Uses of Soybeans as Foods in the West with Emphasis on Tofu and Tempeh

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Soybeans have been used as food in the Orient since ancient times and various methods have been developed to make soybeans as palatable as possible. In recent years, a large number of these simply processed soyfoods are emerging in the West. Tofu and tempeh are the most popular and have the fastest growth rate of any soyfood in America. Tofu is made by coagulating the protein with a calcium or magnesium salt from a hot-water extracted, protein-oil emulsion of whole soybeans. It is a highly hydrated gelatinous product with a bland taste. The texture characteristics of the curds vary from soft to firm, depending on the processing conditions. Thus, tofu can be easily incorporated with other foodstuffs and used in nearly every culinary context from salad to dessert and from breakfast foods to dinner entrees. Tempeh is made by fermenting cooked soybeans with a mold, Rhizopus oligosporus. The white mycelium covers the bean mass and binds it into a firm cake that can be sliced, seasoned, and cooked just like meat. Tempeh is becoming a hamburger alternative for vegetarians.

In the West soybeans have been primarily viewed as an oilseed. As early as 1908, some European countries started to import beans from China to process into oil and meal. Commercial oil mill processing plants, however, were not built in the U.S. until 1922 (1). The oil was then mostly for industrial uses. But, because of declining industrial uses and increasing demand for edible oil in the late 1930s, research on soybean oil for food uses was encouraged. By the seventies, soybean oil became a major edible oil in the United States. Soybean meal, a by-product from the extraction of oil has been widely used as animal feed since the late 1930s. American soybean processors also produce a variety of edible protein products from the meal, such as defatted grits and flours, concentrates, and isolates. These products became known in the fifties and reached the highest popularity as meat extenders in 1973. Since then their use has been static, although the food industry continues to use these products as ingredients in many food systems. The use of these edible soy protein products as direct food, however, is still waiting to be accepted.

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In East Asia, on the other hand, soybeans have traditionally been used directly as foods. Centuries of creative striving have yielded great numbers of protein foods that are versatile, easily digestible and delicious. It has been said that because of the existence of soybeans, the countries of East Asia succeeded in supporting a high population density in those distant days.

Based on processing technology, the soybean foods that have been consumed in East Asia may be classified into two general types: non-fermented and fermented (2-8) as shown in Table I. Names of these foods and the details of preparing and serving such foods may vary from country to country. Among them, soybean curd (tofu) and soy sauce have been the most widely consumed in the Orient.

Table I. Oriental Soybean Foods

<table>
<thead>
<tr>
<th>Foods</th>
<th>Description and Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nonfermented</strong></td>
<td></td>
</tr>
<tr>
<td>Fresh green soybeans</td>
<td>Picked plump, firm, bright green before maturation. Cooked and served as fresh green vegetable.</td>
</tr>
<tr>
<td>Soybean sprouts</td>
<td>Bright yellow beans with 3-5 cm sprouts. Cooked and served as vegetable or in salad.</td>
</tr>
<tr>
<td>Soybean milk</td>
<td>Water extract of soybeans, resembling dairy milk. Served as breakfast drink.</td>
</tr>
<tr>
<td>Protein-lipid film</td>
<td>Cream-yellow film formed over the surface of simmering soybean milk. Cooked and used as meat.</td>
</tr>
<tr>
<td>Soybean curd (tofu)</td>
<td>White or pale yellow curd cubes coagulated from soybean milk. Served as main dish with or without further cooking.</td>
</tr>
<tr>
<td>Soybean flour</td>
<td>Ground roasted dry beans, nutty flavor. Used as filling or coating for pastries.</td>
</tr>
<tr>
<td><strong>Fermented</strong></td>
<td></td>
</tr>
<tr>
<td>Soy sauce</td>
<td>Dark reddish brown liquid, salty taste suggesting the quality of meat extract, a flavoring agent.</td>
</tr>
<tr>
<td>Miso</td>
<td>Paste, smooth or chunky, light yellow to dark reddish brown, salty and strongly flavored resembling soy sauce, a flavoring agent.</td>
</tr>
<tr>
<td>Hamanatto</td>
<td>Nearly black soft beans, salty flavor resembling soy sauce, a condiment.</td>
</tr>
<tr>
<td>Sufu</td>
<td>Cream cheese-type cubes, salty, a condiment, served with or without further cooking.</td>
</tr>
<tr>
<td>Tempeh</td>
<td>Cooked soft beans bound together by mycelium as a cake, clean fresh and yeasty odor. Cooked and served as main dish or snack.</td>
</tr>
<tr>
<td>Natto</td>
<td>Cooked beans bound together by and covered with viscous, sticky polymers produced by bacteria, ammonium odor, musty flavor, served with or without further cooking as main dish or snack.</td>
</tr>
</tbody>
</table>
According to industry statistics gathered in early 1984 by Shurtleff and Aoyagi of the Soyfood Center in California (9), Americans were consuming an average of 2.22 pounds of such traditional soybean foods per year per capita as compared to 1.37 pounds of the modern soy protein foods. The annual production of tofu has increased from 12,020 MT in 1978 to 24,300 MT in 1983, with an average annual growth rate of 15% and the highest growth rate of 27% in 1979. The annual production of tempeh has increased from 511 MT in 1981 to 900 MT in 1983, with the average annual growth rate of 33% and the highest growth rate of 36% in 1982. Soy protein isolates, which had the fastest growth rate among the modern soy products, increased from 11,000 MT produced in 1970 to 41,000 MT in 1982, with an average annual growth rate of 11%. The production figures on soy isolates also include exports. Consequently, the growth rate of consumption in U.S. would be significantly lower than the growth rate of production indicated. Furthermore, there has been little or no growth in the combined U.S. production of soy flour, isolates and concentrates since 1974 based on a survey made by Shurtleff and Aoyagi (9).

