QUALITY CHARACTERISTICS OF SOY AND SOY-WHEAT TEMPEH

by

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INTRODUCTION

An estimated ten million people in the United States are vegetarians (Shurtleff and Aoyagi, 1979). Some so-called vegetarians consume animal products. Ovolactovegetarians eat eggs and dairy products but no meat, fish, or poultry. Some vegetarians include fish or poultry in their diets. Strict vegetarians (vegans) do not consume any animal products. Vegans have difficulty meeting their nutrient needs on such a diet. Special nutritional concerns of the vegan are protein, iron, and vitamin B_{12}.

Soybean products often are consumed by vegetarians throughout the world as a protein source. These products can be either non-fermented as tofu, or fermented as miso or tempeh. Soy or soy-wheat tempeh is a possible meat alternate for vegetarians and other health-conscious people. Protein quantity and quality of tempeh are similar to that of meat products (Shurtleff and Aoyagi, 1979). A 100-gram serving of soy tempeh provides 5 mg of iron (28% of the U.S. Recommended Dietary Allowance) (RDA) and the bioavailability of this iron is reported to be improved over that of soybeans (Moeljopawiro et al., 1987). Tempeh is the only non-animal food known to contain a significant amount of vitamin B_{12}. A specific bacteria must be

present during fermentation to produce this vitamin B_{12}. Tempeh is low in saturated fat and calories, contains no cholesterol, has a high amount of dietary fiber, and is rich in other B-vitamins.

The purposes of this paper are to: 1) discuss the preparation of tempeh; 2) review the sensory and microbiological characteristics of tempeh; and 3) discuss the nutritional contributions of tempeh in the diet.

IMPORTANCE OF SOYBEANS AS A PROTEIN SOURCE

Although soybeans have been used in the Orient since ancient times, their widespread use in the United States (U.S.) has been limited to recent years. Less than 4 million bushels of soybeans were produced in the U.S. in 1922 (Smith and Circle, 1978), while 1.9 billion bushels were produced in 1987. Kansas farm production accounted for 67,520,000 bushels (Thiessen, 1988). Low cost of producing high quality oil and protein is largely responsible for the soybeans success in U.S. markets. Likewise, tempeh provides another inexpensive use of soybeans. Only the hull and solids comprising less than 25% of the soybeans are lost during tempeh manufacture.

Three primary sources of high-quality protein are fish, beef, and soybeans (Shurtleff and Aoyagi, 1979); and legumes are the major source of dietary protein in many

developing countries (Gandjar, 1986). Soybean protein is an abundant and a less expensive protein source than fish or beef. In East Asia soybeans are known as "meat of the fields" (Shurtleff and Aoyagi, 1979).

Soybeans are used as unprocessed whole dry or fresh green soybeans, soy flour, traditional East Asian low-technology processed foods, modern high-technology processed foods (textured vegetable protein, soy isolates, concentrates), and fodder for livestock (Shurtleff and Aoyagi, 1979). Large-scale production and export of soybeans, accompanied by an emphasis on plant materials as foods have renewed interest in fermented foods (Hesseltine and Wang, 1980).

Fermented soybean products are palatable, stable alternatives to the poorly digested, unpalatable cooked soybeans (Platt, 1964). In the Orient fermented foods generally are produced from soybeans and filamentous fungi with lesser amounts of combinations or soybeans and cereals fermented by bacteria, yeast, and fungi (Table 1). Oriental foods such as miso, tofu, soy sauce, and tempeh are becoming more popular among non-Oriental people in the United States (Hesseltine, 1983). Problems with acceptance of fermented foods in the United States are the high-sodium contents of many of these products and the possibility of food-borne illnesses (Hesseltine, 1983).

Table 1-- Oriental fermented soybean foods.

Food	Organisms used	Substrate
Soy sauce	*Aspergillus*, *Pediococcus* *Torulopsis*, *Saccharomyces*	Soybeans, wheat
Miso	*Aspergillus*, *Pediococcus* *Saccharomyces*, *Torulopsis* *Streptococcus*	Soybeans, rice, barley
Hamanatto	*Aspergillus*, *Streptococcus* *Pediococcus*	Soybeans, wheat
Sufu	*Actinomucor*, *Mucor*	Tofu
Tempeh	*Rhizopus*	Soybeans or wheat
Natto	*Bacillus natto*	Soybeans

Source: Wang, 1984

Soaking and heating, salting, acid formation, antibiotic formation, alcohol formation, low surface moisture, and decrease of aflatoxin by Rhizopus and Neurospora contribute to the safety of fermented foods (Wang and Hesseltine, 1981).

Fermentation is a process by which microorganisms or enzymes hydrolyze complex molecules to simpler compounds and utilizes part of the substrate for energy. Wang and Hesseltine (1979) reported the following advantages of producing foods by fermentation: production of desirable enzymes; destruction or masking undesirable flavors and odors; addition of desirable flavors and odors; preservation; synthesis of desirable constituents such as vitamins or antibiotics; improvement in digestibility; and reduction in cooking time.

Fermented foods are used for main course foods, as flavoring or coloring agents, and to change the physical state of the substrate (Hesseltine, 1983). Typical examples of Western fermented foods are cheese, bread, and beer. Cheese is produced with molds and bacteria, and bread and beer are produced with yeasts.

TEMPEH -- A TRADITIONAL FERMENTED FOOD

Tempeh is the only traditional fermented food extensively studied in the West. Indonesians developed a

fermented product, tempeh kedelee, without the aid of modern microbiology or chemistry (Steinkraus, 1983). Tempeh fermentation is similar to cheese fermentation in that hydrolysis of proteins and lipids occurs, flavor intensifies, and free ammonia is released (Steinkraus, 1983). Steinkraus (1980) has described tempeh as a single cell protein grown on an edible substrate.

Tempeh is a fermented cake made of dehulled, partially cooked soybeans bound together by dense cottony mycelia of Rhizopus oligosporus mold (Steinkraus et al., 1960; Shurtleff and Aoyagi, 1979). Tempeh is produced and consumed in Indonesia, Malaysia, Holland, Canada, West Indies, and the United States (Steinkraus, 1983). Tempeh can be used as a main course and prepared in a variety of ways (Shurtleff and Aoyagi, 1979). Protein content and nutritive value make tempeh a good substitute for meat (Steinkraus, 1983).

Fermented tempeh has advantages over unfermented soybeans. During the fermentation process, beany flavor and odor decrease and desirable odors and flavors increase. Nutrititive value and digestibility of tempeh is better than the substrate. Cooking time of tempeh is less than the cooking time required for raw soybeans (Steinkraus, 1983).

Tempeh production in the tropics involves two

distinct fermentations periods. The first which occurs during soaking results in acidification of the soybeans by bacterial fermentation. The second is fungal and results in mycelial growth (Steinkraus, 1983). Tempeh fermentation binds the soybeans into a compact cake, and the soybeans undergo a partial digestion by the mold enzymes (Steinkraus et al., 1960).

