarh. It was subcultured and maintained on potato-dextrose-agar (PDA) slants at 30 °C until it sporulated (5 days) and then it was stored at 7 °C. Mold spores of pure culture were harvested with 2-3 ml of distilled water from the PDA slants. Two to four ml spore suspension was adequate to inoculate 200 g prepared mass for tempeh preparation.

Preparation of tempeh: Tempeh was prepared from soybeans inoculated with pure culture strain of *R. oligosporus* MTCC 556 using traditional Indonesian method with slight modification. The process flow chart for the production of tempeh is given below:

Flow chart for soybean tempeh preparation : 100 g cleaned soybeans ---- Soaked for 16-18 hrs in 500 ml tap water ---- Drained and boiled for 5 min in excess tap water ---- Drained and dehulled manually ------Dehulled bean cooked for 25-30 min in tap water ---- Partially dried on filter paper --- Soaked for 1 h in 0.1% acetic acid ---- Partially dried on filter paper --- Inoculated with 2 ml of *R. oligosporus* suspension --- Placed in container (½" thick layer) --- Incubated at 30 ^{0}C for 24 h --- Fresh tempeh cake (A white cottony mass ½" thick binding the soybean cake).

In place of soybean, field bean, cowpea and bengal gram can be used with little modifications in the procedure. The procedure for preparation of tempehs from bengal gram and cowpea was almost similar to that for soybean tempeh except that 100 g of cleaned and washed bengal gram and cowpea each were boiled in 500 ml tap water for 10 and 5 minutes respectively. Bengal gram was dehulled and split into halves manually after soaking and then cooked for 20 to 25 minutes. The time of cooking also remained same for cowpea after soaking and dehulling. However, field bean was directly dehulled and split into 3 to 4 pieces after soaking with its cooking for 25 to 30 minutes and incubation for 24 to 28 hours. The remaining procedures were same as soybean tempeh.

Design of experiment and diet composition: Albino rats weaned at 28 days of age were selected according to body weight (54-55 g) and were fed the basal diet *ad libitum* for 3 days as a period of adaptation to pulverized diet. The animals were divided into 5 groups of five rats each and were housed individually in screen bottomed cages. The rats of basal group-I were fed raw wheat : bengal gram (WB) (80:20) basal diet. While those of group II, III, IV and V were fed soybean tempeh (ST), bengal gram tempeh (BT), cowpea tempeh (CT) and field bean tempeh (FT) foodstuff for a period of six weeks respectively. The composition of basal and experimental diets fed to rats is sown in Table 1.

A known quantity of each experimental diet was given *ad libitum* in the form of a thick paste. Water was supplied in polythene bottles. Rat weights, diet spillage and refused diet and feces were recorded weekly. The left over diet and feces were dried in oven at 60 ^{0}C, weighed and used for the calculation of diet intake.

At the end of the trial period, the rats were weighed and euthanized in desiccators using diethyl ether after 18 h fast. Blood was collected from the heart directly, allowed to clot and centrifuged to separate the serum. Liver and kidneys, removed from dissected rats, were cleaned and then weighed.

Evaluation of parameters: Protein efficiency ratio (PER) and Feed efficiency ratio (FER) were calculated as per Chapman method [11]. Blood haemoglobin was analyzed by using Hemocor-D reagent kit. The serum was analyzed for alkaline phosphatase (ALP), alanine transaminase (ALT), aspartate transaminase (AST) and lactate dehydrogenase (LDH) by using standard kits supplied by Crest Biosystems, Goa. Serum total protein and albumin were estimated by using kits supplied by Eve's inn diagnostic, Baroda. Liver protein was determined by Lowry et al. [12] method. Serum globulin levels were calculated by subtracting albumin from the total proteins and A/G ratio was worked out. Moisture contents of liver and kidney were determined by the evaporation method [9].

Statistical analysis: The standard SPSS program was run to analyze the data. All the data were tested for significance using the ANOVA and Least Significant Difference Test among means of various parameters [13]. Differences between treatments of $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Tempehs were prepared successfully. Enzyme activity by Rhizopus oligosporus softened the beans and mycelial growth bound the bean mass to form a solid cake. Complex changes to proteins and carbohydrates softened the texture and flavour of beans but has no preservative effect. The product can either be consumed within a few days or preserved by drying.

On the basis of proximate composition of the different foodstuffs used in the preparation of experimental diets, five diets were formulated to isonitrogenous (10 g %) and isocaloric (392 Kcal) Table 1. The cumulative weekly body weight of weaning rats revealed significant variation between groups with highest weight gain in BT and higher in ST groups (Fig. 1).