Tofu

Tofu has long been a source of protein in the Orient. It has much the same importance to the people of the Orient that meats, eggs and cheese have for the people in Western Countries. Tofu is usually sold in the form of a wet cake with a creamy-white color, smooth custard-like texture and a bland taste. It is highly hydrated and, depending on the water content, tofu products with different characteristics can be produced. The typical oriental type of tofu has a water content about 85%. Japanese prefer tofu having a smooth, fragile texture that contains about 88% water. The Chinese, on the other hand, produce many types of firm products with a chewy meat-like texture and a water content as low as 50-60%. Western consumers like tofu with a firm texture; therefore, tofu found in the U.S. supermarkets contains 75-80% water.

Because of its fine texture, bland taste and light color, tofu has been used in nearly every culinary context: desserts, salads, breakfast foods, dinner entrees and burgers. It can be cooked simply with desired flavoring agents or it can be easily incorporated with other foodstuffs.

Preparation. Tofu is made by coagulating the proteins with a calcium or magnesium salt from a hot-water extracted, protein-oil emulsion (soybean milk) of soybeans. The process is simple (Figure 1), but making a reproducible high-quality product is a problem. Many factors, from the quality of the dry beans to pressing the curd can affect the yield and quality of the resultant tofu. In recent years, several studies (10-14) have been made on tofu processing in an attempt to better understand the process and to optimize the processing conditions.

Three main steps are involved in making tofu (Figure 1): Preparation of soybean milk, coagulation of protein, and formation of tofu cakes in a mold. By experience, the Orientals have found that the most suitable ratio of water (including that absorbed during soaking) to dry soybeans is 8:1 to 10:1. Watanabe et al (15)
Dry Soybeans
Seaked in water 16 hr, 20-22°C.
Drained, washed, ground with water.
More water added to make ratio of water to dry beans 10:1

Soybean mash
Boiled for 15 min.
Strained through coarse cloth

Residue (Okara)

Soybean milk
Cooled to 75°C
Added coagulant (powdered gypsum, Ca,
Mg-salt, hydrate,
0.02-0.04M)

Curd
Transferred to a cloth-lined mold
Pressed

Figure 1. Flow diagram for preparation of tofu.
noted a significant reduction in the amount of protein and total solids extracted when the amount of water used was reduced to 6.5 times that of dry beans. Increasing the amount of water over 10:1 increases the extractable materials; however, excess water would result in a soybean milk too low in protein to achieve a proper curd formation.