Tempeh can be made from substrates other than soybeans (Steinkraus, 1983). Other varieties of tempeh are one of five general types: legume tempeh, grain and soy blends of tempeh, grain tempeh, presscake tempeh, and nonleguminous seed tempeh (Shurtleff and Aoyagi, 1979). Tempeh has been made from peanut, peanut and soy, millet, millet and soy, rice and soy, wheat and soy (Shurtleff and Aoyagi, 1979), ground-nut, sunflower-seed, nut or seed and soy (Vaidehi et al., 1985), and bakla (an Indian pulse) and soy (David and Verma, 1981). Soybeans are the most common substrate for the preparation of tempeh, followed by wheat and a soy-wheat blend (Hesseltine et al., 1967; Wang, 1984).

PREPARATION OF TEMPEH

Production

Figures 1 and 2 show flow diagrams of soy and soy-

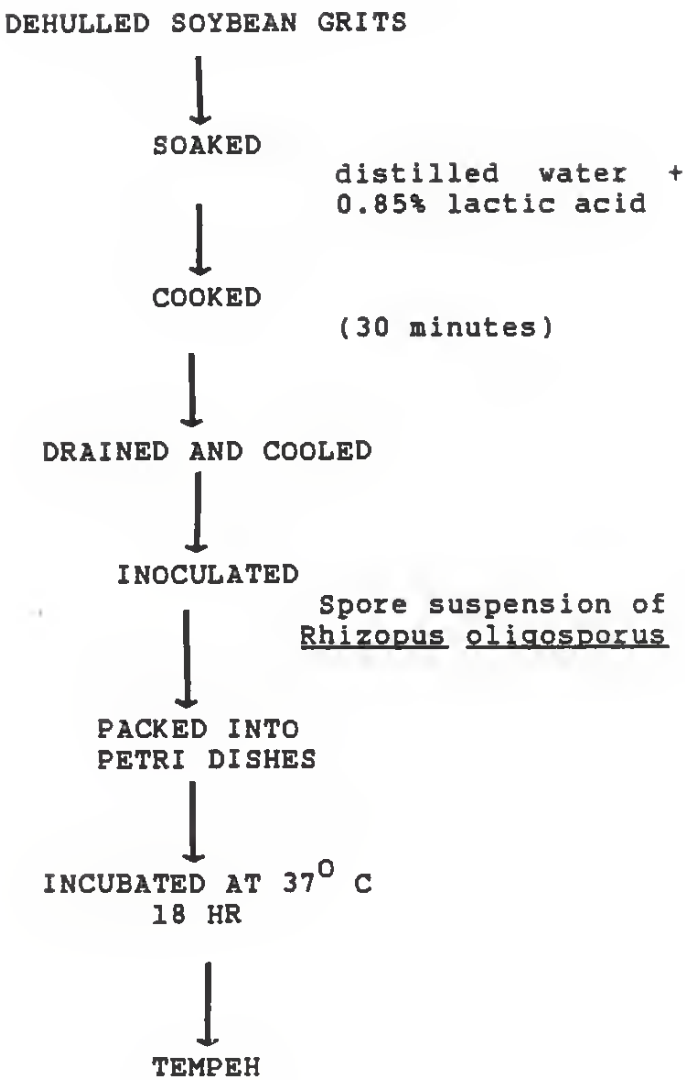

Fig. 1-- Flow diagram of soy tempeh production.

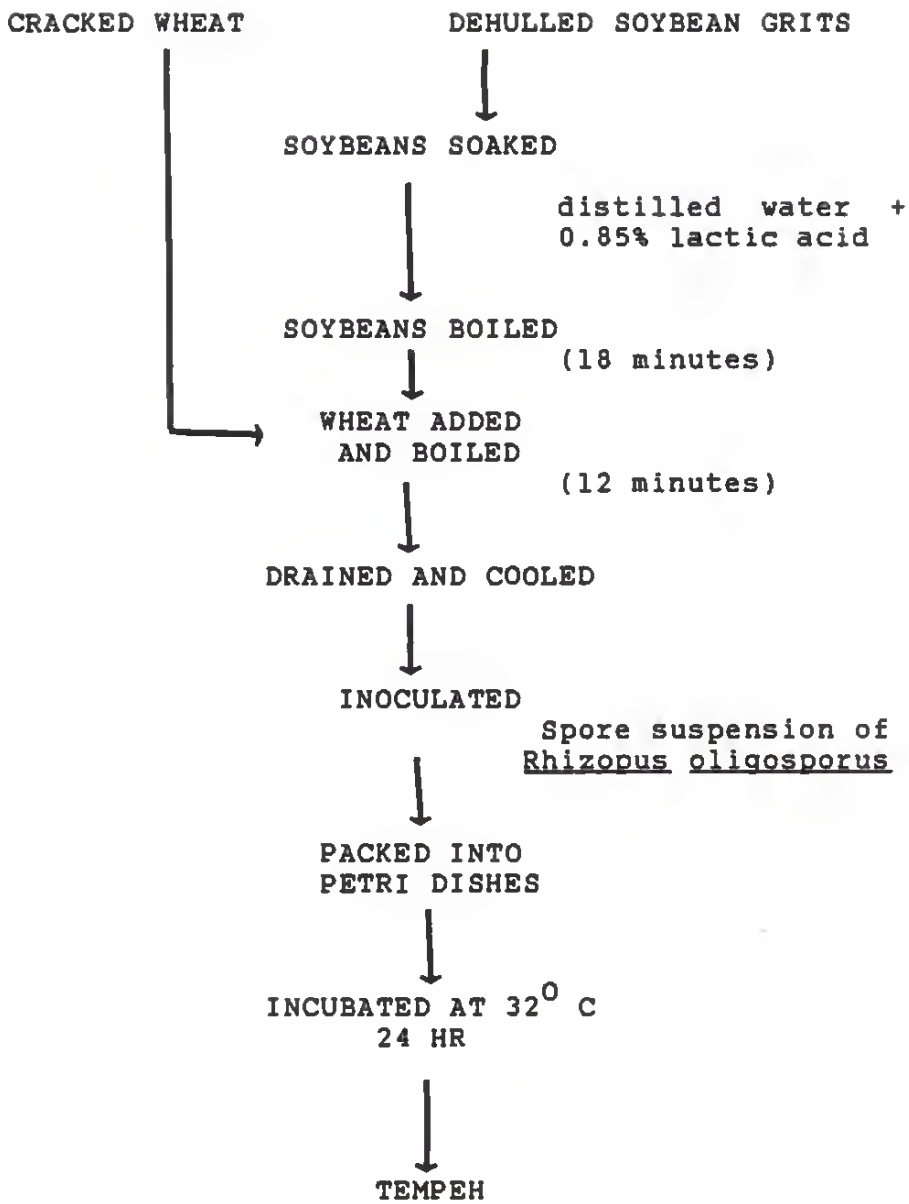

Fig. 2-- Flow diagram of soy-wheat tempeh production.

wheat tempeh production under laboratory conditions. Conditions for tempeh production are flexible as long as the basic requirements for moisture, oxygen, and heat are fulfilled (Steinkraus, 1983). Major steps in soy and soy-wheat tempeh production are preparation of soybeans and wheat, hydration of soybeans, boiling, draining and cooling, inoculation with Rhizopus, and incubation.

