TEMPEH AND MISO FROM CHICKPEA, HORSE BEAN, AND SOYBEAN

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ABSTRACT

Chickpea, horse bean, and soybean are dehulled and ground to different granular sizes. The bean grits are then soaked, autoclaved, and fermented with *Rhizopus oligosporus* or *Aspergillus oryzae* to make tempeh or miso. Our results indicate 0.2- to 0.4-cm diameter grits are best for the fermenting process. Chickpea and soybean tempehs have similar yellowish color and firm texture, while horse bean tempeh is grayish and soft textured. Flavors of all three tempehs are similar, although chickpea and horse bean tempehs have much stronger flavors than soybean tempeh. Chickpea miso is darker than soybean miso; horse bean miso is lightest colored. Fermenting sharply increases reducing sugar, soluble protein, and water-soluble vitamins in tempeh and miso, but essential amino acids remained relatively unchanged.

The rapidly increasing world population and other factors have made food shortage a major crisis in some areas. Diets of more than two-thirds of the world population are protein deficient.

Pulses, oilseeds, and nuts are the main protein-rich vegetable crops. They play a vital role in relieving protein malnutrition in areas where animal products cannot be afforded. Soybean and its products have long been the most important protein source in most densely populated areas, *i.e.*, Oriental countries. Soybean products such as tofu, tempeh, and miso are relishes in daily diets there.

Chickpea (*Cicer arietinum*) and horse bean (*Vicia faba*) are also important leguminous crops in those countries. However, they have not been tried as soybean-based foods. This study substituted chickpea and horse bean for soybean to make tempeh and miso. If these two foods could be prepared from chickpea or horse bean, the demand for soybeans would be greatly relieved.

MATERIALS AND METHODS

Chickpea, horse bean, and soybean samples were from G. B. Ratto Grocery Co., Oakland, CA; The Country Store, Kansas City, MO; and Farmers Co-op Association, Manhattan, KS, respectively. Where and when the crops were produced are not known.

The bean samples were dehulled with roller milling and aspiration. A small portion of the dehulled grits was ground to fine powder for proximate analyses; the rest was ground and sifted to various particle sizes from coarse grits (pass 6-mesh screen and remain on 10-mesh screen) to medium grits (pass 10-mesh, remain on 20-mesh), small grits (pass 20-mesh, remain on 60-mesh), and powder (pass 60-mesh).

Results of proximate analyses of the three samples are shown in Table I.

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Generally, chickpea and cereal grains are similar in composition. Chickpea has high nitrogen-free extract content and low protein content. Soybean has high protein and fat contents and low nitrogen-free extract. The protein and nitrogen-free extract of horse bean fall between those of chickpea and soybean. However, its fat content is extremely low.

TABLE I
Proximate Analyses (%) of Chickpea (CP), Horse Bean (HB), and Soybean (SB)

Sample	Moisture	Ash	Protein[a]	Fat	Fiber	NFE[b]
CP	9.3	2.7	18.8	6.0	2.3	60.9
HB	9.2	3.3	31.2	1.3	1.7	53.3
SB	7.0	5.0	38.0	19.0	4.5	26.5

[a]N × 6.25.
[b]Nitrogen-free extract.

TABLE II
Effect of Bean-Grit Particle Size on Tempeh Fermentation

Bean Characteristic	Mesh Range	CP[a]	HB[a]	SB[a]
Coarse grits	− 6 to +10[b]	+++[c]	+++	+++
Medium grits	−10 to +20	+++	+++	+++
Small grits	−20 to +60	++	+	+
Flour	−60	−	−	−

[a]See explanation of abbreviations, Table I.
[b]Pass through 6-mesh screen and remain on 10-mesh screen.
[c]− to +++ = poor to excellent growth.

TABLE III
Characteristics of Chickpea, Horse Bean, and Soybean Tempehs

Characteristic	CP[a]	HB[a]	SB[a]
Appearance	White	White	White
Texture	Firm	Soft	Firm
Color after frying	Light brown	Gray	Brown
Flavor before frying	Strong alcoholic and ester flavored	Weak alcoholic and ester flavored	Bland
Flavor after overnight storage	Bland	Bland	Bland
Flavor after frying	Very agreeable	Agreeable	Agreeable

[a]See explanation of abbreviations, Table I.

Tempeh was fermented using the procedure described by Hesseltine *et al.* (1) with some modifications. Dehulled bean grits were soaked overnight in tap water at about 25° C. The next morning, the water was drained off, bean grits were put into a perforated aluminum pan covered with a clean cloth, then autoclaved at 121°C for 15 min. The bean grits were removed from the autoclave and were all lumped together. A clean spatula broke up the lump so the grits cooled rapidly. After the grits had cooled to about 40° C, they were inoculated with 2-day-old *Rhizopus oligosporus*[2] spore suspension. This inoculated material was thoroughly mixed and packed in Petri dishes. (*R. oligosporus* does not require much aeration as do many other molds; too much aeration may cause spores to form, which is not desirable in tempeh fermentation. It is, therefore, important to pack the bean grits tightly before incubation). The Petri dishes were then incubated at 37° C with a piece of wet towel under them. After incubating, the bean mass was bound together by pure white mycelia and could be removed from the dish in a whole piece—called raw tempeh cake. This cake was cut into thin (about 0.5 cm) slices, dipped in salt water, and fried in vegetable oil. The fried tempeh was golden brown and judged delicious by taste panel. For chemical analyses, raw tempeh cakes were freeze-dried and ground to fine powder.