Soaking the beans in water facilitates the grinding and it removes some undesirable factors such as the gas-forming oligosaccharides, but it also leaches out soluble proteins. To keep soaking losses at a minimum and to save energy, hydration of soybeans at an ambient temperature, around 20-22°C for 16-18 hr, is most suitable (16). Grinding the soaked beans expedites the extraction and also the formation of the protein-lipid emulsion. A heat treatment is essential, not only for protein denaturation to attain proper curd formation (17), but to improve nutritional value and to reduce off-flavor. Based on in vitro digestibility and amino acid composition (13), the maximum nutritive value of soybean milk can be ensured by boiling for 10-15 min. Excessive heat not only adversely affects the nutritive value and tofu texture, but also reduces the total solids recovery, and thus reduces the tofu yield.

Coagulation is the most important step in terms of reproducible yield and texture of tofu, but it is the least understood. In the Orient, making of tofu has been considered an art, and even today, the relationship between the ion binding to the soybean proteins and the coagulation phenomenon are still not completely understood. According to Fukushima (17), native soy protein molecules are unfolded during heating. Consequently, the free SH groups, disulfide bonds and hydrophobic groups are exposed. In a dilute solution, the unfolded proteins remain soluble, but as the exposed groups are brought closer together through concentration by drying or freezing, or through neutralization of molecular charges, irreversible aggregates result. The bonds responsible for the intermolecular polymerization are the disulfide bonds formed by the sulfhydryl/disulfide interchange reaction and also the interactions among the hydrophobic amino acid residues. Fukushima (17) postulated that the irreversible coagulation in tofu production is brought about by decreasing molecular charges, because added Ca²⁺ or Mg²⁺ ions bind with the negatively charged acidic amino acid residues and the sulfide group of the unfolded protein molecules. Recent studies (10,11,13) have shown that both ionic concentration and type of coagulant affect the quantity and quality of the resultant tofu. Results obtained from our laboratory are shown in Figures 2 and 3 (13). When the concentration of the coagulant is lower than 0.01 M and higher than 0.1 M, there is no curd formation. In studying the binding of unfractionated soybean proteins with calcium ion, Appurao and Rao (18) observed that at higher calcium concentrations the extent of precipitation decreases and the protein becomes soluble again. Our data are consistent with their observations. Data in Figure 2 also show that salt concentrations between 0.02-0.04 M result in the highest nitrogen recovery and that the sensitivity to the concentration shifts is the least. Thus, the use of salt at a level between 0.02 to 0.04 M is most likely to yield a reproducible product with a high nitrogen
Figure 2. Relationship of concentration and type of coagulant to the yield of tofu (13).
Figure 3. Relationship of concentration and type of coagulant to the texture characteristics of tofu (13).
content. Data on texture characteristics (Figure 3) also indicate that there is little sensitivity to concentration changes when a salt level of 0.02-0.04 M is used. The hardness and the brittleness of the curds, however, are influenced by the type of salt used. Calcium chloride and magnesium chloride result in curds with much greater hardness and brittleness than calcium sulfate and magnesium sulfate suggesting that anions have a greater effect on texture than cations. This observation agrees with a study by Aoki (19) on the effect of salt on the gelation of soybean proteins, where anions were found to have a stronger effect on water-holding capacity than cations. The hardness of tofu increases as its water content decreases (14). Tsai et al (11) found that coagulant concentrations between 0.025 and 0.03 M are the most suitable for making Chinese-style tofu.

The temperature of the soybean milk at the time coagulants are added, the modes of mixing and pressing greatly affect the yield and texture of the resulting tofu. Increasing the temperature increases the hardness, but decreases the volume, weight, and water content of tofu (13). Increasing mixing also decreases tofu volume and increases hardness (10,13).

Thus, many factors affect the final product. By knowing the effects that each factor produces, one can choose and establish a set of conditions to reproduce the desired type of tofu.

Soybean Variety. Saio and her coworkers (20) speculated that soybean variety could have an effect on tofu texture, because they found that a gel made from isolated IIS globulin is much harder and more elastic than that made from 7S globulin. They also noted increasing tofu hardness as the amount of phytic acid was increased in soybean milk, but such chemical variations between varieties may not be great enough to have the influence on texture that processing variables do. Recently, Skurray et al (12) made a study with 15 varieties and found no significant correlation between the ratio of 7S to 11S protein or phosphorus content and the quality of tofu. However, they did find that the quality of tofu is more affected by the amount of calcium ion added. Wang et al (14) studied varietal effects with 5 U.S. and 5 Japanese soybean varieties grown under the same environmental conditions, and found that the composition and color of tofu are affected by soybean variety but that yield and texture are not significantly affected. Varieties with a dark brown hilum result in tofu with a less attractive color so that these varieties are not desirable. Tofu made from varieties with a high protein content has a higher protein/oil ratio than tofu made from varieties with less protein (Table II). Therefore, varieties with high protein are preferred.
Table II. Protein to Oil Ratio of Tofu and Soy milk as Affected by Protein and Oil Content of Soybeans (14)