Careful handling of the substrate is important throughout tempeh production. Microbial contamination must be prevented or minimized because tempeh is an ideal medium for growth of many microorganisms. Bacterial growth will overcome the mold resulting in spoilage when boiling time is too short, the beans are still wet during inoculation, incubation temperature is too high, or incubation time is too long (Samson et al., 1987).

Preparation of substrates

Soybeans must be dehulled (Steinkraus, 1983) and wheat must be cracked (Wang and Hesseltine, 1966) before hydration so the mold can reach nutrients in the cotyledons (Steinkraus et al., 1960; Gandjar, 1986). Beans are dehulled mechanically using a roller mill (Wang and Hesseltine, 1979). Martinelli et al. (1964) used full-fat soybean grits (soybean cotyledons that have been mechanically cracked into four to five pieces) in their

study. Since these grits absorb water easily, soaking time was reduced to 30 minutes at 25°C. Whole soybeans or soy grits produce good quality tempeh. When whole soybeans are used, larger spaces exist between the beans which allows for more aeration in the center of the tempeh (Martinelli et al., 1964). Whole wheat kernels produce poor quality tempeh (Wang and Hesseltine, 1966), so cracking is a prerequisite when tempeh is made from wheat.

Hydration

Soybeans are soaked in excess water for 12 to 15 hours at room temperature to facilitate mycelia penetration. Soak water is acidified artificially with 0.85% lactic acid (Steinkraus, 1983). Lowering the initial pH by the addition of acid allows for a longer fermentation time before the mold is killed (Steinkraus, 1983) and limits bacterial contamination (Steinkraus et al., 1965; Tanaka et al., 1985). Mold growth is not inhibited by acidity until the pH falls below 3.5 (Steinkraus et al., 1960). Wheat does not have to be soaked before boiling (Wang and Hesseltine, 1966) to produce good quality tempeh.

Boiling

Soybeans and wheat are heated in water to destroy

microorganisms, to destroy trypsin inhibitor in soybeans, and to release nutrients for mold growth (Steinkraus, 1983). Hydrated soybeans are boiled in an excess amount of water for 25 min for soy tempeh. For preparation of soy-wheat tempeh, soybeans are boiled 13 min, then wheat is added and the mixture is boiled 12 additional minutes. Table 2 contains boiling times for various substrates used in tempeh production.

Draining and cooling

After boiling soybeans or soybeans and wheat are drained and then air-dried on paper toweling. Soybeans or a soybeans-wheat blend are cooled to 37^{o} C before inoculation. Excess water is removed to prevent contaminating bacterial growth which would decrease the shelf life of tempeh (Steinkraus, 1983).

Inoculation

In Indonesia, small pieces of soybean tempeh from a previous batch are kept in the open air to obtain full sporulation. These pieces are ground and used as the inoculum (Wang et al., 1975a; Wang, 1984; Gandjar, 1986). In the United States, pure cultures of Rhizopus oligosporus are used for inoculation. Pure culture inocula of Rhizopus spores are either lyophilized or suspended in

Table 2-- Boiling time required by different substrates for fermentation.

Substrate	Form of substrate	Boiling time (min)
Soybeans	Dehulled, coarse grits	25
Wheat (hard)	Cracked	12
White wheat	Cracked	12
Barley	Dehulled and cracked	12
Oats	Dehulled and cracked	8-10
Rye	Cracked	12
Corn	Cracked	25
Sorghum	Cracked	25
Peanuts	Roasted, dehulled, sliced	25
Rice	Polished and cracked	10

Source: Hesseltine et al., 1967

water on agar slants. Excess inoculum is necessary to insure rapid and uniform fermentation (Martinelli et al., 1964). If too much inoculum is used, the fermentation time becomes critical. If too little inoculum is used, bacteria are allowed to grow (Wang et al., 1975a). Wang et al. (1975a) have recommended 1×10^6 spores per 100 g cooked soybeans or wheat for optimal fermentation.

Rhizopus oligosporus NRRL 2710 (Northern Regional Research Laboratory, USDA, Peoria, Illinois) is the recommended strain of mold for tempeh fermentation (Steinkraus, 1983). Tempeh made of a pure culture of Rhizopus lacks vitamin B_{12}, the vitamin deficient in vegetarian diets. Klebsiella pneumoniae becomes an essential organism for tempeh fermentation if tempeh is to serve as a source of vitamin B_{12} (Steinkraus, 1983).

Steinkraus et al. (1960) originally believed Rhizopus oryzae to be the organism responsible for tempeh fermentation. Later, Steinkraus (1983) reported R. oligosporus was the species responsible. Rhizopus oligosporus NRRL 2710 has many characteristics that make it suitable for tempeh production (Steinkraus, 1983). They include:

1. growth between 30 to 42° C
2. inability to ferment sucrose

3. high proteolytic activity resulting in release of free ammonia after 48 to 72 hr fermentation (Wang and Hesseltine, 1965; Wang and Hesseltine, 1979; Steinkraus, 1983)

4. high lipolytic activity

5. production of a strong antioxidant (Gyorgy, 1961)

6. ability to produce tempeh (Wang and Hesseltine, 1979; Steinkraus, 1983)

7. ability to grow on wheat or other cereal substrates without producing noticeable amounts of organic acids because of minimal amylase activity (Wang and Hesseltine, 1966)

8. inhibition of the growth, sporulation, and production of aflatoxin

9. biosynthesis of B-vitamins (Murata et al., 1968).

Incubation

Incubation temperatures vary between 25 and 37° C. Within this limited range, generally the higher the incubation temperature, the more rapid mold growth will occur (Steinkraus, 1983). Incubation at 37°C favors growth of R. oligosporus over mesophilic molds (Steinkraus, 1983).

During rapid fermentation, internal temperature of the tempeh rises approximately 5 to 7 °C above the incubator temperature (Steinkraus, 1983). Temperature of the fermenting tempeh is indicative of the rate of mold

growth: 1) lag phase--germination of spores 2) slow mold growth 3) rapid growth--temperature of tempeh exceeds incubator temperature 4) temperature peak; temperature gradually falls; sporulation and ammonia production (Steinkraus et al., 1960). If the incubation temperature is 37° C, care must be taken to prevent the temperature of the tempeh from rising above 42° C, which retards mold growth (Steinkraus, 1983). Tempeh is harvested as soon as the soybeans have been overgrown completely and knitted into a compact cake (Steinkraus, 1983).