The average diet and protein intake, body weight gain, PER, FER, total fecal loss and percent feed absorption calculated after 6 weeks of the feeding experiment are presented in Table 2. The values in FT group were significantly lower as compared to all other groups for diet intake (251.67 ± 10.56 vs. 373.91 ± 7.10 to 412.55 ± 16.69 g), protein intake (25.17 ± 1.06 vs. 37.39 ± 0.71 to 42.26 ± 1.67 g) and total weight gain (38.50 ± 4.03 vs. 78.40 ± 5.49 to 100.00 ± 5.09 g).

Table 1: Composition of basal (Group-I) and experimental (Groups-II to V) diets adjusted to provide 10% protein and 7% fat. Source: *AIN-93G-MIX, **AIN-93-VX [10].

Ingredients	Group I	Group II	Group III	Group IV	Group V
WB (80:20) (g)	65.90	--	--	--	--
ST Powder (g)	--	19.25	--	--	--
BT Powder (g)	--	--	42.23	--	--
CT Powder (g)	--	--	--	33.34	--
FT Powder (g)	--	--	--	--	34.63
Mineral Mixture (g)*	3.50	3.50	3.50	3.50	3.50
Vitamin ixture (g)**	1.00	1.00	1.00	1.00	1.00
Vitaminised Oil ml)**	7.00	7.00	7.00	7.00	7.00
Fiber (g)	5.00	5.00	5.00	5.00	5.00
L-Cystine (g)	0.30	0.30	0.30	0.30	0.30
Starch (g)	17.30	63.95	40.97	49.86	48.57
TOTAL	100	100	100	100	100

Among the other four groups, the values of diet and protein intake were non significantly higher for CT group as compared to ST group with intermediate values in BT and basal groups. Further, though the diet and protein intake were apparently higher in rats fed CT group, the total gain in body weight was significantly higher in BT and ST groups as compared to basal and CT groups (Table 2). The PER (1.52 ± 0.12 vs. 1.89 ± 0.06 to 2.47 ±0.06) and FER (0.15 ± 0.01 vs. 0.19 ± 0.01 to 0.25 ± 0.01) were also significantly ($P < 0.01$) lower in FT group than in the other four groups. Further, the values of both parameters were significantly ($P < 0.05$) higher in BT and ST as compared to basal and CT groups. Thus, FT appeared detrimental among the four fermented tempeh groups studied, while BT as well as ST groups appeared superior in terms of weight gain, PER and FER.

Kelbessa [14] concluded that tempeh fermentation improves the nutritive value. Earlier studies [15,16] have also shown an improved weight gain on feeding fermented food. Tempeh flour protein is quite efficient for growth in mice [17]. The PER value

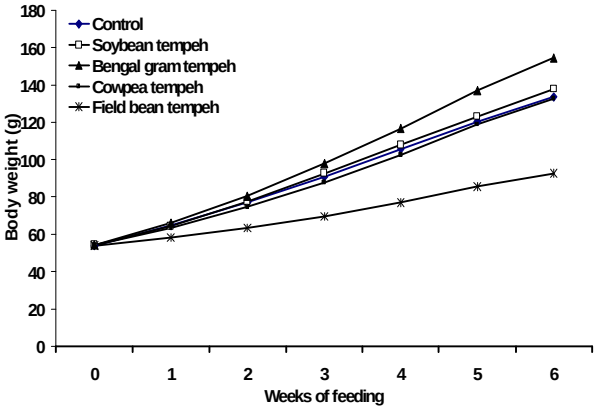

Fig. 1. Cummulative (mean ± SE) weekly body weight of weaning rats fed defferent experimental diets

Table 2: Effect of diet quality on gain in body weight, PER, FER and percent feed absorption (Mean ± SE). ** P < 0.01, NS non-significant.. Means bearing common superscripts within the row do not differ significantly (P > 0.05).