<table>
<thead>
<tr>
<th>Variety</th>
<th>Protein</th>
<th>Oil</th>
<th>Protein/Oil</th>
<th>Soybeans</th>
<th>Soy milk</th>
<th>Tofu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wase-Kogane</td>
<td>45.2</td>
<td>17.4</td>
<td>2.60</td>
<td>2.49</td>
<td>2.07</td>
<td></td>
</tr>
<tr>
<td>Vinton</td>
<td>45.1</td>
<td>17.7</td>
<td>2.52</td>
<td>2.50</td>
<td>2.01</td>
<td></td>
</tr>
<tr>
<td>Toyosuzu</td>
<td>44.1</td>
<td>18.1</td>
<td>2.44</td>
<td>2.13</td>
<td>1.87</td>
<td></td>
</tr>
<tr>
<td>Coles</td>
<td>43.2</td>
<td>18.5</td>
<td>2.34</td>
<td>2.11</td>
<td>1.78</td>
<td></td>
</tr>
<tr>
<td>Yuuzuru</td>
<td>42.3</td>
<td>17.7</td>
<td>2.39</td>
<td>2.30</td>
<td>1.89</td>
<td></td>
</tr>
<tr>
<td>Tokachi-Nagaha</td>
<td>41.8</td>
<td>17.3</td>
<td>2.42</td>
<td>2.12</td>
<td>1.88</td>
<td></td>
</tr>
<tr>
<td>Weber</td>
<td>40.9</td>
<td>19.3</td>
<td>2.12</td>
<td>1.75</td>
<td>1.57</td>
<td></td>
</tr>
<tr>
<td>Hodgson</td>
<td>40.9</td>
<td>19.4</td>
<td>2.11</td>
<td>1.90</td>
<td>1.67</td>
<td></td>
</tr>
<tr>
<td>Corsoy</td>
<td>40.8</td>
<td>18.9</td>
<td>2.16</td>
<td>1.95</td>
<td>1.69</td>
<td></td>
</tr>
<tr>
<td>Kitamusume</td>
<td>40.8</td>
<td>19.4</td>
<td>2.10</td>
<td>1.86</td>
<td>1.57</td>
<td></td>
</tr>
</tbody>
</table>

*Dry basis.*

Composition and Nutritional Value of Tofu. The composition of tofu may vary depending on soybean variety used and method of preparation as exemplified in Tables II and III. Since the method of preparation greatly affects the water content of the product, it influences the percentage of other components (Table III). Tofu available in U.S. supermarkets usually contains 80% or less water so that it may have more than 10% of protein. Other nutrients typically present in 100 g tofu with 84.8% of water are: fiber, 0.1 g; calcium, 128 mg; phosphorus, 126 mg; iron, 1.9 mg; sodium, 7 mg; potassium, 42 mg; thiamin, 0.06 mg; riboflavin, 0.03 mg; niacin, 0.1 mg (21). Tofu has been a source of calcium in the Oriental diet. The calcium content of tofu varies depending on the coagulant used. Tseng et al (22) reported that tofu prepared with a calcium salt has a higher calcium content and higher Ca/P ratio than that prepared by other coagulants. They suggested that tofu can help to correct the imbalanced Ca/P ratio in many American diets. Also, tofu made from calcium salt is a good source of calcium in vegetarian diets.