Miso fermentation procedure was based on that of Shibasaki and Hesseltine (2). Essentially, a steamed rice was fermented with *Aspergillus oryzae*.[3] Then the moldy rice, serving as a starter, was mixed with cooked bean grits, seed culture of *Saccharomyces rouxii*[2], and salt. The ratio of rice:bean:salt is about 3:5:2. Water content of the mixture is adjusted to 52 to 55%. This mixture was incubated at 30°C for 2 weeks, then at room temperature for 3 months. A portion of the product was freeze-dried for analyses of total reducing sugar, water-soluble protein, essential amino acids, and water-soluble vitamins.

When determining total reducing sugar, the sample was first extracted with petroleum ether to remove lipids. One gram of the fat-free sample was extracted with 10 ml 70% alcohol. One milliliter of the alcohol extract was analyzed for reducing sugar content by the Somogyi micro copper method (3). For water-soluble protein analysis, the sample was extracted with distilled water (1). Protein content of the extracted solution was determined by micro Kjeldahl method. Essential amino acids were determined by column chromatography (4)

[2]Obtained from USDA Northern Regional Research Laboratories, Peoria, IL.
[3]Obtained from Department of Agricultural Chemistry, National Taiwan University, Taipei, Taiwan.

TABLE IV
Reducing Sugar Content in Tempeh and Miso (% of Nitrogen-Free Extract)

Sample[a]	Tempeh: Before fermentation	Tempeh: After fermentation	Miso: Before fermentation	Miso: After fermentation
CP	0.86	8.62	0.26	61.50
HB	0.86	13.40	0.26	67.60
SB	2.31	6.80	0.32	65.40

[a]See explanation of abbreviations, Table I.

except that methionine and tryptophan were determined by microbiological methods (5). Water-soluble vitamins were also determined by microbiological methods except that vitamin C was determined by spectrophotometry (6).

RESULTS AND DISCUSSION

Table II shows effects of bean-grit particle size on tempeh fermentation. Tempeh mold *R. oligosporus* successfully fermented coarse and medium grits of all three bean samples. However, fermentation was less in small grits. The mold was unable to ferment flour. After being autoclaved, the flour gummed up to form a solid mass. There was little oxygen below the surface of the mass to support mold growth. The smaller the grits, the larger the loss during soaking, so coarse grits were used to prepare tempeh. Characteristics of the fermented products are summarized in Table III.

Chickpea and soybean tempehs are similar in texture and color, horse bean tempeh has softer texture and is grayish after frying. Both chickpea and horse bean tempehs have alcoholic and ester-like aromas that disappear with frying.

TABLE V
Water-Soluble Protein in Tempeh and Miso Samples (% of Total Kjeldahl Protein)

	Tempeh		Miso	
Sample[a]	Before fermentation	After fermentation	Before fermentation	After fermentation
CP	22.04	56.87	16.67	66.67
HB	19.21	49.60	16.97	69.27
SB	10.28	33.41	9.86	66.20

[a]See explanation of abbreviations, Table I.

TABLE VI
Effect of Tempeh and Miso Fermentations on the Essential Amino Acids of Chickpea, Horse Bean, and Soybean Proteins[a]

	Tempeh[b]			Miso[b]		
Amino Acid	CP	HB	SB	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]After fermentation/before fermentation (based on per 100 mol of total amino acids).
[b]See explanation of abbreviations, Table I.

Table IV shows the total reducing substances in tempeh or miso expressed in % glucose equivalent of total nitrogen-free extract. The reducing substances were extracted with 70% alcohol. Fermenting increased sugar content in both tempeh and miso. However, in miso fermentation about two-thirds of total nitrogen-free extract is hydrolyzed to reducing sugar so misos taste quite sweet.

Table V shows water-soluble protein contents of tempeh and miso. Water-soluble protein increased two to three times after tempeh fermentation, four to six times after miso fermentation. The increases result from proteolytic activity of the molds, which partially hydrolyzed the insoluble protein to soluble protein. In miso, the protein is probably hydrolyzed to peptides and amino acids during the long aging period. The glutamic acid produced may contribute heavily to miso's delicious flavor.

Table VI shows effects of tempeh and miso fermentations on the essential amino acids of chickpea, horse bean, and soybean proteins. If the figure exceeds 1, the amino acid increased after fermentation: values equal to 1 mean no change, less than 1 mean decreased amino acid. Most of the figures are near 1, so most of the essential amino acids remained unchanged after fermentations. However, lysine in chickpea miso and horse bean miso, and tryptophan in all three misos decreased significantly after fermentation.

Table VII shows effects of tempeh and miso fermentations on water-soluble vitamins of chickpea, horse bean, and soybean. Except for thiamin and cobalamin, the water-soluble vitamins increased markedly during fermentations. Tempeh mold, *R. oligosporus,* appeared to be able to synthesize large amounts of water-soluble vitamins from these beans. Chickpea is an excellent substrate for riboflavin, pantothenic acid, and ascorbic acid syntheses. The amount of vitamins synthesized in soybean tempeh was somewhat less than in chickpea tempeh and horse bean tempeh. The increases of water-soluble vitamins in miso were less pronounced than in tempeh. Miso is generally used as flavoring rather than as a main dish: miso may not be an important vitamin source for those who consume it.

TABLE VII
Effects of Tempeh and Miso Fermentations on Water-Soluble Vitamins of Chickpea, Horse Bean, and Soybean[a]

	Tempeh[b]			Miso[b]		
Vitamin	**CP**	**HB**	**SB**	**CP**	**HB**	**SB**
Thiamin (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	TL[c]	TL[c]	TL[c]
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]After fermentation/before fermentation (based on mg per 100 g organic matter).
[b]See explanation of abbreviations, Table I.
[c]Too low to measure.

It is concluded that chickpea can substitute for soybean to make tempeh or miso with similar appearance, texture, and flavor. Horse bean is more suitable for miso-making than tempeh-making.

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