Parameter	Dietary Groups					Calculated 'F' value
	Group I	Group II	Group III	Group IV	Group V	
Total diet intake (g)	387.87^{b} ± 34.34	373.91^{b} ± 7.10	404.33^{b} ± 11.38	412.55^{b} ± 16.69	251.67^{a} ± 10.56	12.33 **
Weight gain (g)	79.50^{b} ± 10.12	83.60^{bc} ± 1.29	100.00^{c} ± 5.09	78.40^{b} ± 5.49	38.50^{a} ± 4.03	14.59 **
Protein intake (g)	38.79^{b} ± 3.43	37.39^{b} ± 0.71	40.43^{b} ± 1.14	41.26^{b} ± 1.67	25.17^{a} ± 1.06	12.33 **
PER	2.05^{bc} ± 0.09	2.24^{cd} ± 0.07	2.47^{d} ± 0.06	1.89^{b} ± 0.06	1.52^{a} ± 0.12	18.02 **
FER	0.20^{bc} ± 0.01	0.22^{cd} ± 0.01	0.25^{d} ± 0.01	0.19^{b} ± 0.01	0.15^{a} ± 0.01	18.02 **
Total fecal loss (g)	58.14^{b} ± 6.07	42.83^{a} ± 1.77	57.65^{b} ± 0.34	56.79^{b} ± 3.66	38.89^{a} ± 1.67	07.60 **
% Feed absorption	85.06^{ab} ± 0.43	88.55^{c} ± 0.37	85.69^{ab} ± 0.50	86.25^{b} ± 0.59	84.48^{a} ± 0.74	08.47 **

Table 3: Effect of diet quality on blood haemoglobin, serum proteins and liver protein (Mean ± SE). * P < 0.05, ** P < 0.01, NS non-significant. Means bearing common superscripts within the row do not differ significantly (P > 0.05).

Parameter	Dietary Groups					Calculated 'F' value
	Group-I	Group-II	Group-III	Group-IV	Group-V	
Haemoglobin (g%)	13.39^{bc} ± 0.20	13.06^{bc} ± 0.42	13.90^{c} ± 0.33	12.76^{ab} ± 0.37	11.93^{a} ± 0.40	4.39 *
Serum total protein (g%)	5.72^{a} ± 0.13	6.80^{c} ± 0.06	6.58^{c} ± 0.15	6.13^{b} ± 0.05	5.86^{ab} ± 0.06	21.81**
Serum albumin (g%)	3.51^{ab} ± 0.12	3.86^{bc} ± 0.14	3.92^{c} ± 0.09	3.55^{ab} ± 0.12	3.43^{a} ± 0.11	3.72 *
Serum globulin (g%)	2.22^{a} ± 0.12	2.94^{b} ± 0.15	2.67^{ab} ± 0.20	2.58^{ab} ± 0.07	2.44^{a} ± 0.14	3.57 *
A : G ratio	1.60 ± 0.10	1.34 ± 0.12	1.51 ± 0.14	1.38 ± 0.09	1.44 ± 0.14	0.80 NS
Liver protein (g%)	20.43^{a} ± 1.82	24.30^{b} ± 0.63	21.07^{a} ± 0.43	20.62^{a} ± 0.63	23.47^{ab} ± 0.92	3.10 *

Table 4: Effect of diet quality on serum profile of certain enzymes (Mean ± SE). * P < 0.05, NS non-significant. Means bearing common superscripts within the row do not differ significantly (P > 0.05).

Parameter	Dietary Groups					Calculated 'F' value
	Group I	Group II	Group III	Group IV	Group V	
Serum ALP (KA unit)	46.48^{b} ± 4.88	35.23^{ab} ± 2.76	32.27^{a} ± 1.75	41.63^{ab} ± 3.94	42.28^{ab} ± 3.56	2.62 *
ALT (U/L 37°C)	27.92 ± 0.31	22.83 ± 0.16	22.24 ± 0.25	23.57 ± 0.43	26.76 ± 0.33	0.67 NS
AST (U/L 37°C)	34.91 ± 4.13	32.69 ± 4.80	31.54 ± 4.33	33.70 ± 2.96	38.27 ± 2.27	0.46 NS
LDH (U/L 37°C)	813.70^{b} ± 37.74	674.60^{ab} ± 51.37	714.15^{ab} ± 25.61	591.94^{a} ± 61.72	632.60^{a} ± 58.35	3.03 *

for the ST observed in the study was similar to earlier reports [18,19]. Kaur [20] and Rema [21] reported a PER of 1.9 for WB (80: 20) diet, which is slightly lower than the value found in the present study. BT showed FER value of 0.25, which was in agreement with the values reported by other workers [21,22]. The total fecal loss was significantly (P < 0.01) higher in rats fed basal, BT or CT diets as compared to the groups fed ST and FT diets (38.89 ± 1.67 to 58.14 ± 6.07 g). Further, the percent feed absorption differed significantly (P < 0.01) among the all groups, being maximum in ST (88.55 ± 0.37) followed by CT, BT and basal groups (85.06 ± 0.43 to 86.25 ± 0.59) and was least in FT group (84.48 ± 0.74). It may be due to presence of some indigestible and antinutritional compounds in field bean that made them unpalatable. FT fed animals showed the lowest values for all the traits studied probably because of poor quality protein in it. These findings very clearly indicated that in the FT group diet was not so palatable and had poor quality protein, which depressed feed/protein intake, body weight gain and PER as well as FER as has been also observed by [1-2].