Table III. Composition of Tofu as Related to Percentage of Water

<table>
<thead>
<tr>
<th>Water %</th>
<th>Protein %</th>
<th>Oil %</th>
<th>Other Solids %</th>
<th>Protein Oil</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>84.8</td>
<td>7.8</td>
<td>4.2</td>
<td>3.2</td>
<td>1.9</td>
<td>21</td>
</tr>
<tr>
<td>85.1</td>
<td>7.5</td>
<td>4.2</td>
<td>3.2</td>
<td>1.8</td>
<td>14</td>
</tr>
<tr>
<td>(84.2-85.7)</td>
<td>(6.8-8.4)</td>
<td>(3.8-4.7)</td>
<td>3.2</td>
<td>(1.6-2.1)</td>
<td></td>
</tr>
<tr>
<td>88.0</td>
<td>6.0</td>
<td>3.5</td>
<td>2.5</td>
<td>1.7</td>
<td>17</td>
</tr>
</tbody>
</table>

*Average of 10 soybean varieties, with ranges in parenthesis.*

Although tofu has been claimed as a low-calorie protein food, the following comparison needs to be considered. One hundred grams of tofu (water, 84.8 g; protein, 7.8 g; oil, 4.2 g) contains about 72 calories, whereas 100 g of cooked hamburger (water, 54.2 g; protein, 24.2 g; fat, 20.3 g) has 286 calories (21). Although the hamburger
meat provides more than three times as much protein as tofu, it has a lower protein/fat ratio (1.2 vs 1.9). Accordingly, hamburger has more calories than tofu based on the weight that provides the same amount of protein. Per 50 g protein, hamburger has 591 and tofu has 461 calories. Hamburger also has more fat in such a comparison. However, protein/fat ratio varies greatly among the various cuts and types of meat. Meat that is well trimmed to arrive at a higher protein/fat ratio could have less calories and fat content than tofu to provide the same amount of protein. Therefore, tofu can not always be considered as low calorie food. However, tofu is a low-density protein food, and is thus more filling. The indisputable nutritional assets of tofu are the absence of cholesterol and lactose, and low amounts of saturated fatty acids.

Microbiological Quality of Tofu. Tofu is a protein-rich substrate with pH around 6, hence it is quite susceptible to microbial growth. Traditionally, tofu has been made and consumed in the same day. However, in the United States, tofu may be held at the supermarkets for many days on produce counters before consumption where temperatures are usually 10-15°C. Thus, microbial deterioration becomes a serious problem (23-25).

Tofu should be relatively free of vegetative microbial cells if it is made under proper sanitary conditions. Cooking the soybean mash at the boiling temperature for 15 min should kill all vegetative cells and leave only the heat-resistant spores as survivors. However, the presence of heat-resistant, spore-forming bacteria observed on soybeans (unpublished data) suggests that, even though contamination may have been prevented during processing, bacterial growth could occur if tofu is stored under conditions suitable for the microbes to grow. Measures then must be taken to prevent the growth of these microorganisms in order to improve the microbiological quality of tofu. In addition to proper storage conditions, the processors should thoroughly clean the beans to reduce the surface microbial load and carry out the processing with a high level of sanitary practices (25,26).

Recently, studies to evaluate the microbiological safety of tofu were made by Kovats et al (27). Water-packed tofu samples were inoculated with such common food pathogens as Clostridium botulinum, Staphylococcus aureus, Salmonella typhimurium, and Yersinia enterocolitica, then held at different temperatures for various lengths of time. They found that all four organisms grew in water-packed tofu. C. botulinum toxin was produced in tofu held at 15°C and 25°C within 3 days and 1 wk, respectively, but not at 5°C and 10°C within 6 wk. S. aureus and S. typhimurium grew at similar rates at 10, 15, 25°C, but neither pathogen grew during storage at 5°C. Staphylococcal enterotoxin was not produced within 4 wk at 10°C even though a population of greater than 10^7/g was present in most samples analyzed. Y. enterocolitica grew at all temperatures evaluated (5, 10, 15 and 25°C). Isolates recovered from tofu samples agglutinated with antiserum (WA-SAA), indicating that the isolates continued to express their virulence-associated determinant after growing in tofu. Thus, like many other foods, the potential of microbial hazards is great for tofu produced under unsanitary conditions and/or stored at improper temperatures. High level
sanitary practices, pasteurization after packaging, and storage and display at 5°C or less by manufacturers, distributors and retailers were recommended by Kovats et al (27).

Tofu can be kept frozen or freeze-dried to prevent microbial deterioration. However intermolecular interactions occur during frozen storage. As a result, the texture of tofu is changed from soft, smooth to sponge-like with a meat-like chewiness.

