EFFECTS OF INOCULA AND INCUBATION TIMES ON SELECTED SENSORY AND PHYSICAL CHARACTERISTICS OF TEMPEH

by

CYNTHIA M. LUND

B.S., Oregon State University, 1985

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

in

Food Science

Department of Foods and Nutrition

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1988

Approved by:

Martha B Stone
Major Professor

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Tempeh, or tempeh kedelee as it is called in Indonesia, usually is made by fermenting dehulled soybeans with a mold of the Rhizopus species. The white mycelia of the mold binds the soybean cotyledons in a cake-like form. Tempeh rarely is eaten raw, but usually is deep-fat fried, stir-fried, or baked.

There are many positive aspects to tempeh. Unlike most soybean products, tempeh is used as a main dish instead of a relish or flavoring agent. Since tempeh does not require a high level of technology, even third world countries can produce it. Soybeans are inexpensive and high in protein which makes tempeh an excellent choice for a meat analogue. These attributes (low cost, high protein, and low technology) were the primary interests of scientists in the United States. Tempeh is important as a good food source in underdeveloped countries according to the pioneers of research on tempeh in the United States (Autret and VanVeen, 1955; Steinkraus et al., 1960; Hesseltine and Wang, 1967b; Shurtleff and Aoyagi, 1979).

Soybeans were accepted slowly in the United States, despite their high protein content. People prefer what they are accustomed to and are not influenced easily by what is better for them (Platt, 1964). There are approximately 10-15 million vegetarians in the United States, and tempeh has been the hamburger alternative for vegetarians since the early 1970's (Wang, 1984). Unlike tofu, tempeh is produced and consumed primarily by non-Orientals. Beany flavor in soybean products is not acceptable to most Westerners. Until scientists determine ways to rid

soybeans of their off-flavors, tempeh is a good alternative as a high protein, inexpensive, acceptable flavor, meat substitute.

The objectives of this study were to: (1) characterize the flavors of tempeh and to compare and contrast these flavors with those found in cooked soybeans; (2) investigate beany flavor reduction during fermentation of tempeh; (3) study beany flavor in relation to the color (amount of whiteness produced by mycelia growth) and texture (degree of firmness) of tempeh.

REVIEW OF LITERATURE

Types of Tempeh

Tempeh is made by fermentation of soybeans, but other substrates can be used. Other substrates include peanuts, coconuts, winged beans, chickpeas, navy beans, fava beans, cowpeas, broad beans, lupin, bakla, and tofu. Many grains are used also, such as, wheat, barley, rice, oats, rye, and buckwheat. Shurtleff and Aoyagi's Book of Tempeh (1979) mentions using the by-product of soymilk for tofu preparation (okara). Some of the Indonesian names for these products include, tempeh kecipir (winged beans), tempeh bongkrek (coconut), tempeh gembus (soybean curd), tempeh benguk (Indonesian legumes), and oncom or ontjom (peanuts/Neurospora) (Steinkraus et al., 1965a; Wang and Hesseltine, 1966; Van Veen et al., 1968; Robinson and Kao, 1977; Shurtleff and Aoyagi, 1979; David and Verma, 1981; Dijen, 1982; Steinkraus, 1983).

Tempeh Production

Village Production of Tempeh

Preparing tempeh can be done using a village method or by a mass production method. In the village method the soybeans are soaked overnight in cool water or for a shorter period in hot water. After soaking, the hulls are removed by treading on them in a basket at the edge of a river or stream. The loosened skins then will float away. After the loosened seedcoats are removed, the beans are transferred into

a cooking pot with water covering the beans by at least 2.54-5.08 cm (1-2 in). Sometimes several tablespoons of hulls are added back to aid in prefermentation, because the hulls contain Lactobacillus or Pediococcus, which acidifies the soak water. The beans are allowed to soak for 24 hr. Next, the beans are brought to a boil in the soak water for 30 min. After cooking, beans are drained and spread on a bamboo tray to cool and dry. Beans are inoculated with scrapings of previously made tempeh. The beans and inoculum are mixed by hand for 6 to 7 min. The beans next are wrapped tightly with banana leaves into packages. They are left to ferment in a warm place for 1 or 2 days (Steinkraus et al., 1960; Martinelli and Hesseltine, 1964; Shurtleff and Aoyagi, 1979).

Mass Production of Tempeh

Steinkraus et al. (1965b) developed a pilot-plant process for the production of tempeh. Dry beans were dehulled by preheating for 10 min at 104°C and passing the cooled beans through a burr mill. Preheating was done to shrink the cotyledons. The space grinder of a burr mill loosened the hulls from the cotyledons, after which the beans were passed over a gravity separator or through a flotation of water to remove the hulls.

Hydration was completed by soaking the dry beans in room temperature water and acidifying with lactic or acetic acid. Acid was added to inhibit bacterial growth. After the overnight soaking, the soak water was saved to cook the beans. Precooking was done in a jacketed steam kettle at 100°C. The beans were drained in a woven wire basket and cooled. When the temperature of the beans had dropped to 35-

38°C, the beans were inoculated with 3 g of powdered lyophilized tempeh mold per kg of dry dehulled beans and mixed for 5 min with a Hobart mixer.

The fermentation process was carried out by spreading approximately 3 kg of inoculated beans on metal dryer trays (35 x 81 x 1.3 cm) that had woven stainles steel 3 mm mesh bottoms. The trays containing the beans were covered with waxed paper to decrease moisture loss, and placed in a fermentation room to incubate for 15-18 hr at 35-38°C and 75-85% relative humidity. Martinelli and Hesseltine (1964) developed shallow trays with perforated bottoms and covers. They also fermented tempeh in perforated plastic bags and tubes. This idea was thought to be good because the tempeh could be fermented in a package and sold in the same container. Perforation is essential because the mold is aerobic. This is also why cakes are usually less than 5 cm (2 in) thick.

Mold for Fermentation

Tempeh originated in Indonesia and is a common everyday food. One reason tempeh originated there was because of its locale near the equator, where the temperature ranges from 20 -30°C. The weather is predictable and rarely ever changes from these temperatures. These conditions are optimal for the growth of many microorganisms. Therefore, it is not suprising that Indonesians prepare several kind of foods using microbes.

In the United States the Rhizopus mold has been studied extensively, especially with fermentation with a pure culture (Steinkraus et al.,

1960; Dijen and Hesseltine, 1961; Hesseltine et al., 1963a; Hesseltine and Wang, 1979). In Indonesia, as mentioned previously, they use fermented tempeh as a starter in conjunction with the Rhizopus mold on the banana leaves. Steinkraus et al. (1960) stated that the mold isolated from crude tempeh scrapings resembled Rhizopus oryzae Went and Prinsen Geerligs. They also found spore-forming Bacillus and a non-sporeforming bacterium. The amount of yeasts and other microorganisms depended on whether or not the culture was dried. These yeasts and bacteria contribute to off-flavors and odors if allowed to develop. In fact Indonesian students felt that tempeh made from the pure culture was "better than tempeh produced in Indonesia." Even though the number of bacteria found on tempeh is small, it is wise to acidify the soak water to a pH of 4.5 to 5.3 to prevent growth. Tanaka et al.(1985) speculated that the presence of acid inhibits growth of pathogens. In the United States most tempeh starter is sold in powder form. Traditionally, the starter can be obtained several ways. One way is by using previous wrappings or pulverized dried tempeh. Other ways are to use broken tempeh pieces mixed with prepared beans or to slice the surface of tempeh where the mold's mycelia exists (Rusmin and Dijen, 1974).