High humidity is necessary for optimal mold growth during incubation (Steinkraus et al., 1960). Humidity can be elevated by placing a tray of water in the bottom of the incubator (Martinelli et al., 1964). If sufficient air is unavailable to the mold, it will not grow. If an excessive amount of air is supplied, the tempeh's surface will dry out before the mold starts to develop spores (Martinelli et al., 1964).

Changes during fermentation

Seventy-five percent of the original soybean solids are recovered in tempeh in the form of fermented products. Three percent solids are lost during fermentation with the remainder lost during dehulling, soaking, and cooking (Smith et al., 1964; Zamora and Veum, 1979). Changes in

solids and protein during tempeh production are presented in Table 3. Soluble solids increased from 13 to 21%. The pH value increased from 5.0 to 7.6 with optimal quality at pH levels from 6.3 to 6.5 (Steinkraus et al., 1960). Optimal tempeh (organoleptically) was obtained when pH reached 6.5 and soluble solids were 21% (Steinkraus et al., 1960). Titratable acidity increases during fermentation (Wagenknecht et al., 1961). A steady increase in pH occurs throughout fermentation because of liberation of ammonia or other end products of protein decomposition (Wagenknecht et al., 1961).

CHARACTERISTICS OF TEMPEH

Sensory

Flavor and texture are derived from the fermentation process (Shurtleff and Aoyagi, 1979). Raw tempeh has a clean, fresh, yeasty or mushroom-like odor (Hesseltine et al., 1963; Wang and Hesseltine, 1979; Steinkraus, 1983) with a slightly cheese-like flavor (Steinkraus et al., 1960). Fried tempeh has a nutty flavor and aroma (Hesseltine et al., 1967). Other quality descriptors for uncooked and fried tempeh are presented in Tables 4 and 5.

Table 3 -- Losses of solids and protein during tempeh preparation.

Material and procedure	Loss of solids (%)	Loss of nitrogen (%)	Protein content (%dry wt)
Whole soybeans			43.0
Dehulling and Soaking	12.6	9.5	
Cooking	11.0	10.0	51.7
Fermentation	3.4	1.7	53.1
Total loss	27.0	19.7	
Dehulled coarse grits			44.7
Soaking and cooking	38.0	31.4	48.6
Fermentation	5.0	2.0	50.0
Total loss	43.0	33.4	

Source: Shurtleff and Aoyagi, 1979

Table 4 -- Evaluation of uncooked tempeh products.

Substrate	Odor	Appearance	Ability to slice
Wheat	Yeasty, fragrant	Brownish-gray with spores	Poor
Soybeans	Slight ammoniacal	White with spores at edge	Good
Soy/Wheat			
3/1	Like soy	Speckled	Good
2/2	Yeasty; like soy	Speckled	Excellent
1/3	Yeasty; like soy	Dark	Poor

Source: Hesseltine et al., 1967

Table 5 -- Evaluation of fried tempeh products.

Substrate	Appearance	Odor	Flavor and acceptability
Soybeans	Excellent	Pleasant	Excellent
Wheat	Surface rough; brown	Pleasant	Like popcorn

Source: Hesseltine et al., 1967

Safety

Microbiological safety of tempeh in the United States at the consumer level is unknown (Tanaka et al., 1985). High numbers of bacteria, yeasts, and molds were found in commercial tempeh in the Netherlands (Samson et al., 1987). Samson et al. (1987) reported the probable origin of these microorganisms to be survival of spore forming bacteria during boiling, recontamination during draining, cooling, or contaminated inoculum. For these reasons eguipment used in tempeh fermenation should be sanitized (Tanaka et al., 1985).

Tempeh can be unsafe if pathogens are present when fermentation begins (Tanaka et al., 1985). Bacteria and yeasts grow along with the *Rhizopus* during and after tempeh fermentation (Samson et al., 1987). Growth of yeasts and bacteria continues during storage at room temperature (Samson et al., 1987).

In a study by Tanaka et al. (1985), tempeh was inoculated with one of the following strains of pathogenic bacteria (*Clostridium botulinum*, *Staphylococcus aureus*, *Salmonella typhimurium*, or *Yersinia enterocolitica*) either before or after fermentation. *Clostridium botulinum* toxin was produced within 2 days of fermentation. The toxin was produced within 5 days of storage in a vacuum pouch when inoculated after fermentation. *S. aureus* grew during

fermentation and when applied after fermentation. Enterotoxins were detected within 2 days. S. typhimurium grew extremely well during fermentation but did not grow well when added after fermentation. Y. enterocolitica grew well both during fermentation and during storage above 5°C.

S. aureus is a concern because of a heat-stable toxin that can be produced (Samson et al., 1987). No toxins were produced during the normal fermentation time of 24 hours or less (Tanaka et al., 1985). Botulinal toxin and staphylococcal enterotoxin were present after 48 hour fermentation indicating that toxins could develop in tempeh produced by a slow or long fermentation (Tanaka et al., 1985). Salmonella, Staphylococcus, and Yersinia should be destroyed during the initial boiling time (Tanaka et al., 1985).

Klebsiella pneumoniae is a species of Gram-negative, facultatively anaerobic bacteria which is widely distributed in soil, water, and the gastrointestinal tract. Klebsiellae are opportunistic pathogens which can lead to bacteremia, pneumonia, urinary tract, and other infections. Many of the Klebsiella strains show multiple antibiotic resistance (Krief and Holt, 1984).

Steinkraus (1983) reported that Klebsiella pneumoniae is a common organism on plant materials and that the

bacterium is probably present during tempeh fermentation. He stated that Klebsiella must be present during tempeh fermentation if the tempeh is to serve as a source of vitamin B_{12}. Unique characteristics of Klebsiella in tempeh fermentation include: does not spoil the tempeh, grows at temperatures from 15 to 45°C, does not interfere with Rhizopus growth, grows at pH as low as 3.5, and produces vitamin B_{12}.

Rhizopus oligosporus produces a compound that inhibits the growth of lactic acid bacteria (Wang et al., 1969). Many Gram-positive organisms were sensitive to the antibiotic; Klebsiella pneumoniae was the only Gram-negative organism sensitive to the antibacterial compound produced by R. oligosporus (Wang et al., 1969). The antibiotic produced by R. oligosporus behaved as many other antibiotics in that at low concentrations it stimulated bacterial growth (Wang et al., 1969). Contradictory findings were reported by Salsman (1987) because Klebsiella growth was evident on plates made with violet red bile agar acidified with acriflavine (Table 6).