**Tempeh**

Tempeh, originating in Indonesia, is made by fermenting dehulled and briefly cooked soybeans with Rhizopus mold; the mycelium binds the soybean cotyledons together in a firm cake. Freshly fermented tempeh has a clean, yeasty odor. When sliced and deep-fat fried, it has a nutty flavor and pleasant aroma. Tempeh is used as a main dish and meat substitute in Indonesia. Vegetarians in the West have used tempeh as hamburger patties. Unlike most other fermented soybean foods which usually involve more than one microorganism, long brining, and an aging process, tempeh fermentation is short and simple and requires only one mold.

**Preparation.** Traditionally, soaked, hand-dehulled and briefly boiled soybeans are inoculated with small pieces of tempeh from a previous fermentation, wrapped in banana leaves which also serve as a source of inoculum, then left at room temperature for 1-2 days.

Studies carried out by Hesseltine et al (28) resulted in a pure culture fermentation as shown in Figure 4. To save time and labor, mechanically dehulled, full-fat grits have replaced the traditional whole soybeans. A tempeh starter containing spores of Rhizopus oligosporus NRRL 2710 (29) is now used in the West in place of traditional inocula. Not only are Petri dishes the most convenient laboratory container, they also are used commercially in preparing tempeh patties. Other containers such as shallow aluminum foil or metal trays with perforated bottoms and perforated plastic film covers, and perforated plastic bags and tubings have been used successfully for tempeh fermentation. Rhizopus oligosporus requires air to grow, but too much aeration will cause spore formation and also may dry up the beans, resulting in poor mold growth. Therefore, both properly perforating the containers and packing the beans for fermentation are important.

**Tempeh Products from Grains and Other Beans.** Traditionally tempeh is made from soybeans known as tempeh kedele. However, copra (pressed coconut cake) and the by-product from making soybean milk have also been used in Indonesia to make tempeh known as tempeh bongkrek and tempeh genbus, respectively. Recently, attempts have been made (30) to make tempeh-like products from grains such as wheat, oats, barley, rice, mixtures of cereal and soybeans, and from beans other than soybeans, such as broad beans, cowpeas, mung beans and winged beans. In the United States, tempeh made from a mixture of wheat and soybeans (31) has been available commercially since 1970.

**Biochemical Changes During Fermentation.** The effects of R. oligosporus on soybeans have been studied by several investigators (30,32). As shown in Table IV, the fermentation process does not
Dehulled full-fat soybean grits

\[ \text{\downarrow} \rightarrow \text{Tap water} \]

Soaked 30 min. at 25 C

\[ \text{\downarrow} \]

Drained

\[ \text{\downarrow} \rightarrow \text{Tap water} \]

Cooked \( [30 \text{ mm.}] \)

\[ \text{\downarrow} \]

Drained and cooled

\[ \text{\downarrow} \]

Inoculated \( \rightarrow \) Spore suspension of \( \text{Rhizopus oligosporus Saito} \)

\( \text{NRRL 2710} \)

Tightly packed in petri dishes

\[ \text{\downarrow} \]

Incubated 31 C for 20-24 hr

\[ \text{\downarrow} \]

Tempeh cake

Figure 4. Flow diagram for tempeh fermentation.
greatly affect the proximate composition of soybeans. The slight
increase in the percentage of protein reflects the decrease of other
constituents that the mold might have consumed for growth.

Table IV. Proximate Composition of Soybeans and Tempeh

<table>
<thead>
<tr>
<th>Food</th>
<th>Protein</th>
<th>Oil</th>
<th>Fiber</th>
<th>Ash</th>
<th>Carbohydrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybeans</td>
<td>47.8</td>
<td>26.8</td>
<td>3.9</td>
<td>3.4</td>
<td>18.1</td>
</tr>
<tr>
<td>Tempeh</td>
<td>48.1</td>
<td>24.7</td>
<td>3.1</td>
<td>3.3</td>
<td>20.8</td>
</tr>
</tbody>
</table>

Treated similarly as for fermentation except the inoculation step
was omitted.

As the mold begins to grow rapidly, the temperature of fermenting
beans rises a few degrees above the incubator temperature, then
falls as the growth of mold subsides. The pH increases steadily to
above 7, presumably because of protein break-down. After 69 hrs. of
incubation, soluble solids rise from 13 to 28%, soluble nitrogen
increases from 0.5 to 2.0%, but total nitrogen remains fairly constant
and reducing substances decrease slightly, probably due to utilization
by the mold. The mold does not utilize the carbohydrates in the
soybeans; instead, it uses the soybean oil as its energy source.