Hesseltine (1965) isolated 40 strains of Rhizopus from tempeh (Table 1). From this study Hesseltine found Rhizopus oligosporus, especially NRRL strain 2710, was the optimum mold used in making tempeh. Rhizopus oligosporus has the ability to produce spores in large quantities at a rapid pace. Wang et al. (1972a) proved R. oligosporus grown in a milk medium produced high antibacterial activity, especially against gram-positive microorganisms including both microaerophilic and anaerobic

Table 1. Strains of Rhizopus species which make acceptable tempeh

Name	No. of Strains
Rhizopus oligosporus Saito	25
R. stolonifer (Ehren) Vuill	4
R. arrhizus Fischer	3
R. oryzae Went and Geerligs	3
R. formosaensis Nakazawa	3
R. achlamydosporus Takeda	2
Total	40

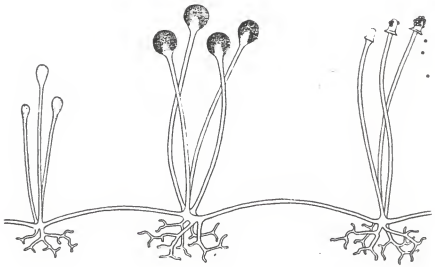
(Hesseltine, 1965)

bacteria. This antibacterial agent occurs frequently in Oriental fermented foods, which explains why disease and infections are minimized in Oriental cultures. Antibacterial compounds are thought to stimulate growth (Wang et al., 1969; Wang et al., 1972a; Tanaka et al., 1985). Perhaps part of this hypothesis could be explained by the rapid growth of R. oligosporus giving little chance for the bacteria to multiply before tempeh fermentation is complete.

Description of Rhizopus

The genus Rhizopus is a member of the class Phycomycetes. Rhizopus are referred to as bread molds, although they grow on fruit and vegetables, also. Morphologically, these molds have nonseptate, cottony, coenocytic mycelia with sporangiophores that develop and arise at the nodes, where thick tufts of rhizoids form. The sporangia are usually large and black. The columnella is hemispherical and the base of the large globose sporagium (apophysis) is cup-shaped. The molds produce clusters of root-like holdfasts called rhizoids, as well as stolons, which are capable of rooting and can give rise to a new organism. When the spores mature, the sporangial membrane will rupture (Figure 1)(Banwart, 1981).

R. oligosporus is grown on potato-dextrose agar slant at 28°C for 5-7 days (Hesseltine et al., 1963b). Wang et al. (1975a) developed a tempeh inoculum having high viable spore counts that could retain viability with minimal attention. The spores were made by fermenting rice for 4 or 5 days at 32°C and 40% moisture level. The fermented cakes were blended into a slurry with sterilized water then freeze-dried. Viability was maintained even after 6 months' storage.



(Stanier et al., 1963)

Biochemistry of Rhizopus

Rhizopus oligosporus gets its source of carbon from common sugars such as glucose and galactose, as well as from trehalose, cellibose, xylose, soluble starch, and soybean oil (Hesseltine et al., 1963b). The mold does not obtain carbon from principal carbohydrates of soybeans, raffinose, stachyose, and sucrose. Since these soybean sugars are not utilized by R. oligosporus, it is likely that the lipid material, especially fatty acids, is the primary energy source (Wagenknecht et al., 1961).

The mold does not use any single amino acid for its source of nitrogen. Ammonium salts and amino acids such as glycine, leucine, proline, and aspartic acid are good nitrogen sources (Sorenson and Hesseltine, 1966). Tryptophan supports no growth (Wang and Hesseltine, 1979a; 1979b). R. oligosporus is proteolytic, a positive factor since soybeans are high in proteins. Since the mold depends primarily on lipid material for its energy, its lipase activity is strong to hydrolyze the soy lipids (Wagenknecht et al., 1961). Amylase activity is low and there is no detectable pectinase activity (Hesseltine et al., 1963b).

Conditions for Rhizopus

Tempeh mold grows well at 30-42^{o}C. Mycelia growth becomes visible after 12 hr and can be completed after 18-24 hr. If the temperature is above 42^{o}C the mold's temperature will rise higher with the release of heat from metabolism. The high temperature will eventually kill the

mold. If the temperature is below 30°C, the mycelia will be produced at a slower rate. For example, at 25°C it requires 80 hr and 28°C requires 26 hr to complete fermentation. The best medium for Rhizopus growth is potato dextrose agar (PDA). An alternate medium is yeast extract-malt extract peptone-glucose agar (YM) (Ellis and Hesseltine, 1983). Breaking the cotyledons into four or five pieces increases rapidity of mold growth. This gives the mold more surface area for attachment and still permits enough oxygen for growth. These cotyledon pieces, also called grits, absorb water faster, and therefore, decrease soaking time (Hesseltine and Wang, 1979). Grits also increase soluble solid losses. High humidity (75-85%) is essential for tempeh fermentation because it will help prevent the cotyledons from drying out and getting hard. Development of hardness hinders the binding of mycelia to the cotyledons. Although the humidity should be high, the surface area of the beans should have low moisture content. A high moisture content on the bean's surface can give rise to bacteria spoilage (Wang and Hesseltine, 1981). Because this mold is fast growing and requires high moisture for growth and for enzyme synthesis, the danger of contamination by a toxin-producing fungi would be minimal (Wang and Hesseltine, 1974). Rathburn and Shuler (1983) developed fermentation chambers that had an inlet gas pass through a humidifier to prevent drying of the beans.

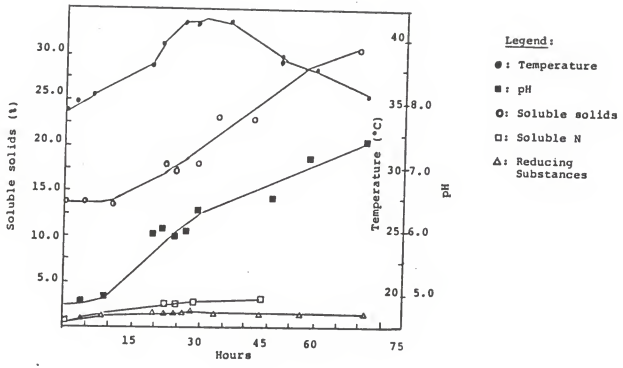
The correct amount of oxygen is essential for the growth of Rhizopus. If there is too little oxygen the mycelia would not be able to grow sufficiently to bind the cotyledons and form a cake-like product. However, if there is an excess of oxygen the mold will

sporulate causing an undesirable black color, or the cotyledons will dry out before the mold can develop.