Nutritional value of tempeh

Nutritive value of soy and soy-wheat tempeh makes them good substitutes for meat. Soy tempeh contains 157

Table 6--Growth of microorganisms in tempeh inoculated with *Klebsiella pneumoniae*.

Fermentation time (hrs)	Potato dextrose agar	Violet red bile agar with 0.6% acriflavine
0	-	-
1	-	-
2	-	-
3	-	-
4	-	-
5	-	+
6	-	+
7	+	+
8	+	+
9	+	+
10	+	+
11	+	+
12	+	+
13	+	+
14	+	+
15	+	+
16	+	+
17	+	+
18	+	++
19	+	++
20	+	++
21	++	++
22	++	++
23	++	++
24	++	++

- no growth
+ growth -- isolated colonies
++ growth -- plate completely covered with organisms

Source: Salsman, 1987

calories per 100 g, is low in cholesterol and saturated fat, is high in fiber and most B-vitamins including B_{12}, and has good quality protein (Shurtleff and Aoyagi, 1979). Digestibility has been reported to increase (Shurtleff and Aoyagi, 1979) but data by Hackler et al. (1964) did not support improved digestibility.

Proximate composition

During tempeh fermentation proximate composition changes slightly (Table 7). Crude fat and protein increase in soy and soy-wheat tempeh while the percentage of nitrogen-free extract decreases (Zamora and Veum, 1979; Wang, 1986). Carbohydrates decrease in wheat and soy-wheat blends of tempeh and increase in soy tempeh. Wang (1986) has been suggested that the mold utilizes carbohydrates as an energy source which causes a reduction in carbohydrates in tempeh made from wheat. Rhizopus apparently utilize a non-carbohydrate source of energy, i.e. triglycerides, for growth (Wang et al., 1968; Wang, 1986).

Protein

Fresh soy tempeh contains an average of 19.5% protein, comparable to the protein content of meat products. On a dry-solids basis tempeh contains over 40%

Table 7-- Effect of 24-hr fermentation on the proximate composition of wheat and soybeans.

	Ash	Ether extract	Protein	Fiber	Carbohydrates
	%				
Wheat, control	1.7	1.9	17.4	2.6	76.5
Wheat, fermented	1.8	2.0	18.2	3.1	74.9
Soybeans control	3.4	26.8	47.8	3.9	18.1
Soybeans fermented	3.3	24.7	48.1	3.1	20.9
Blend[a] control	2.5	12.5	31.6	2.8	50.7
Blend[a] fermented	2.6	12.1	33.1	2.7	49.6

[a] wheat : soybean (1:1)

Source: Wang, 1986

protein (Steinkraus, 1983). Soy tempeh is a complete protein in that it contains all the essential amino acids. Soy protein is rich in lysine which is an essential amino acid lacking in cereal grains. The first limiting amino acid in soybeans is methionine (Shurtleff and Aoyagi, 1979). Thus, combining soybeans with cereals can form a complete protein (Gandjar, 1986). Table 8 consists of the amino acid pattern of unfermented and fermented soy-wheat blend. Protein quality analysis of soy-wheat tempeh supports the complementarity of this tempeh combination.

Amino acid profile. Fermentation does not alter the amino acid profile of cereals or soybeans (Table 9) but can make them more biologically available (Hesseltine, 1983). This is shown by the improvement in net protein utilization (Zamora and Veum, 1979). Net protein utilization is slightly less for tempeh than for chicken or beef. Amino acid content decreases slightly or remains the same during tempeh fermentation except for tryptophan which significantly increased in tempeh fermented 24 hr (Stillings and Hackler, 1965; Murata et al., 1967). After 5 min of deep-fat frying, lysine and cysteine decreased, with few changes in the other amino acids (Stillings and Hackler, 1965).

Table 8-- Amino acid pattern of soy-wheat tempeh.

Amino acid	Control	Fermented
	mg/g	
Cystine	53	56
Isoleucine	190	176
Lysine	138	154
Methionine	45	45
Phenylalanine	132	106
Threonine	91	99
Tryptophan	24	25
Tyrosine	90	93
Valine	123	127

Source: Wang et al., 1968

Table 9-- Effect of fermentation on the amino acid composition of a mixture of wheat and soybeans (1:1).

Amino acid	Control	Fermented
	g/16 g N	
Alanine	3.53	5.28
Arginine	5.70	4.50
Aspartic acid	8.00	8.30
Cystine	1.83	1.92
Glutamic acid	21.08	14.82
Glycine	3.56	3.46
Histidine	2.15	2.24
Isoleucine	3.96	4.02
Lysine	4.82	5.26
Methionine	1.58	1.58
Phenylalanine	4.59	3.61
Proline	6.63	3.72
Serine	4.32	3.81
Threonine	3.16	3.40
Tryptophan	0.85	0.85
Tyrosine	3.12	3.20
Valine	4.30	4.34

Source: Stillings and Hackler, 1965

Protein efficiency ratio (PER). Murata et al. (1971) found the PER of soy tempeh to be similar to the PER for that of unfermented soybeans. Gyorgy (1961) reported the PER of tempeh superior to soybeans at a 10% protein level but not at 20%. A low PER for tempeh could be caused by a loss of sulfur-containing amino acids during fermentation (Smith et al., 1964). The PER of tempeh supplemented with lysine, methionine, and threonine was improved to the same level as tempeh with added egg (Murata et al., 1971). Hackler et al. (1964) studied the effects of deep-frying and steaming on PER and apparent digestion coefficients (Table 10). PER declined following 3 or more min deep-frying but steaming showed no significant effect. Apparent digestion coefficients indicated decreased nitrogen absorption with increased deep-frying time but no change was noted with steaming.

PER of soy-wheat tempeh is significantly higher than the PER of either substrate fermented alone (Table 11). This increased PER cannot be explained by the amino acid composition since the amino acid profile was not changed significantly. Increased PER has been explained by an increased availability of lysine in wheat (Wang et al., 1968). Pepsin and pancreatin digestion indicated a 15% increase in lysine during fermentation of wheat with

Table 10-- Effect of steaming time on PER and apparent digestion coefficient of tempeh.

Steaming time (min at 100°C)	PER	Digestion coefficient (%)
Casein control	2.76	93.1
15	2.21	88.7
30	2.10	88.6
60	2.19	87.0
120	2.10	86.1

Source: Hackler et al., 1964

Table 11-- PER changes of soybeans, wheat, and soy-wheat blend during tempeh fermentation.

Protein source	PER	
	Cooked, unfermented	Raw tempeh
Soybeans	2.17	2.27
Soybeans and wheat	2.49	2.79
Wheat	1.28	1.71
Casein (reference)	2.81	

Source: Wang et al., 1968

little change in the other essential amino acids (Wang et al., 1968).

