TEMPEH: NUTRITIVE VALUE IN RELATION TO PROCESSING[1]

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ABSTRACT

Tempeh, an Indonesian food of good flavor and texture, is made by fermenting soybeans with a species of *Rhizopus*. The fermented food is high in protein and unsaturated oil. Rats fed tempeh showed a small reduction in growth and protein efficiency compared with autoclaved and dehulled full-fat soybean meal. This reduction in nutritive value may not be serious when one considers the improved edibility of soybeans for human consumption by fermentation. Loss of solids and protein in dehulling, soaking, washing, and cooking of soybeans before fermentation did not reduce the nutritive value of either cotyledons, or full-fat grits (chips), used to make tempeh. Since pancreatic hypertrophy did not occur in rats fed tempeh, the heat used in normal preparation of tempeh is sufficient to destroy the factors in raw soybeans responsible for poor growth and pancreatic hypertrophy. Methionine supplementation of tempeh significantly increased rate of rat growth and protein efficiency values.

Tempeh (1,2), known also as *témpé kédelé* and *témpé*, is a popular Indonesian food made by fermenting soybeans with *Rhizopus*, and small amounts are consumed in Holland. Tempeh is high in protein and unsaturated oil; when fried in oil, it has a pleasing flavor and texture. These desirable qualities have created an interest in evaluating tempeh as a low-cost product for use in worldwide food programs (3) and suggest that it may have a place in our own domestic foods. Recent research on the preparation of tempeh has been described by Steinkraus *et al.* (4); Hesseltine *et al.* (5); Ko Swan Djien and Hesseltine (6); and Martinelli and Hesseltine (7).

VanVeen and Schaefer (2) have claimed that tempeh is more easily digested than unfermented soybeans and that tempeh protein is excellent in quality. György (8) reported that the nutritive value of one lot of freeze-dried tempeh prepared from Seneca soybeans was equivalent to that of skimmilk and much higher than the unfermented soybean control. Steinkraus *et al.* (4) reported that the nutritive value of tempeh decreased with increased fermentation time.

This paper reports on processing losses in preparation of tempeh; its nutritive value when prepared from whole beans and chips (full-fat

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grits) with and without added methionine; its effect on rat pancreas; and changes in its amino acid composition before and after fermentation.

Materials and Methods

In these and other studies at the Northern Laboratory, tempeh has been prepared by three methods, referred to as 1) cotyledon or traditional tempeh, 2) chip tempeh, and 3) inhibited tempeh. Hawkeye soybeans, 1961 crop, were used unless otherwise indicated.

Cotyledon Tempeh. Whole soybeans were soaked in excess water overnight, the seed coat was removed by hand, excess tap water was added, and the cotyledons were boiled 30 min.; then the water was drained. The well-drained cotyledons were inoculated with *Rhizopus oligosporus* NRRL 2710 and fermented at 32°C. for 20 to 25 hr.

Chip Tempeh. In this method, the traditional procedure of making tempeh was modified so as to introduce a mechanical method of removing the seed coat (hulls). Dry whole beans were cracked between corrugated rolls into six to eight parts. This treatment loosened the seed coat, which was removed by screening and aspirating in a seed cleaner. The chips (grits) obtained were soaked and treated similarly to the cotyledons.

Inhibited Tempeh. In making tempeh from either cotyledons or chips, substantial material losses occurred in removing the seed coat and in discarding the water used in soaking, washing, and cooking. Total losses on a dry basis of the whole beans varied from 24.0 to 43.0%, including as much as 5% loss in fermentation (Table I). To

TABLE I

PROCESSING LOSSES FOR TWO METHODS OF TEMPEH PRODUCTION

MATERIAL AND PROCEDURE	LOSS OF SOLIDS		LOSS OF NITROGEN		PROTEIN CONTENT[a] (N × 6.25) HAWKEYE
	Harosoy	Hawkeye	Harosoy	Hawkeye	
	%	%	%	%	%
Whole soybeans					43.0
Full-fat meal					47.5
Dehulling and soaking[b]	12.6	9.5	8.0	3.9	...
Cooking	11.0	14.0	10.0	3.0	51.7
Fermentation	3.4	1.0	1.7	0.8	53.1
Total loss	27.0	24.5	19.7	7.7	...
Dehulled chips					44.7[c]
Soaking and cooking[d]	38.0	...	31.4	..	48.6
Fermentation	5.0	...	2.0	..	50.0
Total loss	43.0	...	33.4	..	...