Biochemical Aspects of Tempeh

Steinkraus et al. (1960) reported increased temperature of the soybeans as mold growth increased. When the substrate reached 43-44^{o}C, the mold growth subsided and the temperature began to decrease. When rapid mold production occurred, ammonia was produced as a result of protein breakdown. This caused the pH to rise from 5.0 to nearly 7.6 (Figure 2). The protein breakdown resulted from the release of the proteases from the mold. This and other rapid chemical changes occurring during tempeh fermentation is explained in a study by Jurus and Sundberg (1976). The hyphae infiltration of the mycelia has measured as much as 25% of the average width of a soybean cotyledon. These authors speculated that the hyphae can mechanically push the bean cells apart, which allows the beans to become soft. This can occur before or at the same time as enzyme digestion takes place. Therefore, enzyme activity would be enhanced, because the distance that the enzyme has to diffuse is reduced.

Enzyme digestion also affected the soluble solids content (Jurus and Sundberg, 1976). During the first 30 hr when the mold growth is the most rapid, the soluble solids rose from 13 to 21%. After this stage the quality of the tempeh began to deteriorate and the soluble solids increased to 27.5% after 70 hr (Figure 2). When enzyme digestion started ammonia production, the result was an increase in soluble nitrogen from 0.5 to 2.0% even though the total remained constant at 7.5% (Sudarmadji, 1975) (Figure 2).



(Sudarmadji, 1975)

Van Buren et al. (1972) reported an increase in fiber content from 3.7 to 5.85% between raw dehulled soybeans and tempeh. This was attributed to an increase in mycelia. The authors also reported increased levels of nitrogen free extract (NFE) although there was a decrease in 66% ethanol-soluble NFE. They speculated the contrast was attributable to the water soluble pectic and hemicellulose-type material solubilized by mold enzymes, which apparently is responsible for the softening effect of the mold on the cooked soybeans. This softening effect also aids in keeping the cells of the beans intact after violent agitation. Steinkraus et al. (1960) conducted cytological studies on soybeans cooked in water, soybeans steamed for 90 min, and fermented soybeans (tempeh). Beans cooked in water and tempeh remained intact after blending in a Waring blender, but the steamed beans contained relatively few intact cells.

Changes in Amino Acids

The effect of fermentation on the amino acid content has produced conflicting data. Steinkraus (1983) reported lysine decreased by 10% and more than 25% after fermentation for 36 and 60 hr, respectively, while methionine decreased 3% and 8%, respectively. Stillings and Hackler's data (1965) are supportive of this while Smith et al. (1964) and Murata et al. (1967) showed only a $\pm$5% change in amino acid content between the unfermented soybeans and tempeh (24 hr fermentation). In Murata's et al. (1967) data for 48 hr fermentation showed increases of free amino acids over a range of 1 to 85 times from D-1 (unfermented) to D-3 (most palatable tempeh) (Table 2). The differences in the data

Table 2. Amino acid composition of tempeh and unfermented soybeans (mg/g N)

	Indonesian soybeans		Harosoy soybeans	
	Unfermented control	Tempeh[a]	Unfermented control	Tempeh[b]
	No. of sample		No. of sample	
	A-1	A-2	D-1	D-3
Asp	744	756	704	673
Thr	278	282	241	218
Ser	270	268	323	273
Glu	1050	1000	1100	974
Pro	342	309	342	307
Gly	292	275	263	257
Ala	250	228	280	338
Cys	113	121	98	80
Val	328	345	336	319
Met	77	81	61	61
Ile	338	356	301	310
Leu	525	565	518	492
Tyr	171	161	188	178
Phe	302	302	384	307
Try	67	87	63	66
Lys	392	410	384	330
His	160	167	187	171
Arg	491	440	392	373

[a]Tempeh made in Indonesia.

[b]Tempeh fermented for 48 hr in laboratory.

(Murata et al., 1967)

could be attributed to not dehulling soybeans before hydration. Murata et al. (1967) and Smith et al. (1964) did not dehull the soybeans before hydration. Essential amino acid levels did not change throughout the 24 hr fermentation period. Stillings and Hackler (1965) also reported that deep-fat frying of tempeh for five and seven min produced a decrease in amino acid content. The data show a slight decrease of lysine and cysteine at five min, but a 20% decrease of these amino acids at seven min of deep-fat frying. Ammonia values decreased as frying time increased. These scientists also reported that steaming for two hr at 100°C had little effect on the amino acid composition of tempeh.

Changes in Carbohydrates

The principle carbohydrates of soybeans, sucrose, stachyose, and raffinose, are decreased after soaking. They are solubilized and discarded with the cooking water. Therefore, the tempeh production process allows the flatulent causing sugars to be discarded. As mentioned previously, common sugars, such as glucose, fructose, galactose, and maltose are used as a carbon source to growth.

Nutritive Value of Tempeh

Many scientists have studied the nutritional advantages of fermenting soybeans. Contradictory findings in these experiments might be attributed to the methodology used, the variety of soybeans, or the use of pure or mixed culture (see Table 3). Protein quality and growth rate were evaluated using rats. Smith et al. (1963) found that rats fed tempeh showed a slight reduction in protein efficiency and growth

Table 3. Effects of fermentation on protein efficiency ratio (PER) and digestibility

Product	PER[a]	Digestibility[a]	Reference
Soybean tempeh	nd	nd	Wang et al. (1968)
	nd	nd	Hackler et al. (1964)
		+	Murata et al. (1967)
	nd	+	Zamora and Veum (1979)
	nd	+	vanVeen and Steinkraus (1970)
Wheat tempeh	+		Wang et al. (1968)
Wheat soybean tempeh	+		Wang et al. (1968)

[a]+ = increase; nd = no difference from unfermented product

(Teutonico and Knorc, 1985)

compared to the rats fed autoclaved full-fat soybean meal. Smith et al. (1964) stated that this reduction could be insignificant if considering the improved edibility of soybeans for human consumption. In fact, they found supplementation with methionine, a limiting amino acid of soybean protein, actually increased growth rate and protein effiency ratios (PER). Stillings and Hackler (1965) found a close relationship in PER values and amino acid composition in fermented and heat processed soybean. Even though the amino acid composition was similar, Murata et al. (1967) noted an increase in free amino acids after fermenting soybeans with the Rhizopus mold. Murata et al. (1971) continued his investigation and later reported PER values were not significantly different in tempeh versus unfermented soybeans, but addition of a whole egg to the tempeh diet increased PER values. Supplementation with a whole egg added more protein, but the same results could be accomplished by supplementing with lysine, methionine, and threonine. If tempeh powder could be supplemented with low levels of sulphur containing amino acids, it could be useful as a substitute for milk powder in developing countries.