Lipids

Soybeans are rich in lipids containing 18% in mature seeds and 20-26% by dry weight (Wagenknecht et al., 1961). Glycerides are the primary lipids in soybeans. Sudarmadji and Markakis (1977) reported a 24.5% decrease in fat during fermentation of soybeans with R. oligosporus. Wagenknecht et al. (1961) reported total fat remained constant during fermentation with R. oryzae.

Mold exhibited strong lipase activity hydrolyzing over one-third of the soybean neutral fat during three days of fermentation (Sudarmadji and Markakis, 1977). Glycerides in soybeans are broken down into free fatty acids during the first 30 hrs of tempeh fermentation (Wagenknecht et al, 1961; Sudarmadji and Markakis, 1977). Cooked soybeans contained only 1% free fatty acid compared to 30% in tempeh. Linoleic and linolenic acid were not found in the free form in soybeans but linolenic acid was found to be 53% of the total free fatty acids of tempeh (Table 12). Free amino acids could improve nutritional value.

Sudarmadji and Markakis (1977) suggested bacteria accompanying the mold during fermentation could be

Table 12-- Distribution of free fatty acids during soy tempeh fermentation.

mg/100 g tempeh	Cooked soybeans	24-hour tempeh	69-hour tempeh
Palmitic	41	420	863
Stearic	31	175	367
Oleic	127	713	1671
Linoleic	0	2510	5032
Linolenic	0	293	302

Source: Wagenknecht et al., 1961

responsible for the lipolytic activity rather than the Rhizopus because fatty acids are liberated even after mold growth has stopped. Total fat content of tempeh increased by 300% during deep-fat frying in coconut oil. After frying, free fatty acid content decreased in tempeh and increased in the coconut oil (Sudarmadji and Markakis, 1977).

Murakami et al. (1984) found tempeh oil to be stable to oxidation. Gyorgy (1961) was the first to report the anitoxidant produced during the tempeh fermentation process which was measured by peroxide values. The lipid of tempeh was more stable against autoxidation than unfermented soybeans, and antioxidant activity of tempeh increased with fermentation time (Ikehata et al., 1968). Comparison between boiled soybeans and tempeh seems to support the presence of antioxidants in tempeh (Gyorgy et al., 1964). Stahl and Sims (1986) questioned the presence of an antioxidant in tempeh oil because of sensory evaluations and measurement of oxygen absorption rate.

Carbohydrates

Soybeans contain 34% carbohydrates which include sucrose and other sugars, stachyose, raffinose, pentosans, and galactans but contains little or no starch. Carbohydrates are the primary solids lost during washing,

soaking, dehulling, and cooling. Shurtleff and Aoyagi (1979) cited Shallenberger et al. (1976) who reported raffinose decreased by 52% and stachyose by 49% during heating of soybeans. Reduction in oligosaccharides contribute to better digestibility of tempeh as compared to cooked soybeans. Stachyose and raffinose are oligosaccharides that contribute to flatulence in unfermented soybeans (Shurtleff and Aoyagi, 1979). Tempeh has less flatulence potential than soybeans because of the reduction of raffinose and stachyose and/or because the antibiotic formed by Rhizopus oligosporus kills intestinal bacteria. These bacteria, such as Clostridium perfingens, usually break down oligosaccharides to produce sucrose and intestinal gas (Shurtleff and Aoyagi, 1979).

During tempeh fermentation other carbohydrates decrease. Hemicellulose (galactans and pentosans) decrease by 50%. Hexoses contained in small amounts in soybeans also decrease rapidly (Shurtleff and Aoyagi, 1979). Fiber content increases during tempeh fermentation as the mold mycelium develops, but there is a loss of other solids.

Some strains of Rhizopus produce lactic, fumaric, and other organic acids. When wheat or other high-starch substrate is used, the amylolytic enzymes produced by these strains hydrolyze starch to sugars which are then

fermented to organic acids (Wang and Hesseltine, 1966).

Phytic acid

Uncooked soybeans and wheat contain a significant amount of phytic acid, the principle source of phosphorus in seeds. Phytates represent over 70% of the total phosphorus in whole soybeans (Sutardi and Buckle, 1985a). Phytates adversely affect nutritional status by chelating minerals and making them unavailable for use by nonruminant animals. During tempeh fermentation, Rhizopus molds produce phytase which decrease the phytic acid content of whole soybeans from 1% to 0.7%, a 30% decrease (Sutardi and Buckle, 1985a; 1985b). Phytic acid in tempeh decreases during boiling, refrigeration, and deep-fat frying (Shurtleff and Aoyagi, 1979; Sutardi and Buckle, 1985b).

Trypsin inhibitor

Raw soybeans contain trypsin inhibitors which inhibit the proteolytic enzyme trypsin when consumed by monogastric animals. Inhibition of trypsin in humans leads to a loss of sulfur-containing amino acids and pancreatic hypertrophy. At least five trypsin inhibitors have been isolated from the 2s fractions of soy globulin proteins (Shurtleff and Aoyagi, 1979).

Heating soybeans before fermentation reduced the trypsin inhibitor in the soybeans (Wang et al., 1972). Trypsin inhibitor increased significantly in tempeh apparently because of the release of trypsin inhibitor bound in the soybeans by the enzymatic secretion by the R. oligosporus. The mold apparently does not synthesize the trypsin inhibitor because when the mold is grown on other substrates, no trypsin-inhibiting activity was found (Wang et al. 1972; 1975b). This trypsin-inhibiting activity is caused by the presence of three unsaturated fatty acids: oleic, linoleic, and linolenic with linoleic acid having the most inhibitory activity (Wang et al., 1975b). Presence of trypsin inhibitors in raw tempeh could explain the low PER values reported. Trypsin inhibitor is destroyed readily by heat.

Vitamin content

Soy tempeh is a good source of many vitamins, especially B-vitamins (Table 13). During fermentation thiamin decreases and riboflavin, niacin, biotin and folate increase (Roelofsen and Talens, 1964; Murata et al., 1967; 1970). The reported increase in vitamin B_{12} during tempeh fermentation is an interesting occurrence.

Murata et al. (1970) and Sanke et al. (1971) reported the increase in folate compounds from soybeans to

TABLE 13-- B-complex vitamins of tempeh versus soybeans.

Vitamin	Soybeans (100g)	Tempeh (100g)
Thiamin	0.48 mg	0.28 mg
Riboflavin	0.15 mg	0.65 mg
Niacin	0.67 mg	2.52 mg
Pantothenic acid	430.00 mcg	520.00 mcg
Pyridoxine	180.00 mcg	830.00 mcg
Folacin	25.00 mcg	100.00 mcg
Cyanocobalamin	0.15 mmcg	3.90 mcg
Biotin	35.00 mcg	53.00 mcg

Source: Shurtleff and Aoyagi, 1979

tempeh was caused by de novo formation by Rhizopus oligosporus and not because of the presence of a bound form of folate compounds. Murata et al. (1968; 1970) reported that Rhizopus produces riboflavin and biotin also. Vitamin B_{12} is synthesized by bacteria (Shurtleff and Aoyagi, 1979) but not by mold (Okada et al., 1983).