[a] Dry basis.
[b] Hulls separated by hand from both varieties of soybeans represent 7.9% of the whole bean and contain 1.87% nitrogen. Hulls separated mechanically account for 9.5% of the bean and contain 2% nitrogen. Soaking losses are usually 1–2%.
[c] Harosoy variety.
[d] Soaking losses are about 8%.

reduce these losses, chips were cooked, one part to two parts of water, for nearly 2 hr. at 100°C. When all the water was absorbed, the chips were inoculated directly and then fermented. When this modified procedure was used, there was no loss of solids, except during fermentation.

In rat-feeding tests reported in Tables II and III, the cooked cotyledons and chips, and their corresponding fermented products, were dehydrated in a freeze-dryer to 5–8% moisture. Soybean products used in diets 5, 7, 14, and 16 were subjected to additional moist heat (see footnotes in tables) and then dehydrated in a freeze-dryer to determine

TABLE II

EFFECT OF PROCESSING AND TEMPEH FERMENTATION OF SOYBEANS ON GROWTH, PROTEIN EFFICIENCY, AND PANCREAS WEIGHT OF RATS

Diet No.	Dietary Constituent	Mean Weight Gain ± SE [a]	PE [b]	Mean Pancreas Weight ± SE
		g.		*g./100 g. B.W.* [c]
1	14% Casein [d]	193.2 ± 6.31	2.81 ± 0.06	0.39 ± 0.01
2	Full-fat meal [e]	190.6 ± 7.13	2.62 ± 0.05	0.39 ± 0.02
3	Cooked chips	193.0 ± 10.16	2.55 ± 0.08	0.39 ± 0.01
4	Chip tempeh	157.0 ± 10.63*	2.36 ± 0.09*	0.41 ± 0.03
5	Chip tempeh [e]	154.2 ± 11.42*	2.40 ± 0.06*	0.41 ± 0.01
6	Cotyledon tempeh	172.4 ± 11.32	2.48 ± 0.03*	0.40 ± 0.01
7	Cotyledon tempeh [e]	162.0 ± 10.71*	2.35 ± 0.04*	0.42 ± 0.03
8	14% Casein [d]	165.8 ± 6.80	2.99 ± 0.008	0.50 ± 0.03
9	Full-fat meal [e]	167.2 ± 12.46	2.78 ± 0.08	0.44 ± 0.02
10	Cooked cotyledon	159.0 ± 7.67	2.63 ± 0.07	0.49 ± 0.02
11	Cotyledon tempeh	160.0 ± 5.88	2.64 ± 0.02	0.51 ± 0.01
12	Cotyledon tempeh with 0.3% methionine	203.2 ± 5.23*	3.09 ± 0.07*	0.48 ± 0.02

[a] Standard error.
[b] Protein efficiency.
[c] Body weight.
[d] Diets 1 through 7, 32-day assay; diets 8 through 12, 28-day assay.
[e] Autoclaved 40 min. with steam at atmospheric pressure.
*P <0.05.

TABLE III

GROWTH, PROTEIN EFFICIENCY, AND PANCREAS WEIGHTS OF RATS FED INHIBITED TEMPEH

Diet No.	Dietary Constituent [a]	Mean Weight Gain ± SE [b]	PE [c]	Mean Pancreas Weight ± SE
		g.		*g./100 g. B.W.* [d]
13	Chips, steamed 30 min.	161.0 ± 7.76	2.50 ± 0.04	0.51 ± 0.02
14	Chips, steamed 60 min.	159.8 ± 5.46	2.47 ± 0.05	0.48 ± 0.02
15	Inhibited tempeh from chips steamed 30 min.	152.2 ± 4.56	2.51 ± 0.04	0.48 ± 0.02
16	Inhibited tempeh from chips steamed 60 min.	127.2 ± 8.32 [e]	2.31 ± 0.05 [e]	0.48 ± 0.02

[a] All were steamed in pressure cooker at 5 p.s.i. Time of cooking is indicated for each diet.
[b] Standard error, 30-day assay.
[c] Protein efficiency.
[d] Body weight.
[e] P <0.05.

whether the cooking conditions used in normal tempeh preparation were adequate for maximum nutritive value. It has been previously reported (9) that raw soybean meal requires as little as 15 min. of steaming for maximum nutritive value.