The original studies by Murata et al. (1967) showed no large differences in protein and ash content in tempeh, but a slight increase in fiber and a slight decrease in fat content from the unfermented soybeans. The increase in fiber probably was caused by the mycelia produced by the mold and the decrease in fat was attributed to the lipase activity of the mold for energy. Zamora and Veum (1979) conducted experiments on feeding rats diets of unfermented dehulled soybeans (HUS), heated dehulled soybeans fermented with Aspergillus

oryzae (AOS), or heated dehulled soybeans fermented with Rhizopus oligosporus (ROS). Results from their studies contrasted somewhat with the results of Murata et al. (1967). Zamora and Veum (1979) found a slight increase in crude fat and crude protein as a result of fermentation, while crude fiber showed no significant difference. The percentage of nitrogen increased 5% in the diet containing fermented soybeans when compared to the diet with unfermented soybeans. Data also indicated that soybean protein quality and utilization were improved with fermentation. In the first part of the experiment each of the diets contained 20.46% of the various soy products (HUS, AOS, ROS) and in the second part the diets contained 14.89%. This allowed the scientists to observe that the higher quality fermented soybean protein was used more efficiently by the rats as the dietary level decreased. Zamora and Veum (1979) also examined the average daily weight gain of the rats for a four-week period. Rats fed a tempeh had a greater weight gain than those fed unfermented soybeans. These data conflicted with experiments conducted by Hackler et al. (1964) and Smith et al. (1964). However, data from Hackler et al. (1964) were dependent on acceptability of tempeh. They reported that the acceptability of tempeh decreased after 12 hr increments of fermentation. Therefore, a decrease in rate of growth is attributed to a decrease in food consumption because there were no significant differences in PER values.

Stillings and Hackler (1965) studied the effects of deep-fat frying on amino acid content and biological data. The results indicated the amino acid content decreased with increased frying time from five to seven min. Biological data, such as PER, EAAI (essential amino acid

index), and RI (requirement index) were also decreased with increased exposure to heat. This was unexpected because biological data usually would reflect the availability of amino acids before destruction. Hackler et al. (1964) examined the effects of deep-fat frying in an earlier study and found no change in digestibility, growth, feed consumption, and PER with deep-fat frying for three min or less or steaming for two hr or less.

Wang et al. (1968) examined adding a cereal product (wheat) to the soybean as a substrate to make tempeh. The PER of wheat increased with fermentation. This was attributed to the availability of lysine in wheat by fermenting. According to the PER values, the 24 and 72 hr fermented products were not as acceptable to the rats as the products fermented for 48 hr. The amount of food consumed and the weight gain of the rats fed the diet of fermented wheat were greater than in the wheat control group.

Wang et al. (1968) stated that the mixture of wheat and soybeans (1:1) gave a good pattern of amino acids and supported growth as well as casein. Since amino acid composition was not significantly altered, the scientists looked at the availability of the individual amino acids in the protein and found the total essential amino acids released from wheat by enzymatic digestion increased about 10% after 24 hr of fermentation. Wang and Hesseltine (1965) postulated that the proteolytic enzyme produced by the mold attacked the protein in such a way that more lysine and histidine could be made available to the digestive enzymes of animals.

B-Vitamins in Tempeh

Niacin, riboflavin, pantothenic acid, and pyridoxine contents in soybeans increased after fermentation, but no significant difference in thiamin has been found (Table 4)(Roelofsen and Talens, 1964; Murata et al., 1967). Roelofsen and Talens (1964) found higher levels of thiamin, riboflavin, and nicotinic acid. Murata et al. (1967) discovered that although thiamin decreased with fermentation, it actually increased during the first 24 hr of incubation. The same researchers reported an increase in riboflavin from 8 to 47 times, pyridoxine from 4 to 14 times, and niacin from 2 to 5 times in tempeh manufactured and sun dried in Indonesia compared to unfermented soybeans. Higher values were found after 48 to 72 hr of fermentation. Wang and Hesseltine (1966) noticed the amounts of niacin and riboflavin in wheat tempeh were greater than that of the unfermented wheat, but thiamin appeared to be less. Therefore, Rhizopus oligosporus has a great synthetic capacity for niacin, riboflavin, pantothenic acid, and pyridoxine, but not thiamin (Wang et al., 1979a). All fungi are autotrophic with respect to riboflavin and nearly all are autotrophic with respect to niacin. Many fungi require either thiamin or its building blocks, but even though this is true thiamin should not decrease because Rhizopus oryzae is autotrophic (Roelofsen and Talens, 1964).

Scientists were interested in the presence of vitamin B-12 because vegetable products do not contain this vitamin. Vitamin B-12 is essential for proper formation of erythrocytes and prevention of pernicious anemia. Hesseltine and Wang (1979) stated that Rhizopus does

Table 4. A comparison of certain vitamins in soybeans and in tempeh

Vitamin	Concentration	
	In soybeans per gram	In tempeh per gram
Riboflavin	3.0 µg	7.0 µg
Pantothenate	4.6 µg	3.3 µg
Thiamin	10.0 µg	4.0 µg
Niacin	9.0 µg	60.0 µg
B_{12}	0.15 µg	5.0 µg

(Steinkraus, 1985)

not synthesize vitamin B-12 in tempeh, but a bacterium present in the mold does. Using a lactic acid fermentation to kill unwanted microorganisms, will not influence the vitamin B-12 in tempeh. In fact, the presence of the mold did not inhibit or enhance the production of vitamin B-12. This vitamin's production microbiologically is limited to Propionibacterium, Pseudomonas, Clostridium, and some Streptomyces (Liem et al., 1977). Steinkraus (1985) reported the bacterium present in tempeh is a gram negative rod, identified as a nonpathogenic strain of Klebsiella pneumoniae. This bacterium was found to lengthen the fermentation time from 18 - 20 hr to 25 - 30 hr (Liem et al., 1977). The amount of vitamin B-12 production in tempeh differs. Soybean varieties (such as Harasoy, Rampage, Indonesian yellow, and Indonesian black) have been found to vary in levels of B-12 produced. The cause has not entirely been identified, but it is related to the cobalt concentration. Vitamin B-12 requires about 4% of its molecular weight in the form of cobalt.

The recommended daily allowance of vitamin B-12 is 3 μg a day according to a report by the Committee on Dietary Allowance in 1977 (Liem et al., 1977). They can reach this RDA by consuming approximately 60 g (2 oz.) of tempeh (Liem et al., 1977).

Trypsin Inhibitors in Tempeh

Raw soybeans contain trypsin inhibitors. Smith et al. (1963) stated there was no evidence of pancreatic hypertrophy, indicating normal production of tempeh destroyed these inhibitors. Trypsin is an enzyme secreted by the pancreas. When trypsin is inhibited, the

pancreas enlarges. Wang et al. (1972b) discovered that fermented boiled soybeans showed higher trypsin inhibitory activity than unfermented especially at 48 hr of fermentation. This increase of trypsin inhibitory activity was not synthesized by the mold because there was no inhibitory acivity when the mold was grown in a milk or wheat media. The reason for the increase is most likely due to the breakdown of the soybean substrate by enzymes produced by the mold. Wang et al. (1972b) found that when heated soybeans were combined with proteases from the mold, there was trypsin inhibitory activity. Once the inhibitor is released, heat will destroy it immediately. These scientists felt proteins must be denatured before the proteases can liberate bound soybean trypsin inhibitors. Kakade et al. (1974) supported this finding and hypothesized that proteases of Rhizopus oligosporus solubilized the heat-denatured trypsin inhibitors and exposed amino acid residues to inhibitors by interaction with trypsin. When heated there are some denaturation changes in trypsin inhibitors, such as the amino acid residues at the reactive sites become inaccessible for the formation of the trypsin-trypsin inhibitor complex (Kakade et al., 1974).