Liem et al. (1977) reported a significant amount of vitamin B_{12} was found in commercial tempeh from Canada. This tempeh was found to be contaminated with a bacterium later identified as Klebsiella. Researchers have isolated (Okada et al., 1985a) and identified (Okada et al., 1985b) Klebsiella as the bacterium producing B_{12} in Indonesian tempeh. It has been reported in the literature that pure culture-produced tempeh contained insignificant amounts of B_{12} confirming Rhizopus does not produce the vitamin (Liem et al., 1977; Okada et al. 1983). Tempeh produced with the addition of Klebsiella had 150 ng of vitamin B_{12} per gram of tempeh (Liem et al., 1977). Neither presence of mold nor soaking soybeans in lactic acid interfered with vitamin production.

Liem and his coworker's (Liem et al., 1977) results indicated that tempeh produced under hygenic food conditions in the U. S. has no significant quantity of vitamin B_{12}. Okada et al. (1983) reported that Indonesian tempeh prepared by pure culture contained less than 0.1

mcg B_{12}/100g tempeh. Trudesdell et al. (1987) found 0.12 mcg B_{12}/100g in commercial tempeh. Herbert et al. (1984) analyzed commercial tempeh by radioassay of cyanocobalamin. His results confirmed the lack of B_{12} activity in tempeh produced by a pure culture method when he reported only 0.03 ng B_{12} per gram of tempeh.

Vitamin B_{12} is an essential nutrient necessary for the formation of red blood cells. This vitamin is required in only small amounts. Herbert (1987b) presented evidence that 2 mcg of cobalamin daily will maintain adequate nutriture. Non-vegetarians receive vitamin B_{12} from milk, meat, or other meat products; but vegetarians who consume no animal products must find alternate sources of this important vitamin, usually in the form of vitamin supplements (Steinkraus, 1983).

Cyanocobalamin officially is termed vitamin B_{12} according to chemists. In nutrition and pharmacology, B_{12} refers to cobamides that show vitamin activity in humans. Cyanocobalamin is a member of the cobalamins which are a group of compounds with a central cobalt atom bound to four pyrrole groups.

Cyanocobalamin is heat stable (Voigt and Eitenmiller, 1978; Shurtleff and Aoyagi, 1979; Chin, 1985). Cyanide can be split off on exposure to light producing hydroxocobalamin (Lindemans and Abels, 1985). For this

reason all analytical work involving organcorrinoids should be performed in the dark or under dim-red light (Lindemans and Abels, 1985). Farquharson and Adams (1976) found adenosylcobalamin, which is light sensitive, to be in unexpected high amounts in food.

The cyano group attached to the cobalt atom can be replaced by other ions to yield other cobalamins such as hydroxocobalamin, chlorocobalamin, nitrocobalamin, and thiocyanatocobalamin which are readily converted back to cyanocobalamin with cyanide (Voigt and Eitenmiller, 1978). The forms of vitamin B_{12} found in food are adenosylcobalamin, methylcobalamin, hydroxocobalamin, sulphitocobalamin, and cyanocobalamin (Farquharson and Adams, 1976). The metabolically active forms of vitamin B_{12} are adenosylcobalamin and methylcobalamin which are formed from hydroxocobalamin (Lindemans and Abels, 1985). In living tissues, the cobalamins always are bound with high affinity to the enzymes for which they function as cofactors (Lindemans and Abels, 1985).

Although cyanocobalamin officially is called vitamin B_{12}, the molecule is actually an artifact of the isolation and extraction processes. Similar compounds containing bases other than dimethylbenzimidazole are called B_{12} analogs (Chin, 1985).

Vitamin B_{12} analogs. Vitamin B_{12} analogs are

produced by altering the nucleotide moiety (Voigt and Eitenmiller, 1978). Examples of B_{12} analog are deoxyribosides. These compounds are 4000 times less biologically active that cyanocobalamin (Voigt and Eitenmiller, 1978). Deoxyribosides and other compounds can invalidate microbiological assays of the vitamin if the test substance is high in nucleic acids (Voigt and Eitenmiller, 1978).

Measurement of vitamin B_{12}. Many methods are available for measuring vitamin B_{12}. These methods do not measure the same compounds. Some measure all cobalamin-like compounds, some measure cobalamins, and others measure cobalamins that can be utilized by organisms.

Methods include chromatography, atomic absorption spectrophotometry of cobalt, measurement of B_{12}-dependent enzymes, radioimmunoassay, and microbiological assays (Chin, 1985). Microbiological and radioimmunoassay methods are the most commonly used. The British Analytical Methods Committee, Association of Official Analytical Chemists (AOAC), and United States Pharmacopeia (USP) recommend microbiological assay to measure vitamin B_{12}. Herbert and Drivas (1982), Herbert et al. (1984), and Herbert (1987a) recommended radioimmunoassay because of its specificity for B_{12} utilized by humans.

Radioimmunoassay and microbiological assays using the same extraction procedure gave similar results according to Bennink and Ono (1982). Beck (1979) compared two methods of extraction before radioassay and found results to vary based upon the extraction method used. Newmark et al. (1976) stated that low B_{12} for foods reported by Herbert was caused by inadequate extraction of B_{12}.

Voigt and Eitenmiller (1978) report that nearly all B_{12} contents of food have been determined by microbiological methods, and that these methods over-estimate the levels of cyanocobalamin (Herbert and Drivas, 1982). Assay organisms for vitamin B_{12} are an alga (Ochromonas malhamensis), a protozoan (Euglena gracilis), or a bacterium (Lactobacillus leichmanii). Ochromonas and Lactobacillus are the most widely used B_{12} assay organisms. The Analytical Methods Committee (1956) of Great Britain recommend use of Ochramonas malhamensis. The AOAC and USP recommend use of Lactobacillus leichmanii for assay of vitamin B_{12} (Chin, 1985).

Lactobacillus leichmanii assay is more widely accepted than O. malhamensis because of its rapid growth, uniformity of response, and precision of results (Voigt et al., 1979). The major disadvantage of L. leichmanii is that the organism is less specific than O. malhamensis (Lichtenstein et al., 1959).

B_{12} sparing ability of deoxyribosides toward L. leichmanii is a concern for the method. Contribution of deoxyribosides and other noncobalamins to the growth of L. leichmanii can be determined and corrected in the assay by adjusting the pH of the extracts to 12 and autoclaving 30 min at 121° C. This procedure destroys true vitamin B_{12}. Remaining microbiological activity can be taken as noncobalamin growth factors for the organism. Less than 20% of the B_{12} activity in diet samples is caused by noncobalamins. In most samples this is not a significant consideration (Chin, 1985).