The haemoglobin concentration, serum and liver protein profile of rats fed basal and different experimental diets have been furnished in Table 3. Blood haemoglobin was significantly (P < 0.05) lower in FT and CT groups than in the other three groups (11.93 ± 0.40, 12.76 ±0.37 vs. 13.06 ± 0.42 to 13.90 ± 0.33 g%). Low haemoglobin may be due to high tannin contents of diets. Tannin content increases during mould fermentation, probably due to release of assayable tannins formerly bound to proteins and other organic substances [23]. Tannins depress the absorption of iron [24]. Fermentation by lactic acid producing organisms and *R. oligosporus* increases the bioavailability of iron in BT and ST [25-27]. Serum total protein was significantly (P < 0.01) higher in all tempeh groups compared to the basal group (5.72 ± 0.13 g %), being significantly higher in the ST, BT and CT groups. Similar increase in serum protein has been reported in earlier studies on feeding fermented foods from bean and grain tempeh [28]. Serum total protein levels were improved in all fermented food groups. *Rhizopus* spp. solid state fermentation to produce tempeh has been reported to enhance the nutritional quality including the protein quality, bioavailability and the reduction of levels of antinutritional factors in foods [29]. Shekib [30] and Kiers [31] revealed that fermentation improves protein

Table 5: Effect of diet quality on liver and kidney weights and their different profiles (Mean ± SE). * P < 0.05, ** P < 0.01, NS non-significant. Means bearing common superscripts within the row do not differ significantly (P > 0.05).

Parameter	Dietary Groups					Calculated 'F' value
	Group I	Group II	Group III	Group IV	Group V	
Body Weight (g)	$133.60^{b} \pm 11.87$	$137.80^{bc} \pm 0.92$	$154.20^{c} \pm 4.21$	$132.60^{b} \pm 6.12$	$92.60^{a} \pm 2.87$	12.58 **
Fresh liver weight (g)	$3.21^{a} \pm 0.31$	$4.33^{b} \pm 0.46$	$4.26^{b} \pm 0.29$	$3.83^{ab} \pm 0.33$	$2.94^{a} \pm 0.18$	3.65 *
Liver moisture (%)	$68.17^{b} \pm 0.68$	$64.39^{ab} \pm 2.40$	$68.44^{b} \pm 1.27$	$65.47^{ab} \pm 1.54$	$60.88^{a} \pm 1.51$	3.85 *
Liver dry weight (%)	$31.83^{a} \pm 0.68$	$35.61^{ab} \pm 2.40$	$31.56^{a} \pm 1.27$	$34.53^{ab} \pm 1.54$	$39.12^{b} \pm 1.51$	3.85 *
Fresh kidney weight (g)	$0.84^{ab} \pm 0.06$	$0.88^{bc} \pm 0.03$	$1.00^{c} \pm 0.07$	$0.80^{ab} \pm 0.04$	$0.72^{a} \pm 0.02$	4.96 *
Kidney moisture (%)	$73.20^{a} \pm 0.51$	$74.28^{abc} \pm 0.38$	$75.46^{c} \pm 0.40$	$73.95^{ab} \pm 0.39$	$74.68^{b} \pm 0.25$	4.56 *

content, quality and digestibility of tempeh foods. Serum albumin (3.43 ± 0.11 to 3.92 ± 0.09 g %) and globulin (2.22 ± 0.12 to 2.94 ± 0.15 g %) levels also followed an almost similar trend to that of serum total protein and differed significantly (P < 0.05) between the different experimental groups. A non significant difference was observed in A:G ratio among the five groups (1.38 ± 0.09 to 1.60 ± 0.10). It was due to change of protein composition during fermentation. The microbial growth is known to cause complex changes to nutritive values of fermented foods by changing the composition of proteins and improves serum albumin and globulin levels [31,32]. In general, the average albumin concentration was the lowest in FT group, while globulin was the lowest in basal group. Liver total protein content was greater in ST group (24.30 ± 0.63 g%), although it did not differ significantly from FT group (23.47 ±0.92 g%), the values of the other three groups were at par statistically (20.43 ± 1.82 to 21.07 ± 0.43 g%).