Wang et al. (1975b) found that free fatty acids, oleic, linoleic, and linolenic acids, were primarily responsible for the increase in trypsin inhibitors in tempeh. These are some of the unsaturated free fatty acids hydrolyzed by the mold's extracellular lipase activity on the soybean oil (Wang and Hesseltine, 1966). Wagenknecht et al. (1961) identified the free fatty acids of tempeh as palmitic, stearic, oleic, linoleic, and linolenic, with linolenic predominating. No linoleic or linolenic and only small amounts of the others were found in cooked

unfermented soybeans. Oleic and linoleic acid are more inhibitory than linolenic, but these long chain unsaturated fatty acids are more inhibitory than saturated fatty acids. The inhibition of trypsin by fatty acids is attributed to the detergent properties of fatty acid salts. Zamora and Veum (1979) reported an increase in antitrypsin activity in fermented soybeans, but these scientists felt the inhibitors were proteinaceous in nature. Roozen and DeGroot (1985) agreed with the theory of Wang et al. (1972b and 1975b) that trypsin inhibitory activity was accomplished by unsaturated free fatty acids, but also indicated that nonprotein, water soluble, low molecular weight molecules contained inhibitory effects. These low molecular weight material would be leached out during soaking or else removed in the dehulling and washing steps. These scientists also stated the protein-type inhibitors are inactivated by steaming the beans. They discovered the trypsin inhibitor in tempeh contains about 5% residual trypsin inhibitory activity. These release and inactivating soybean trypsin inhibitors could improve the beans nutritionally.

Phytic Acid in Tempeh

Phytate is an organic form of phosphorus that is poorly utilized by humans because they have low phytase activity to catalyze the hydrolysis of phytates to inositol and phosphoric acid in their intestine. Also, the phytate ion chelates with several mineral elements including copper, zinc, cobalt, magnesium, iron, and calcium to form insoluble phytate-mineral and protein-phytate mineral complexes. As a result, there is a reduction of the absorption of these elements from the intestinal tract.

Therefore, phytates in high concentrations are undesirable (Wang et al., 1980).

Phytate occurs in high concentration in soybeans where up to 90% of phophorus can be in this form. Several scientists have tried to examine ways to alleviate this nutritional problem. Rackis (1974) found autoclaving soy isolates for 4 hr at 115°C destroyed most of the phytates. Sudarmadji and Markakis (1977) looked at how the tempeh process allows hydrolysis of phytates to inositol and inorganic phosphorus. This process reduced mineral deficiencies and increased the nutritive value of tempeh. Sutardi and Buckle (1985) examined the phytate content of soybeans during tempeh production, fermentation, storage, and deep-fat frying. The dehulling and washing steps decreased phytic acid content (% dry weight) of soybeans, but the first soak actually increased phytic acid levels. After the soak the phytic acid content was halved during tempeh's fermentation and further reduced when stored for 72 hr at 5°C and 30°C. Deep-fat frying of tempeh in peanut oil halved it again. In fact, less than 10% of the original phytic acid content was recorded after fermentation, storage, and deep-fat frying (Sutardi and Buckle, 1985).

Antioxidant Activity in Tempeh

Indonesians have been using tempeh to preserve fish for centuries. They would place a piece of tempeh with fish until the tempeh was sold at the market. Early research on the presence of an antioxidant in tempeh showed that dried, pulverized, and stored soybeans had peroxide values that range from 18.3 to 201.9, while tempeh's peroxide values

ranged from 0 to 1.1 (Gyorgy, 1961). Gyorgy et al. (1964) showed rats fed tempeh had better growth and greater resistance of red blood cells to dialuric acid-induced hemolysis than rats fed plain boiled soybeans. Hemolysis of red blood cells, that is caused by dialuric acid or low concentration of hydrogen peroxides, indicates a deficiency of vitamin E or an effective antioxidant. Gyorgy et al. (1964) stated tempeh's antioxidant prevented hemolysis of red blood cells of vitamin E deficient rats with dialuric acid. They isolated tempeh's antioxidant by paper chromatography and identified it as 6,7,4-trihydroxyisoflavone ("Factor 2"). Ikehata et al. (1968) supported this theory and stated the lipids of tempeh were more stable against autoxidation than the lipids of unfermented soybeans. These scientists substantiated that "Factor 2" was a potent antioxidant for vitamin A and linoleic acid in an aqueous solution at pH 7.4. "Factor 2" also was combined with soybean oil or powder and it did not prevent autoxidation. This compound was given orally to rats with deficient vitamin E diets, but it did not prevent red blood cells against hemolysis in the dialuric acid test. Ikehata et al. (1968) postulated these results could be attributed to poor absorption from the intestinal tract of the rats or a low affinity to tissue. Other problems with "Factor 2" were its insolubility in oil and its difficulty to disperse in powder (Ikehata et al., 1968).

Gyorgy et al. (1974) continued the study and found most oils combined with tempeh oil would not become oxidized when exposed to the air and temperatures up to 60°C for many weeks. Most oils will develop off flavors in a few months, but will last indefinitely when mixed with

tempeh oil. They stated "Factor 2" was the most active antioxidant among the natural flavenoids. Murakami et al. (1984) deduced the main isoflavones responsible for antioxidant activity were daidzein and genistein. These isoflavones only appeared to be effective antioxidants when tempeh oil was added to refined oils or when they were in aqueous suspension of linoleic acid. Murakami et al. (1984) reported the stability of tempeh to oxidize was generated by the liberation of lipophilic isoflavones from glucoside to B-glucosidase.

Stahl and Sims (1986) disagreed with Gyorgy because he only measured peroxide values, which indicate hydrogen peroxide concentrations. They measured the rate of oxygen absorption because this measured resistance to oxidation and is not limited by the degree of stability of intermediate oxidation products, such as hydrogen peroxides. Stahl and Sims (1986) found that tempeh oil added to safflower oil actually increased O_2 absorption. Tempeh appeared to be a good antioxidant on the basis of peroxide values. Stahl and Sims concluded that Gyorgy was in error because when only peroxide values are considered, there appears to be a protective effect. He indicated the rapid decomposition of hydrogen peroxides was caused by the presence of free fatty acids. Wagenknect et al. (1961) reported Rhizopus oligosporus possessed strong lipase activity and caused hydrolysis of about one-third of the neutral fat during the fermentation process. Stahl and Sims also suggested the strong fermentation odor masked the development of rancidity when evaluated by organoleptic methods.

Flavor and Texture of Tempeh

Besides affecting the nutritional value, fermentation alters the flavor of raw soybeans. Soybeans are not as acceptable in the Western world as they are in the East. Many scientists have tried to determine the compounds and reactions that contribute to these off-flavors. Oxidation of unsaturated fatty acids in soybeans is the principal source of the off-flavor. The enzyme, lipoxygenase, is the major cause of off-flavor development in soybeans (Rackis et al., 1970). Lipoxygenase attacks linoleic acid to liberate intermediates or hydrogen peroxides, which further breakdown to aldehydes, ketones, alcohols, furans, or to epoxy which forms hydroxy acids. These compounds give soybeans off-flavors that have been described as beany, grassy, bitter, oxidized, stale, cardboardy, cerealy, roasted, or even fishy (Table 5). The major cause of lipid oxidation is when the lipoxygenase in the soybean is activated by imbibing water or disrupting the seed. Since lipid oxidation is the principal cause of the major off-flavors Wilkens and Lin (1970) examined 80 compounds of off-flavored soy milk. The major components were hexanal (25%), hexanol, hexenal, ethyl-vinyl-ketone, and 2-pentyl furan, all of which impart a grassy, beany flavor. The green beany flavor is associated with soybeans during development whereas the bitter component seems to develop upon maturation (Rackis et al., 1970).