Rat Bioassay. Weanling albino male rats, purchased from Simonsen Laboratories, Gilroy, California, averaging 48 g. body weight, were separated into groups of five, housed individually in wire-bottomed cages, and allowed food and water *ad libitum*. The composition of the basal casein diet is given in Table IV. This diet was used only as a

TABLE IV
COMPOSITION OF BASAL DIET

INGREDIENT	PERCENT OF DIET
Crude casein[a]	17.2 (N × 6.25 = 14% protein)
Cerelose	50.8
Corn starch	20.0
Soybean oil	4.0
Salt, USP XIV	4.0
Vitamin mix[b]	2.0
Powdered cellulose	2.0

[a] Soybean ingredients were substituted for all of the casein and cellulose plus sufficient cerelose, in amounts necessary to maintain the protein level (N × 6.25) at 14%.
[b] Vitamin mixture supplied the following nutrients per 100 g. of diet: 1,800 units vitamin A, 200 units vitamin D, 10 mg. alpha-tocopherol, 90 mg. ascorbic acid, 10 mg. inositol, 150 mg. chlorine chloride, 4.5 mg. menadione, 10 mg. *p*-aminobenzoic acid, 9 mg. niacin, 2 mg. riboflavin, 2 mg. pyridoxine hydrochloride, 2 mg. thiamine hydrochloride, 6 mg. calcium pantothenate, 0.04 mg. biotin, 0.18 mg. folic acid, and 0.0027 mg. vitamin B_{12}.

general control. For statistical analysis of the data in Table II, a diet containing heated full-fat soybean meal was used. It was prepared by flaking dehulled chips between smooth rolls to a thickness of about 0.012 in., treating the flakes in an autoclave with live steam for 40 min. at atmospheric pressure, and then air-drying. Chips heated in a pressure cooker at 5 p.s.i. for 30 min. (diet 13) served as the control for statistical analysis of the data in Table III. The various soybean products were substituted in the basal diet at the expense of entire amounts of casein and cellulose plus sufficient cerelose to maintain the protein level (N × 6.25) at 14%. Protein efficiency (PE) was calculated from body weight gain divided by protein intake. Pancreas weights were determined by the method of Booth *et al.* (10). All feeding experiments were repeated at least once with fresh preparations, and no significant differences in the results were obtained.

Amino Acid Analyses. Dehulled soybeans and tempeh were defatted with hexane and hydrolyzed according to previously described procedures of Rackis *et al.* (11). Amino acid content of soybean meal and of tempeh, except for cystine and tryptophan, was determined by the procedures of Spackman, Stein, and Moore (12). Cystine was determined as cysteic acid according to the method of Schram, Moore, and Bigwood

(13); a forerun of 100 ml. of 0.01*N* acid was used to remove interfering substances. Tryptophan was determined by the method of Spies and Chambers (14). Defatted soybean meal and defatted tempeh were extracted with a mixture of methanol-chloroform (vol./vol.) and then with 80% ethyl alcohol to remove substances that interfere with tryptophan analysis (11).

Results and Discussion

Processing Losses in Making Tempeh. The dehulling, soaking, washing, cooking, and fermenting steps employed in the preparation of tempeh all contribute to loss of meal constituents. Average values for these losses obtained from a number of tempeh preparations are given in Table I.

In the mechanical dehulling operation, 90% or more of the hulls were removed with a Eureka seed cleaner. This hull fraction accounts for about 9.5% of the beans and contains small amounts of hypocotyl and cotyledon fines. Nitrogen content of the mechanically separated hulls was about 2%. The hulls, when separated by hand from Hawkeye and Harosoy soybeans, accounted for about 7.9% of the whole bean and contained 1.87% nitrogen.

