NUTRITIONAL ASPECTS OF FERMENTED FOODS FROM CHICKPEA, HORSEBEAN, AND SOYBEAN[1]

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ABSTRACT

Soybean, chickpea, and horsebean are three major leguminous seeds cultivated by mankind. The antitryptic activities of soybean are much stronger than those of chickpea or horsebean, but almost all such activities can be destroyed by autoclaving the dehulled seed grits for 15 min. When autoclaved grits were fermented to prepare tempeh or miso, essential amino acids of their proteins were virtually unchanged, but water-soluble vitamins increased considerably. No aflatoxins were produced. Diets were prepared from freeze-dried tempehs or autoclaved (unfermented) grits for protein efficiency ratio (PER) tests. Rats on freeze-dried tempeh diets ate more, gained more weight, and had higher PERs than did rats eating autoclaved (unfermented) grits. The PER of chickpea tempeh was slightly higher than that of soybean tempeh. The PER of horsebean tempeh was significantly lower than that of either of the other two tempehs, but the PER of horsebean tempeh diet enriched by 0.2% L-methionine and 0.1% L-tryptophan exceeded that of soybean tempeh. The poor performance of horsebean tempeh resulted from amino acid imbalance rather than from toxic material. Misos contained about 25% salt db—too much to be acceptable rat diets.

The rapidly increasing human population makes food shortage a major world problem. In some areas, two-thirds of the population suffer from protein malnutrition. Soybean and its products are a vital protein source in many densely populated areas, i.e., the Oriental countries. Soybean products such as tofu (protein isolate), tempeh (*Rhizopus oligosporus* fermented product), and miso (*Aspergillus oryzae* fermented product) are indispensable in the daily diets.

Although chickpea (*Cicer arietinum*) and horsebean (*Vicia faba*) are also important leguminous crops in those countries, they have not been substituted for soybean in soybean-based foods. Methods of preparing tempeh and miso from chickpea, horsebean, or soybean have been described (1). Nutritional changes in rats fed those legumes after fermentation are discussed in this article.

MATERIALS AND METHODS

Commercial samples of chickpea, horsebean, and soybean were obtained from G. B. Ratto Grocery Company, Oakland, CA; The Country Store, Kansas City, MO; and Farmers Co-op Association, Manhattan, KS; respectively.

All samples were dehulled with a roller mill, aspirated, and ground to particles approximately 2–4 mm in diameter. The dehulled grits were used to make tempeh and miso.

Tempehs were prepared according to the procedure that Hesseltine et al. (2) described. They were fermented, freeze-dried, and finely ground for chemical analyses or for preparing rat diets. Miso preparation was based on the method

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that Shibasaki and Hesseltine (3) proposed. After aging, misos were freeze-dried and ground for chemical analyses. Because misos contain about 25% salt db, they were not fed to rats. Such high salt concentration would cancel the beneficial effect of the protein. Only tempeh samples were used in the rat-feeding study.

Methionine and tryptophan levels were determined by a microbiological method (4) because they decompose during acid hydrolysis of protein, but all other amino acid levels were determined by column chromatography (5). Vitamin C levels were determined by spectrophotometry (6), but other water-soluble vitamins were evaluated by microbiological methods.

TABLE I
Effect of Tempeh and Miso Fermentations on Essential Amino Acids of Chickpea, Horsebean, and Soybean Proteins[a]

	Tempeh			Miso		
Amino Acid	**CP[b]**	**HB[c]**	**SB[d]**	**CP**	**HB**	**SB**
Lysine	0.98	1.21	0.91	0.63	0.77	0.98
Threonine	1.18	1.06	0.93	1.00	0.88	0.95
Valine	1.16	0.96	0.96	1.05	1.15	1.07
Methionine	1.18	1.24	1.06	0.99	1.05	0.97
Isoleucine	1.08	0.96	0.91	1.12	1.09	1.07
Leucine	0.98	0.89	0.93	1.01	1.02	0.98
Phenylalanine	0.91	0.89	1.15	1.19	0.89	0.96
Tryptophan	0.95	0.83	1.23	0.63	0.68	0.55

[a]Figures show molar ratios (based on 100 mol of total amino acids) of fermented and unfermented beans. Ratios exceeding 1 indicate increased amino acid; 1, no change; less than 1, decreased amino acid.
[b]CP = chickpea.
[c]HB = horsebean.
[d]SB = soybean.