Sudarmadji and Markakis (1978) examined the effects of lipids on tempeh's organoleptic properties. Tempeh developed its most appealing flavor, color, and texture after incubation of 30 hr at 32°C. It retained these desirable attributes for 24 hr. After this period tempeh

Table 5. Compounds contributing to off-flavors in soy products

Furans	Green beany
Aldehydes	Green beany, grassy, stale, cardboard
Alcohols	Oxidized, grassy/beany
Trihydroxy fatty acids	Bitter
Fatty acid dimers	Bitter
Phenolics	Sweet-nauseating, astringent
Furfurals (-ols)	Cerealy
Browning products	Roasted
Oxidized phosphatidylcholine	Bitter
Volatile amines	Fishy

(Kinsella and Damodaron, 1980)

lost the pleasant flavor, darkened in color, smelled of ammonia, became sticky, and the texture deteriorated. Sudarmadji and Markakis (1978) described tempeh as going through three phases: a fermentation phase, the first 30 hr, during which mold growth, lipolysis, and temperature increase along with good sensory results; transition phase, the next 24 hr, during which mold growth and lipolysis subside and the temperature decreases and sensory results remain the same; and the third phase, the deterioration phase, during which bacteria growth begins and lipolysis reappears and adverse organoleptic changes occur. In these experiments the sensory work was done in the United States because many Indonesians consider a strong flavored tempeh contaminated with bacteria good tempeh, but tempeh consumers in the West consider such a product spoiled and obnoxious (Wang and Hesseltine, 1981).

Hesseltine and Wang (1979) stated, "In tempeh fermentation the beany flavor of the soybeans disappears." Steinkraus (1978) attributes the improved flavor to the hydrolysis of residual fats to free fatty acids. Hesseltine et al. (1967a) describes the odor as "pleasant yeasty and mushroom odor". Djurtoft and Nielsen (1983) describe the flavor after frying, "Served this way the flavor and texture of tempeh is very acceptable, somewhat similar to fried chicken or other light meat." Gyorgy et al. (1974) felt the appearance of tempeh resembled that of Camembert cheese.

No reports were found in the literature that used trained sensory panelists to identify flavor or texture characteristics of tempeh. A sensory panel was used in a study by Sudarmadji and Markakis (1978) but only acceptability was reported.

MATERIALS AND METHODS

Culture Propagation and Storage

Freeze-dried spore preparation of Rhizopus molds (NRRL 1526, NRRL 2710, NRRL 2549) obtained from the Northern Regional Research Center, USDA, Peoria, IL, (see Table 6) were transferred asceptically and streaked on Potato Dextrose Agar (PDA) slants (DIFCO Lab., Detroit, MI). Cultures were grown on slants and incubated for 2 days at 30°C to allow for sporulation, then stored at refrigerator temperature (4-7°C) until needed. Monthly culture transfers were made to keep Rhizopus organisms viable.

Tempeh Preparation

Soybean Preparation

Whole certified seed grade Williams 82 soybeans were purchased locally, and dehulled in the Department of Grain Science and Industry. Dehulled soybeans were rinsed with tap water (22 -25°C) and drained. A 0.05% lactic acid solution (1.5 L tap water at 45°C and 12.8 ml lactic acid) was added to each 500 g of beans (Steinkraus, 1983). After soaking beans for 15 hr, the soaking water was retained and used to cook beans in a 6.6 L (6 qt) cooking vessel on a gas burner (149°C, 300°F). When heating was initiated a white foam exuded from the mixture and changed to light brown color after approximately 10 min. When the foam dissipated, the water began to rapidly boil (100°C). The heat was

Table 6. Description of three strains of Rhizopus mold

Strain	Description
NRRL 1526	Rhizopus arrhizus Fischer. Used in Holland to produce tempeh. Originally isolated from Texas soil by Dr. M.B. Morrow and received in the ARS Culture Collection in 1940. This strain has the ability to form fumaric acid.
NRRL 2710	Received as Rhizopus sp. from Geneva Experiment Station, New York. Originally brought from Indonesia by Miss Yap B. Hwa, where it came from tempeh.
NRRL 2549	Received as Rhizopus oryzae Went and Geerligs isolated from tempeh by Prof. L.M. Olah, Laboratorium Treub, Bogor, Indonesia, in 1956.

(Hesseltine et al., 1963b)

reduced and the beans were heated at 90±2°C for 20 min. Cooked beans were drained and dried on paper toweling for 30 min.

Inoculation of Cooked Soybeans

An ultraviolet light in a bacterial hood was turned on 30 min before inoculation to insure death of all environment organisms. Dispersion of organisms was done under a bacterial hood. Each Rhizopus slant was aseptically combined with 9 ml sterilized water to allow mold spores to be dispersed. Dispersions were used to inoculate the cooked beans (500 g/slant). Next, approximately 1000 g inoculated beans were packed tightly into two plastic containers (7.5 x 7.5 x 2.5 cm) with perforated holes (diam = 3 mm) made 2.5 cm apart. Containers of inoculated beans were placed in an incubator maintained at 30°C for the predetermined incubation periods.

Frying Procedure

Samples were removed from the incubator and cut into 1.25 cm (0.5 in) cubes. The cubes to be fried were placed in a wire sieve and heated in a deep-fat fryer (Sunbeam Model) with vegetable oil filled to a depth of 2.5 cm. Oil was held at 180°C and samples were submerged for 3 min.

Analysis of Samples

Samples were evaluated at the following stages: cooked beans without inoculation and after inoculation and incubation for 12, 18, 24, and 30 hr. Three types of inocula (NRRL 1526, NRRL 2710, NRRL 2549) were examined after the four incubation periods. Selected measurements

were made on "raw" tempeh (following incubation but without frying) and tempeh cubes that were deep-fat fried.

Instrumental Measurements

Raw and fried tempeh samples made from three different Rhizopus strains were examined for textural attributes after incubation times of 12, 18, 24, and 30 hr with the Instron Universal Testing Machine Model 1122. Standard cubes (1.25 x 1.25 x 1.25 cm) were sheared with a Warner-Bratzler attachment (full scale load = 0.01; crosshead speed = 50 mm/min; chart speed = 200 mm/min). Four peak heights were measured for each sample and mean values were used to determine the degree of firmness for each sample. Firmness was expressed in kg of force.

A HunterLab Model D54 Spectrophotometer was used to determine the whiteness of each sample. Six randomly selected areas (two on the top, two on the bottom, two on the side) were measured on each 7.5 x 7.5 x 2.5 cm sample after removal from its container. Cooked beans and raw tempeh prepared from each of the Rhizopus strains (NRRL 1526, NRRL 2710, NRRL 2549) for each of the incubation time (0, 12, 18, 24, and 30 hr) were evaluated for color parameters. L, a, b-values were determined, but only L-values indicating whiteness were reported.