The hull fraction, when removed after soaking from Harosoy soybeans, accounted for nearly 13% of the whole bean and contained 4.64% nitrogen on a dry basis. For Hawkeye soybeans the hull fraction accounted for 9.5% and contained 3% nitrogen. With both soybean varieties, however, up to 10% of the hull fraction may remain with the cotyledons.

The 1961 crop of Harosoy contained many hard beans (15), whereas only a few were in the Hawkeye variety. The hard beans have an average protein content of about 42%, and since in the wet method of separation they are eliminated with the hulls, this fraction has an abnormally high protein content. Thus, the wet dehulling procedure resulted in a greater loss of bean solids and of protein, particularly with Harosoy. When dehulled chips are used, the problem of hard beans is overcome, but much larger amounts of solids and protein are lost in soaking and cooking. Fermentation losses are about 1–5%. Changes in the protein content of the soybean products obtained during processing are given in Table I. The products contain approximately 18–22% oil and nearly 3% fiber.

Nutritive Value of Tempeh. The results of the rat-feeding experiments are presented in Table II. Diets 3 and 10 containing cooked chips and cotyledons, unfermented, gave approximately the same growth rate and PE values as the soybean control diets 2 and 9, respectively; therefore, little loss in nutritive value occurred before fermenta-

tion. However, fermentation had a variable effect on growth in that the mean weight gains of rats fed tempeh diets 4 through 7 were decreased in comparison with the soybean control group (diet 2), whereas the growth of rats on diet 11 containing cotyledon tempeh was not reduced. The decreases in growth on diets 4, 5, and 7 were statistically significant ($P < 0.05$). The PE values for diets 4 through 7 were significantly lower than the full-fat soybean control.

Fermentation losses in preparing tempeh used in diets 4 through 7 were greater than for diets 11 and 12. Changes in availability of essential growth substances during fermentation may be a factor affecting nutritive value of tempeh. Since methionine supplementation greatly enhanced both the growth response and the PE values of tempeh (diet 12), it was considered unlikely that any growth-inhibiting factors were formed during mold fermentation. Also, these results are consistent with the amino acid compositional data of soybean products (11), including amino acid data given in Table V. In all these prod-

TABLE V

AMINO ACID COMPOSITION AND PROTEIN SCORE OF DEHULLED SOYBEAN MEAL, COOKED COTYLEDONS, AND TEMPEH

Amino Acid	Dehulled Soybeans	Cooked Cotyledons	Tempeh [a]	Amino Acid	Dehulled Soybeans	Cooked Cotyledons	Tempeh [a]
	g./16 g. N [b]				*g./16 g. N* [b]		
Arginine	7.2	7.6	7.2	Leucine	7.9	7.9	8.0
Histidine	2.4	2.4	2.4	Isoleucine	4.9	4.7	4.9
Lysine	6.5	6.4	6.3	Valine	5.2	5.4	5.2
Tyrosine	3.6	3.8	3.7	Glutamic acid	18.5	19.5	17.5
Tryptophan	0.85	1.02	0.95	Aspartic acid	11.5	11.7	11.4
Phenylalanine	5.4	5.1	5.0	Glycine	4.3	4.3	4.3
Cystine	1.2	1.3	1.2	Alanine	4.4	4.4	4.6
Methionine	1.5	1.4	1.5	Proline	5.3	5.5	5.4
Serine	4.7	4.8	5.1	Ammonia	10.0	9.6	9.5
Threonine	3.8	3.9	3.9	Protein score [c]	63	65	63

[a] Cotyledons fermented 22 hr.
[b] Defatted with hexane before analysis; 24-hr. acid-hydrolysis period.
[c] Calculated from the provisional amino acid pattern of FAO (2).

ucts, methionine plus cystine are the most limiting amino acids for growth. Steinkraus *et al.* (4) fermented tempeh for longer periods up to 60 hr. and reported that the nutritive value decreases with increasing time of fermentation.