TABLE II
Effects of Tempeh and Miso Fermentations on Water-Soluble Vitamins of Chickpea, Horsebean, and Soybean[a]

	Tempeh			Miso		
Vitamins	**CP[b]**	**HB[c]**	**SB[d]**	**CP**	**HB**	**SB**
Thiamine (B_1)	1.01	1.06	0.69	1.55	1.28	1.61
Riboflavin (B_2)	2.82	9.12	4.90	6.19	2.43	2.13
Pyridoxine (B_6)	3.99	4.15	2.47	2.00	1.21	1.69
Cobalamin (B_{12})	1.07	1.76	1.25	...[e]	...[e]	...[e]
Niacin	16.80	5.45	4.87	3.80	1.30	3.18
Pantothenic acid	1.92	20.05	2.84	2.37	1.96	1.68
Ascorbic acid (C)	4.50	16.45	3.29	7.20	7.51	3.77

[a]Figures are ratios (fermented/unfermented) of vitamin. Ratios exceeding 1 indicate increases during fermentation; 1, no change; less than 1, decrease.
[b]CP = chickpea.
[c]HB = horsebean.
[d]SB = soybean.
[e]Concentration too low to determine.

The procedure for aflatoxin analysis was based on an AOAC method (7). Aflatoxin standards were obtained from the Food and Drug Administration, Washington, DC. Antitryptic activity was determined by the method that Kakade et al. (8) proposed, in which synthetic substrate benzoyl-DL-arginine-P-nitroanilide is used to react with trypsin.

Protein efficiency ratio (PER) and apparent digestibility coefficient (DC) of autoclaved (unfermented) and tempeh (fermented) samples were evaluated by AOAC methods (9). The test animals were 3-week-old weaned white male albino rats purchased from Charles River Breeding Laboratories (Wilmington, MA). They were fed in two batches: first batch diets contained autoclaved (unfermented) bean samples; second batch, tempeh (fermented) meals. Casein control diet was included in each batch to permit calculation of PER based on a value of 2.50 for casein. During the first two weeks, rats on autoclaved

TABLE III
Antitryptic Activity and Aflatoxin Analyses of Raw Beans, Autoclaved Beans, Tempehs, and Misos

	Raw			Autoclaved			Tempeh			Miso		
	CP[a]	HB[b]	SB[c]	CP	HB	SB	CP	HB	SB	CP	HB	SB
TUI[d]	14.7	6.7	82.2	1.2	0.0	1.2	2.4	1.2	0.0	0.0	0.0	0.0
Aflatoxin	ND[e]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

[a]CP = chickpea.
[b]HB = horsebean.
[c]SB = soybean.
[d]TUI (percent trypsin unit inhibited) 30% acetic acid-treated trypsin has a TUI of 100% (8).
[e]ND = not detectable.

TABLE IV
Weight Gains, Food Consumptions, Protein Efficiency Ratios, and Digestibility Coefficient of Rats Fed Autoclaved (Unfermented) Compared with Those Fed Tempeh (Fermented) Bean Samples as Protein Sources

	Gains (g)		Food Consumed (g)	
Diet	Unfermented	Fermented	Unfermented	Fermented
CP[a]	92.8	113.6	345.6	393.0
HB[b]	19.4	41.0	155.6	205.0
HB + Met. + Try.[c]	64.0[d]	60.5[d]	206.0[d]	173.5[d]
SB[e]	79.0	105.9	319.6	367.6
Casein	150.8	132.1	397.4	377.7
LSD[f] (0.05)	...	...	...	...

[a]CP = chickpea.
[b]HB = horsebean.
[c]HB diet enriched with 0.2% L-methionine and 0.1% L-tryptophan, fed on third and fourth weeks.
[d]Average, two rats.
[e]SB = soybean.
[f]LSD = least significant difference, 0.05.

(unfermented) horsebean and those on horsebean (fermented) tempeh grew extremely poorly. The essential amino acid composition of horsebean protein was extremely low in methionine and tryptophan, so both diets were enriched with 0.2% L-methionine and 0.1% L-tryptophan.

RESULTS AND DISCUSSION

The effects of tempeh and miso fermentations on essential amino acids of chickpea, horsebean, and soybean proteins are shown in Table I. Values near 1 indicate that amino acids remained virtually unchanged during fermentation. However, lysine and methionine in horsebean and tryptophan in soybean increased considerably during tempeh fermentation, but lysine in chickpea and horsebean, and tryptophan in all three legumes, decreased markedly during miso fermentation. Explanation of the opposite changes is difficult. Misos, however, developed some brownishness as they aged, probably from reactions between amino acids and reducing sugars, which may partially explain the decrease of some amino acids in misos.