All the reported B_{12} values for tempeh have been assayed by L. leichmanii without the alkali boil except for a report by Herbert et al. (1984) who found no B_{12} activity in tempeh.

Soy-wheat tempeh. No studies have been conducted on the vitamin content of soy-wheat tempeh.

Minerals

Little research has been conducted on the mineral content of tempeh. Ash is reported to decrease during production possibly because of a loss of solids. Table 13 contains calcium, iron, and phosphorus content of soy tempeh. Relative bioavailability of iron in soybeans is reported to increase during tempeh production

Table 14-- Mineral content of soy tempeh.

Mineral	Amount per 100 grams fresh tempeh (mg)	100 g tempeh as a percent of RDA (%)
Calcium	142	14
Phosphorus	240	24
Iron	5	28

Source: Shurtleff and Aoyagi, 1979

(Moeljopawiro et al., 1987). This improved bioavailability of iron could be explained by the reduction of phytic acid. For this reason, bioavailability of other minerals could be expected to improve also.

SUMMARY

Tempeh is a fermented soybean food which originated in Indonesia and is formed by the fermentation of hydrated soybeans with Rhizopus oligosporus mold. Tempeh usually is consumed as a meat substitute. Flavor and texture are derived from the fermentation process. Uncooked tempeh has a yeasty odor and a mild cheese-like flavor. Fried tempeh has a nutty flavor and odor.

Tempeh can be made with other substrates, either alone or in combination with soybeans. A soy-wheat blend of tempeh is produced in the United States. Production of tempeh requires a shorter preparation and fermentation time compared to the production of other fermented soybean products. Major steps are preparation of the substrate(s), hydration of soybeans, boiling, draining and cooling, inoculation with Rhizopus, and incubation.

Nutritive value of soy and soy-wheat tempeh makes them good substitutes for meat. Soy tempeh contains 157 calories per 100 g, has good quality protein, is low in

cholesterol and saturated fat, and is high in fiber and B-vitamins. Digestibility of the protein and bioavailability of minerals are reported to increase. Phytic acid decreases during fermentation and storage of tempeh.

Soy tempeh contains about 20% protein which is comparable to the protein content of meat products. Soy tempeh is limiting in methionine but is rich in lysine. Combining soybeans with wheat, which is rich in methionine but limiting in lysine, forms a complete protein. This complementarity is demonstrated by the improvement in net protein utililization and protein efficiency ratio over that of either substrate alone. Tempeh fermentation does not alter the amino acid profile but can make the amino acids more biologically available.

Rhizopus or a bacterium present during fermentation exhibits a strong lipase activity hydrolyzing the glycerides into free fatty acids. Most notable is the increase in free linoleic and linolenic acids.

Carbohydrates decrease during tempeh fermentation. Stachyose and raffinose are decreased by about 50%. Reduction in these oligosaccharides can contribute to the improved digestibility and less flatulence potential of tempeh compared to soybeans.

Thiamin decreases during fermentation with Rhizopus.

Niacin, riboflavin, biotin, folate, and cyanocobalamin increase. The reported increase in vitamin B_{12} (cyanocobalamin) is an interesting occurrence. Klebsiella must accompany the mold during fermentation to produce vitamin B_{12}.

Although tempeh fermentation was discovered centuries ago, the process could play an important role in production of protein-rich meat analogs in the future as the world population continues to increase. Tempeh fermentation demonstrates one way of producing protein-rich meat substitutes that are easily digestible, nutritionally adequate, and inexpensive. Tempeh is growing in popularity in the United States and could spread to other areas of the world where suitable substrates are available.

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APPENDIX

Table A-1-- Estimated cost of making tempeh.

Soybeans (assuming 10% dehulling loss)	
For soy tempeh	$0.12
For soy-wheat tempeh	$0.06
Wheat	
For soy-wheat tempeh	$0.02
Water	< 0.01
Potato Dextrose Agar	< 0.01
Lactic Acid	< 0.01
Soy tempeh =	$0.12/ 10 servings
Soy-wheat tempeh =	$0.08/ 10 servings
Ground beef at $1.59/lb	$3.18/ 10 servings

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QUALITY CHARACTERISTICS OF SOY AND SOY-WHEAT TEMPEH

by

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AN ABSTRACT OF A MASTER'S REPORT

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Tempeh is a fermented soybean food which originated in Indonesia and is formed by the fermentation of hydrated soybeans with Rhizopus oligosporus mold. Tempeh usually is consumed as a meat substitute. Flavor and texture are derived from the fermentation process. Uncooked tempeh has a yeasty odor and a mild cheese-like flavor. Fried tempeh has a nutty flavor and odor.

Tempeh can be made with other substrates, either alone or in combination with soybeans. A soy-wheat blend of tempeh is produced in the United States. Production of tempeh requires a shorter preparation and fermentation time compared to the production of other fermented soybean products. Major steps are preparation of the substrate(s), hydration of soybeans, boiling, draining and cooling, inoculation with Rhizopus, and incubation.

Nutritive value of soy and soy-wheat tempeh makes them good substitutes for meat. Soy tempeh contains 157 calories per 100 g, has good quality protein, is low in cholesterol and saturated fat, and is high in fiber and B-vitamins. Digestibility of the protein and bioavailability of minerals are reported to increase. Phytic acid decreases during fermentation and storage of tempeh possibly contributing to improved bioavailability of minerals.

Soy tempeh contains about 20% protein which is

comparable to the protein content of meat products. Soy tempeh is limiting in methionine but is rich in lysine. Combining soybeans with wheat, which is rich in methionine but limiting in lysine, forms a complete protein. This complementarity is demonstrated by the improvement in net protein utililization and protein efficiency ratio over that of either substrate alone. Tempeh fermentation does not alter the amino acid profile but can make the amino acids more biologically available.

Rhizopus or a bacterium present during fermentation exhibits a strong lipase activity hydrolyzing the glycerides into free fatty acids. Most notable is the increase in free linoleic and linolenic acids.

Carbohydrates decrease during tempeh fermentation. Stachyose and raffinose are decreased by about 50%. Reduction in these oligosaccharides can contribute to the improved digestibility and less flatulence potential of tempeh compared to soybeans.

Thiamin decreases during fermentation with Rhizopus. Niacin, riboflavin, biotin, folate, and cyanocobalamin increase. The reported increase in vitamin B_{12} (cyanocobalamin) is an interesting occurrence. Klebsiella must accompany the mold during fermentation to produce vitamin B_{12}.

Although tempeh fermentation was discovered centuries

ago, the process could play an important role in production of protein-rich meat analogs in the future as the world population continues to increase. Tempeh fermentation demonstrates one way of producing protein-rich meat substitutes that are easily digestible, nutritionally adequate, and inexpensive. Tempeh is growing in popularity in the United States and could spread to other areas of the world where suitable substrates are available.