There was no evidence of pancreas enlargement in any of these groups (diets 1 through 12). Nor was there any improvement in growth or PE values when autoclaved tempeh (diets 5 and 7) was fed. Therefore, the normal heat-treatment in preparing tempeh is apparently adequate to inactivate the growth inhibitor(s) originally present in soybeans (9,10).

Nutritive Value of Inhibited Tempeh. Inhibited tempeh made from

either cotyledons or chips (5) had a very unpleasant odor and an astringent taste, and was, therefore, unsuitable as a food. The mycelium growth was very poor and contained a large amount of sporulation. In spite of poor mycelium growth, solids losses of 1 to 4% were obtained during fermentation.

Rat-feeding tests were made on freeze-dried inhibited tempeh (Table III) to determine whether the altered fermentation process would adversely affect growth. Weight gains and PE values for inhibited tempeh (diet 15) prepared from chips steamed 30 min. were comparable to those from autoclaved chips in diets 13 and 14. However, rats fed inhibited tempeh (diet 16) prepared from chips autoclaved for 60 min. showed reduced weight gains and PE values. Diet 13 was used as a control for statistical analysis of data in Table III, since there were no significant differences in weight gains and PE of diets 13 and 14. All pancreas weights were within the normal range. Inhibited tempeh made from cotyledons affected growth and PE in a similar manner and, therefore, the data were not included in Table III.

Changes in Amino Acid Content of Tempeh. The amino acid content of tempeh was determined on Hawkeye soybeans (1961 crop) after 22 and 30 hr. of fermentation; the cystine values were also determined separately after 18, 24, and 30 hr. Results for the 22 hr. of fermentation, compared with amino acids of defatted meal and cooked cotyledons from the same beans, are shown in Table V as g. per 16 g. nitrogen. The 30-hr. fermentation was not significantly different from that of the 22-hr. period. The cystine values after 18, 24, and 30-hr. fermentations were 1.2, 1.3, and 1.2 g. per 16 g. nitrogen, respectively.

The amino acid values for tempeh, fermented up to 30 hr., do not show any significant change from the controls within the error of our amino acid assays of about ±5%. Thus, we are unable to explain the reduction in nutritive value of tempeh by our amino acid assays. However, we do not believe the results exclude the possibility that a small loss of methionine or cystine, or both, may, since they are limiting amino acids, be the cause of lower PE for the tempeh. The animal may be more sensitive to a small loss of sulfur amino acid than the method of assay. Since Steinkraus *et al.* (4) showed losses of methionine of about 4 and 11% in tempeh after 36 and 60 hr., respectively, and losses of lysine of 11 and 24% for the same periods, we believe the evidence would indicate possible losses of sulfur amino acids during 24–30 hr. of fermentation might be significant with respect to animal growth. Changes in the availability of sulfur amino acids for rat growth may also be a factor.

Although a substantial loss of solids occurs during the production

of tempeh, much of the soluble protein can be precipitated and recovered (16) by adjusting the pH of the washings to 4.5. Of more importance is the variation in the amount of fermentation which may occur within a given period of incubation; these differences in extent of fermentation may vary the nutritive value of the final product. As a result, some measurement of the extent or rate of fermentation will have to be devised to have a uniform nutritive value for tempeh.

The fermentation must also be controlled sufficiently to give enough mycelium growth to bind the cotyledons or chips together so that tempeh can be sliced thin and fried in vegetable fat or cut into small pieces to put in soup. These are the common forms in which tempeh is usually consumed. The results obtained with inhibited tempeh indicate that the heat-stable, water-soluble factor (or factors) which inhibits mycelium growth may also affect the normal metabolic pathways of the mold, resulting in end products that impart an undesirable odor and flavor. The nutritive value of inhibited tempeh varied similarly to that of tempeh made traditionally.

Preliminary tests with cotyledons and chips indicated that at least 30-min. steaming at 100°C. was required to destroy completely the growth inhibitor and pancreatic hypertrophic factors in soybeans, whereas only 15 min. of steaming are required for flakes (9). Particle size, therefore, influences the amount of heat-treatment needed. Rackis *et al.* (17) previously reported that because the pancreatic hypertrophic factor in certain of the meal fractions is relatively more heat-stable, changes in processing conditions to achieve maximum PE are required.

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