Sensory Analysis

Sensory analysis of tempeh was done by a seven-member trained sensory panel practicing Attribute Scaling using Descriptive Analysis. Panel members consisted primarily of graduate students attending Kansas State University. Panel members were chosen by interest and

availability. Ten hours were spent training the panel for flavor analysis of tempeh. During training, panelists were given a list of terms drawn from the literature. They deleted and added terms to the list after tasting the samples. Panelists identified parameters for odor, taste, and texture (firmness) during ballot forming stage (see Form A-1 for ballot). Once attributes were determined, panelists were trained with references (see Form A-2 for references) for each sensory characteristic. Panelists also agreed to evaluate firmness as the first bite using the back molars. Firmness was determined on the entire cube, indicating how tight or compact the beans were in the sample cube, rather than the firmness of the individual beans.

During the preliminary setup, cutting of the 1.25 cm cubes and frying was done the morning before each afternoon tasting session, then four cubes of each sample were placed in coded styrofoam cups (142 ml or 5 oz) with lids. All samples were tasted at room temperature ($24\pm2^{o}C$). Each panelist was served four or five samples per session. Raw samples were served first to the panelists. After waiting 15-20 min, panelists evaluated four or five fried samples. Order of sample presentation was assigned randomly by computer. Each of the ten attributes (odor -- nutty, mushroom-like, yeasty, beany, ammonia-like; taste -- nutty, mushroom-like, beany, cerealy; or texture -- firmness) was evaluated on a 60 digit unstructured linear scale, with anchors of "none" to "extremely" (see Form A-1) (Stone and Sidel, 1981). Data were collected by computer and scored on paper ballot. Plastic spoons were provided to aid in serving. Panelists received four 1.25 cm cubes in each cup. Distilled deionized water, apples, and soda crackers with unsalted tops were provided to cleanse palates between samples.

Statistical Analysis

A randomized complete block design was used for data collection. Since there were thirteen samples for each method for each week, on two days the panelists would receive four samples and on the third day they would receive five. Sample preparation and distribution were randomized for four replications. Data were subjected to analysis of variance with procedures of the Statistical Analysis System (SAS Institute, 1979). Means were compared and differences were separated using LSD procedures. ANOV for instrumental measurements, sensory attributes for the cooked beans and tempeh were as follows:

Source of Variation for Instron Data	DF		
Treatment	25		
Inoculum (IO)		2	
Incubation (IC)		3	
Method (M)		1	
Inoc x Incub (IOxIC)		6	
Inoc x Method (IOxM)		2	
Incub x Method (ICxM)		3	
Inoc x Incub x Method (IOxICxM)		6	
Replication (R)		3	
		26[a]	Subtotal
Replication (R)	3		
	28	Total	

[a]standard 3-way ANOV because of the presence of two controls (no inoculation or incubation for raw and fried samples).

Source of Variation for Color Data	DF	
Treatment	12	
Inoculum (IO)		2
Incubation (IC)		3
Inoc x Incub (IOxIC)		6
Replication (R)		3
		14[b] Subtotal
Replication (R)	3	
	15	Total

[b]Not a standard 3-way ANOV because of the presence of one control (no inoculation or incubation for raw sample; color measurements were not determined on fried samples).

Source of Variation for Sensory Data	DF	
Treatment Sensory (TS)	25	
Error Treatment (TSxR)	75	
Panelist (P)	6	
Error Panelist (TSxP)	150	
Replication (R)	3	
	259	Total

RESULTS AND DISCUSSION

Instrumental Analysis

Firmness

F-values and probabilities from ANOV for Instron measurements are presented in Table 7. There were significant differences among treatments, but no differences existed among replications. Mean values for Instron data for type of inoculum and incubation time as influenced by preparation method are shown in Table 8. For the control sample (no inoculum or incubation) beans were not held intact by mycelia into a cake-like network, therefore, no instrumental values for firmness could be obtained. NRRL 1526 produced the lowest mean value for firmness in comparison to the other inocula. Slower growth was produced in the tempeh containing the NRRL 1526 organism. NRRL 2710 produced the firmest samples (see Figure A1) because the mycelia were more tightly bound. NRRL 2710 is the typical inoculum used in tempeh production (Hesseltine et al., 1963b).

The Instron mean values for firmness for the effects of incubation time are shown in Table 8. Instron firmness was greatest in samples with the longest incubation times. Firmness values increased as incubation time was increased. No incubation time indicates no mold production, which accounts for a 0 value. Incubation times of 24 and 30 hr were not significantly different ($p<0.05$), but were different ($p<0.05$) from the other incubation periods. Firmness markedly decreased between the 12 and 18 hr incubation times. This indicates that the

Table 7. F-values and probabilities from ANOV for Instron measurements for firmness of raw and fried tempeh

Source of Variation	DF	F-value	Probability
Treatment	25	18.42	0.0001
Inoculum	2	16.45	0.0001
Incubation	3	64.36	0.0001
Method	1	98.55	0.0001
IO x IC	6	0.92	NS
IO x M	2	3.28	0.0428
IC x M	3	11.64	0.0001
IO x IC x M	6	0.58	NS
Replication	3	2.54	NS

Table 8. Instron mean values for firmness for inoculum[a], incubation time[b], and preparation method[c] for tempeh

Inoculum	Firmness (kg)
0	0.0d
NRRL 1526	9.8c
NRRL 2710	17.1a
NRRL 2549	13.2b
LSD (0.05)	3.21
Incubation Time (hr)	
0	0.0c
12	2.1c
18	12.7b
24	17.9a
30	20.9a
LSD (0.05)	3.31
Method	
Raw	7.6b
Fried	17.2a
LSD (0.05)	1.91

[a]Each control (0) value is a mean for eight determinations; each value for an inoculum is a mean for 24 determinations. Means with the same letter in a column within inoculum parameters are not significantly different ($p<0.05$).

[b]Each control (0) value is a mean for eight determinations; each value for an incubation time is a mean for 32 determinations. Means with the same letter in a column within incubation time parameters are not significantly different ($p<0.05$).

[c]Each value is a mean for 52 determinations; means with the same letter in a column are not significantly different ($p<0.05$).

mold's mycelia would not have enough time to bind the cotyledons during the 12 hr incubation.

Fried samples were firmer than the raw tempeh (Table 8, Figure A1). This increase in firmness upon exposure of tempeh to heat is attributable to several factors including protein coagulation and moisture reduction. Steinkraus (1983) reported an increase of 62% moisture from an unfermented control to raw tempeh. No values for moisture were reported after frying.