Although total nitrogen remains fairly constant during
fermentation, free amino acids increase in tempeh. The essential
amino acid index, on the other hand, is not significantly changed by
fermentation. Perhaps the amount of mycelial protein present in
tempeh is not high enough to alter greatly the amino acid composition
of the soybeans, nor does the mold depend upon any specific amino
acid for growth.

Niacin, riboflavin, pantothenic acid and vitamin B₆ contents
are greatly increased in tempeh during fermentation, whereas thiamin
exhibits no significant change. R. oligosporus appears to have a
great synthetic capacity for niacin, riboflavin, pantothenic acid,
and vitamin B₆, but not for thiamin.

The most interesting and important finding was the presence of
vitamin B₁₂ in tempeh because foods derived from plant materials are
deficient in this essential nutrient. Vitamin B₁₂ is known to be
synthesized by microorganisms; however, molds have not been reported
to produce Vitamin B₁₂. Lien et al (33) found a fairly high amount
of vitamin B₁₂ in commercial tempeh bought from Canada and
subsequently confirmed that the major source of the vitamin was a
result of a contaminating bacterium which the authors isolated and
identified as Klebsiella. They reported that tempeh made from pure
mold isolated from commercial tempeh contained nutritionally
insignificant amounts of vitamin B₁₂, confirming that the tempeh
mold does not produce the vitamin. On the other hand, tempeh made
with the mold and the bacterium, Klebsiella, isolated from commercial
tempeh, had 150 ng of vitamin B₁₂ per gram of tempeh. The presence
of the mold does not interfere with the production of vitamin B₁₂ by
the bacteria, but presence of the bacteria requires longer
fermentation time. Lien and his co-workers also demonstrated that
soaking soybeans either with or without an acid did not increase the
vitamin B₁₂ content. The results indicated that tempeh made with
pure mold fermentation under hygienic conditions adopted for food processing in this country has no nutritionally significant amount of vitamin B₁₂. However, there is a great potential to make vitamin B₁₂-enriched tempeh with an inoculum containing *Rhizopus oligosporus* and a vitamin B₁₂-producing bacterium.

**Nutritional Value.** Although enzymes produced by the mold have acted upon the substrate and partly hydrolyzed its constituents into small molecules, the digestibility coefficient of tempeh tested by the rat-assay method is not significantly different from that of unfermented substrate. It is also not surprising to learn that the protein efficiency ratio (PER) of tempeh (8) as determined by rat assay is not significantly different from that of unfermented but properly heat-treated soybeans, because the fermentation process does not significantly change the total nitrogen and the amino acid composition. However, tempeh made from a mixture of wheat and soybeans has been shown (8) to have a better protein value than that made from soybeans alone, because of the complementary effect of mixed proteins and the increased availability of lysine in wheat from fermentation.

Increase in vitamins, such as niacin, riboflavin, pantothenic acid, Vitamin B₆, and Vitamin B₁₂, is of great nutritional significance, especially where fortifying foods with synthetic vitamins is not practiced.

**Microbiological Quality of Tempeh.** Like tofu, tempeh should normally be relatively free of contaminated vegetative cells, but may not be free of heat-resistant spores. Failure of fermentation caused by bacterial contamination has been reported by tempeh producers. In order to assure successful fermentation, a starter with high viability is as important as a high level of sanitary practices. To maintain the microbiological quality of tempeh, steaming after fermentation and then freezing are recommended. Results obtained from studies on the safety of tempeh inoculated with different bacterial pathogens (34) indicated that tempeh should be steamed after fermentation and then kept at 5°C or below until it is used.

**Conclusions**

Whole soybean foods have been the major source of protein in East Asia since ancient times and various methods have been developed to make soybeans more palatable. Among these simply made soybean foods, tofu and tempeh have recently become increasingly popular in the West. The production processes may not improve the nutritional value of soybean protein, but they reduce the cooking time, improve the organoleptic characteristics and increase the versatility of soybean uses. With the recently increasing interest in protein foods other than those from animal origin, the consumption of tofu and tempeh has been on an upsurge in the West and is expected to continue its steady growth in the years to come.
Literature Cited


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