Effects of tempeh and miso fermentations on water-soluble vitamins of chickpea, horsebean, and soybean are shown in Table II. Thiamine and cobalamin either decreased or slightly increased; all the rest increased markedly in either tempeh or miso. Tempeh mold *R. oligosporus* seemed to synthesize water-soluble vitamins from all three legumes. Chickpea is an excellent substrate for niacin synthesis; horsebean, an excellent substrate for riboflavin, pantothenic acid, and ascorbic acid syntheses. Amounts of vitamins synthesized in soybean tempeh were less than in chickpea tempeh or horsebean tempeh, but relative amounts did not vary widely. The increase of water-soluble vitamins in miso was less than in tempeh. Miso generally is used as food flavoring rather than as a food staple, so miso is much less important than tempeh as a vitamin source.

Results of antitryptic activity and aflatoxin analyses are summarized in Table III. All three raw legumes showed some antitryptic activity, but antitryptic activity of soybean was much stronger than that of chickpea or horsebean. When soybean had 82.2% antitryptic activity, chickpea had 14.7% and horsebean 6.7%.

PER ± Standard Deviation		**Digestibility**		**PER (casein = 2.50)**	
Unfermented	**Fermented**	**Unfermented**	**Fermented**	**Unfermented**	**Fermented**
2.92 ± 0.30	2.80 ± 0.14	79.28	72.35	1.95	2.11
1.34 ± 0.20	2.00 ± 0.15	85.86	81.80	0.89	1.51
3.34[d]	3.45[d]	85.86[d]	81.80[d]	2.22[d]	2.60[d]
2.66 ± 0.20	2.70 ± 0.18	84.92	81.06	1.77	2.03
3.75 ± 0.22	3.32 ± 0.34	93.50	93.03	2.50	2.50
0.317	0.200	...	...	...	...

Autoclaving at 121°C for 15 min almost completely destroyed antitryptic activity. No evidence of any aflatoxin was found in any sample.

Table IV gives results from feeding rats diets prepared from autoclaved (unfermented) and tempeh (fermented) samples. In first batch diets (unfermented), the PER of casein was highest. PER of unfermented chickpea protein was slightly higher than that of unfermented soybean protein, while PER of unfermented horsebean protein was lowest. Fisher's least significant difference test showed that the PERs of both casein and unfermented horsebean protein differed significantly from that of any other protein. PER of unfermented chickpea protein did not differ significantly from that of unfermented soybean protein. Both differed significantly, however, from those of casein and unfermented horsebean protein.

Enriching the horsebean unfermented diet with methionine and tryptophan significantly increased its PER, so the poor growth of rats on the horsebean unfermented diet resulted from low methionine and tryptophan contents rather than from toxic material.

The DC of legume proteins appeared to be closely related to the amount of food consumed. The more food consumed, the lower the DC. Thus, chickpea diets had the highest food consumption among the test diets and the lowest DC.

In second batch diets (fermented), the PER of casein was significantly higher than those of chickpea, soybean, and horsebean proteins, and PERs of chickpea and soybean proteins were significantly higher than that of horsebean protein. PERs of fermented chickpea and soybean proteins did not differ significantly from each other, however.

Enriching the fermented horsebean diet with methionine and tryptophan also significantly increased PER (from 2.00 to 3.45).

When unfermented and fermented diets were compared, average weight gains of rats on tempeh diets were higher than those on the unfermented bean diets. Tempeh fermentation apparently increased palatability of the beans markedly so that rats ate much more on tempeh diets than on unfermented bean diets.

When PERs based on casein at 2.50 were compared, the fermented bean proteins had higher values than did their unfermented counterparts. This may be related to the increases of most water-soluble vitamins and some essential amino acids.

All rats on either autoclaved (unfermented) or tempeh (fermented) diets appeared healthy and active and behaved as those on the casein control diet. Necropsy showed no pancreatic, hepatic, or nephritic abnormalities. In rats on horsebean diets no symptoms similar to those of favism developed, and blood and urine appeared to be normal. All rats on test diets developed gaseous cecum, but those on the casein control diet did not. Carbohydrates such as raffinose and stachyose in the test diets probably could not be absorbed in rats' small intestines and were fermented by microorganisms in the cecum.

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