The serum enzyme profile analyzed in rats at the end of 6 weeks of feeding (Table 4) revealed non significant differences in ALT (22.24 ± 0.25 to 27.92 ± 0.31 U/L) and AST (31.54 ± 4.33 to 38.27 ± 2.27 U/L) activity among all the groups. However, ALP was significantly (P < 0.05) higher in basal, FT and CT groups (41.63 ± 3.94 to 46.48 ± 4.88 KA unit) than in ST (35.23 ± 2.76 KA unit) and BT groups (32.27 ± 1.75 KA unit). No pertinent report on the effect of different legume tempeh on serum enzyme profile could be traced in the literature reviewed. However, Owu [33] and Edem [34] observed a dose dependent decrease in plasma AST and ALT levels and an increase in ALP activity with increase in dietary palm oil intake compared to the basal diet containing oil when fed to rats. LDH activity was significantly (P < 0.05) higher in basal group (813.70 ± 37.74 U/L) followed by BT (714.15 ± 25.61 U/L) and ST groups (674.60 ± 51.37 U/L) than in the other groups, being lower in CT (591.84 ± 61.72 U/L) and FT groups (632.60 ± 58.35 U/L). ALP and LDH enzymes did not differ within the tempeh groups.The effect of diet quality on liver and kidney weights and their different parameters have been presented in Table 5. The weights of liver and kidney differed significantly in different groups. The weight of liver ranged from 2.94 ± 0.18 g in FT to 4.33 ± 0.46 g in ST groups. Whereas the weight of kidney varied from 0.072 ± 0.02 g in FT to 1.00 ± 0.07 g in BT groups. Liver moisture content differed significantly (P < 0.05) between the five dietary groups, being the highest in BT (68.44 ± 1.27 %) and the least in the FT group (60.88 ± 1.51 %). Kidney moisture content also differed significantly (P < 0.05) between the five dietary groups, being the highest in BT (75.46 ± 0.40 %) and the least in the basal group (73.20 ± 0.51 %). Moreover, the values for kidney moisture contents were relatively higher than the liver moisture content in all the groups. Liver and kidney dry weight percent differed significantly (P < 0.05) between the five dietary groups, being the least in BT (31.56 ± 1.27 and 24.56 ± 0.40 %) and the highest in FT group (39.12 ± 1.51) for liver dry weight and in basal group (26.80 ± 0.51 %) for kidney dry weight, respectively, the values of other groups being intermediate. The values for the basal diet observed in the study were similar to those reported earlier [20,21]. It indicates that all the components studied appeared to affect the nutritional parameters and organ weight. Although the biological alterations have been mainly induced by lectin and trypsin inhibitor, the contribution of other factors such as Soya toxin and urease can not be excluded. These negative effects, however, can be partially eliminated or inactivated with adequate fermentation.

As compared to unfermented basal WB (80:20) diet, tempeh prepared from bengal gram and soybean had better protein quality as well as palatability in terms of increased weight gain and PER as well as FER. All tempeh improve serum proteins. ALP, AST, ALT and LDH enzymes reveal to be safer to tempeh consumption. Tempeh prepared from cowpea had

better palatability in view of diet intake, while tempeh prepared from field bean had both poor palatability and protein quality in terms of diet intake, weight gain and PER. The result indicated that bengal gram and soybean tempeh proteins were quite efficient for growth in rats.

Continued scientific research will provide a better understanding and further knowledge on the identification of the nutritional aspect of tempeh. Contribution from nutrition and the food science community from all over the world to develop tempeh from a variety of legumes as a raw material that are nutritious and affordable will help us to meet the challenge of protein-calories malnutrition for all in the 21st century.

REFERENCES

[1] Paredes-Lopez, O. and Harry, G.I.: J. Fd. Sci., 54(4): 968-970 (1989).

[2] Kebede, B., Urga, K. and Nigatu, A.: Ethiopian J. health Develop., 9(2): 97-103 (1995).

[3] Steinkraus, K.H. *Indonesian Tempeh and Related Fermentations*. In: Handbook of Indigenous Fermented Foods. (Steinkraus, K.H. et al., ed), Marcel Dekker, New York, pp 1-94 (1983).

[4] Baumann, U., Bisping, B., Rehm. H.J.: Content and release of amino acids during the tempe fermentation. In: *Second Asian Symposium on Non Salted Fermentation*, (Hermana, M. Mahmud K.M.S. and Karyadi D. eds), 13-15 February, 1990, Jakarta, Indonesia. Ministry of Health, Nutrition Research and Development Centre, Bogor, Indonesia, pp 33-47 (1990).

[5] Hering, L., Bisping, B. and Rehm, H.J.: Fat Sci. Technol., 93: 303-308 (1991).

[6] Baumann, U., Bisping, B.: Food Microbiol., 12: 39-47 (1995).

[7] AOAC, Official Methods of Analysis, 14th edn., Associa-tion of Official Analytical Chemists, Washington, DC (1984).

[8] Oser, B.L.: *Hawk's Physiological Chemistry*, 14th ed., T.M.H. Pub. Comp. Ltd. New Delhi, pp 1373-1376 (1976).

[9] AOAC, Official Methods of Analysis, 17th ed., Associa-tion of Official Analytical Chemists, Washington, DC (1990).

[10] Reeves, P.G., Forrest, H., Nielsen and George.: J. Nutr., 123: 1929-1951 (1993).

[11] Chapman, D.G., Catillo, R. and Campbell, J.A.: Can. J. Biochem. Physiol., 39: 679- 681 (1959).

[12] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J.: J. Biol. Chem., 193: 265-275 (1951).

[13] Steel, R.G.D. and Torrie, H.H.: *Principles and Procedures of Statistics*. McGrew Hill Co., New York (1980).

[14] Kelbessa-urga, Binyam-kebede and Ayele-nigatu: Ethopian J. Sci. 19(2): 195-206 (1996).

[15] Khader, V.: J. Plant Foods, 5:31- 36 (1983).

[16] Zamora, R.G. and Veum, T.L.: J. Nutr., 109(7): 1333-1339 (1979).

[17] Tavares, S.G. and Kiyan, C.: Alimentos e Nutricao, 13: 23-34 (2002).

[18] Bai, R.G., Prabha, T.N., Rao, T.N.R., Sreedhara, V.P.and Sreedhara, N.: J. Fd. Sci. and Techno., India, 12(3):135-138 (1975).

[19] Kao, C. and Robinson, R.J.: Cereal Chem., 55(4): 512-517 (1978).

[20] Kaur, T.: *Nutritional Studies on Fermented Foods*. Ph.D. Thesis, S. P. Univ. V.V. Nagar (1990).

[21] Rema, K.B.: *Nutritional Studies on Germinated Seeds and Seedlings*. Ph.D. Thesis, S. P. Univ. V.V. Nagar (1993).

[22] Eskeland, B. and Nordal, J.: J. Fd. Sci., 45(5): 1153-1156 (1980)

[23] Deshpandey, S.S., Sathe, S.K., Sakunkhe, D.K. and Cornforth, D.P.: J. Fd. Sci., 47: 1846-1650 (1982).

[24] Reddy, N.R., Pierson, M.D., Satthe, S.K. and Salunkhe, D.K.: J. Ass. Off. Chem. Soc., 62: 541-549 (1985).

[25] Moeljopawiro, S., Gordon, D.T. and Fields, M.L.: J. Fd. Sci., 52(1): 102-105 (1987).

[26] Macfarlane, B.J., Riet, W.B., Bothwell, T.H., Baynes, R.D., Siegenberg, D., Schmidt, U., Tal, A., Taylor, J.R.N. and Mayet, F.: Am. J. Clin. Nutr., 51(5): 873-880 (1990).

[27] Tawali, A.B. and Schwedt, G.: Nahrung., 42(1): 29-31 (1998).

[28] Sood, N. and Kokke, R.: *Tempeh Traditional Biotechnology from Indonesia*. Biotechnology and food industries. Proceeding of the International Symposium held in Budapest, Hungary, 5-9 October 1987-1988, pp 569-585 (1988).

[29] Steinkraus, K. H.: *Handbook of Indigenous Fermented Foods*. 2nd ed. New York: Marcel Dekker (1996).

[30] Shekib, L.A.: Plant Foods Hum. Nutr., 46, 201-205 (1994).

[31] Kiers, J.L., Nout, M.J.R., Rombouts, F.M.: J. Sci. Agric., 80:1325-1331 (2000).

[32] Dworschak, E.: *Handbook of Nutritive Value of Processed Food*. Vol. 1, CRC press. Boca Paton, Florida (1982).

[33] Owu, D.U., Osim, E.E. and Ebong, P.E.: Acta Tropica, 65: 65-73 (1998).

[34] Edem, D.O.: Plant Foods Hum. Nutr., 57: 319-341 (2002).