When comparing the interactions among inocula and incubation times for raw and fried tempeh (Table 9), the control samples for both raw and fried have values of 0 for firmness because no mycelia were present to bind the cotyledons. NRRL 1526 at 12 hr incubation had low firmness values, but raw tempeh was slightly firmer when compared to the fried sample. An exception was NRRL 2549 which appeared to be a faster growing strain (Figure A1). Exposure to convection currents during frying disrupted what little mold growth had started. NRRL 2710 tempeh fermented for 30 hr produced firmest tempeh, regardless of whether it was raw or fried. Tempeh from this inoculum, incubated for 24 hr, and fried, was firmer than tempeh from the other two inocula, even after additional incubation time (Figure A1). All inocula after 12 hr incubation, and NRRL 1526 and NRRL 2549 after 18 hr incubation, were generally crumbly and contained minimal, mycelia growth to bind the cotyledons. Whereas, NRRL 2710 and NRRL 2549 after both 24 or 30 hr incubation periods and after frying were firmer ($p<0.05$) than the other fried samples (Table 9).

Table 9. Instron mean values[a] for firmness for the interaction between inoculum and incubation time for preparation of raw and fried tempeh

Preparation	Inoc. x (NRRL)	Incub. (hr)	Firmness (kg)
Raw	0	0	0.0m
	1526	12	0.7m
	2710	12	3.9lm
	2549	12	1.5m
	1526	18	4.9jklm
	2710	18	11.0hij
	2549	18	5.6jklm
	1526	24	8.4ijkl
	2710	24	11.7hij
	2549	24	10.8hijk
	1526	30	12.1ghi
	2710	30	16.1fgh
	2549	30	12.5fghi
Fried	0	0	0.0m
	1526	12	0.0m
	2710	12	3.6lm
	2549	12	2.8lm
	1526	18	8.8ijkl
	2710	18	25.5bcd
	2549	18	19.2def
	1526	24	18.5efg
	2710	24	31.9ab
	2549	24	26.2abc
	1526	30	24.7cde
	2710	30	33.0a
	2549	30	26.9abc
LSD (0.05)			6.88

[a]Each value is a mean for four determinations; means with the same letter in a column are not significantly different ($p<0.05$).

Sample Whiteness

Table 10 shows the F-values and probabilities from ANOV for the HunterLab spectrophotometer L-values for raw tempeh. Treatments again produced significant differences. Table 11 contains mean values for sample whiteness (L-values) for raw tempeh separated for each inoculum and for each incubation time. Significant differences occurred depending on the type of inoculum used, and the control had a significantly lower L-value than the inoculated samples ($p<0.05$). NRRL 2710 produced the highest L-value or the whitest cakes. It was not significantly different from that produced by NRRL 1526, but both samples were whiter than tempeh from NRRL 2549 ($p<0.05$). NRRL 2549 had a problem with sporulation in the 30 hr incubation period, causing lower L-values because of dark spores. Wang and Hesseltine (1979) stated that sporulation is undesirable to the consumer. Whiteness increased as incubation time increased. There was no difference ($p<0.05$) in sample whiteness between 24 and 30 hr of incubation. All of the other incubation periods were significantly different ($p<0.05$) from one another.

In Figure A2 NRRL 2710 was the whitest cake for all incubation times, except for the 30 hr where sporulation must have occurred for it to have decreased L-values. NRRL 2549 tempeh was lower ($p<0.05$) than that from the other two inocula at 30 hr incubation (Table 12). Steinkraus et al. (1960) indicated for the most optimum tempeh, the cake must be overgrown with white mycellium without excessive sporulation. According to these criteria, the optimum product for each inoculum was

Table 10. F-values and probabilities from ANOV for HunterLab Spectrophotometer L-values for raw tempeh

Source of Variation	DF	F-value	Probability
Treatment	12	12.86	0.0001
Inoculum	2	3.72	0.0369
Incubation	3	25.36	0.0001
IO x IC	6	1.07	NS
Replication	3	1.20	NS

Table 11. HunterLab Spectrophotometer's mean values for sample whiteness for inoculum[a] and incubation time[b] for raw tempeh

Inoculum	L-value
NRRL 1526	73.27ab
NRRL 2710	75.91a
NRRL 2549	72.25b
0	59.33c
LSD (0.05)	3.48
Incubation Time (hr)	
30	78.23a
24	78.92a
18	72.36b
12	66.91c
0	59.33d
LSD (0.05)	3.63

[a]Each control (0) value is a mean for four determinations; each value for an inoculum is a mean for 12 determinations. Means with the same letter in a column within inoculum parameters are not significantly different ($p<0.05$).

[b]Each control (0) value is a mean for four determinations; each value for an incubation time is a mean for 16 determinations. Mean with the same letter in a column within incubation time parameters are not significantly different ($p<0.05$).

Table 12. HunterLab Spectrophotometer's mean values[a] for sample whiteness for the interaction between inoculum and incubation time for raw tempeh

Inoculum (NRRL)	x Incubation Time (hr)	L-value
0	0	59.33f
1526	12	66.57e
2710	12	67.95e
2549	12	66.21e
1526	18	70.70de
2710	18	75.54bcd
2549	18	71.38cde
1526	24	76.64abc
2710	24	81.83a
2549	24	78.10ab
1526	30	81.13a
2710	30	78.23ab
2549	30	75.33bcd
LSD (0.05)		5.46

[a]Each value is a mean for four determination; means with the same letter in a column are not significantly different ($p<0.05$).

from NRRL 1526 after 30 hr incubation, NRRL 2710 after 24 hr incubation, and NRRL 2549 after 24 hr incubation (see Figure A2). The control was darker (less white) ($p<0.05$) than all other samples (Table 12).

Incubation time and type of inoculum produced parallel trends for instrumental firmness and whiteness data. NRRL 2710 produced the firmest and whitest cakes of all inocula tested. The control produced the least white and the least firm of all the combinations (Figure A3). In Figure A4 the firmest sample was produced after 30 hr incubation while the whitest sample resulted after 24 hr incubation period.

Sensory Analysis

F-values and probabilities from ANOV for sensory parameters are presented in Table 13. Highly significant differences ($p<0.0001$) existed among treatments for all sensory parameters.

Odor Attributes

Nutty Odor. Fried tempeh had more nutty odor compared to the raw samples (Table 14), because a cooked, toasted flavor developed during frying. Steinkraus et al. (1960) stated deep-fat fried tempeh had a nutty flavor acceptable to nearly everyone who tested it. In the fried samples, the control had the lowest nutty odor score other than the 18 hr incubated sample of NRRL 1526, which did not have much mycelia growth and would taste similar to the control. The 12 hr incubated sample of NRRL 1526 had low nutty odor which was not significantly different from the fried or from the 18 hr incubated sample. Samples from the three

Table 13. F-values and probabilities from ANOV for sensory parameters for raw and fried tempeh[a]

	Source of Variation				
	Treatment	Error Treatment	Panelist	Error Panelist	Replication
DF	25	75	6	150	3
SENSORY PARAMETERS					
Odor					
Nutty	4.97 (0.0001)	1.22 (NS)	287.31 (0.0001)	4.70 (0.0001)	1.18 (NS)
Mushroom-like	55.68 (0.0001)	1.03 (NS)	186.45 (0.0001)	2.48 (0.0001)	2.90 (NS)
Yeasty	47.83 (0.0001)	1.49 (0.008)	50.83 (0.0001)	1.94 (0.0001)	1.44 (NS)
Beany	40.32 (0.0001)	0.93 (NS)	35.36 (0.0001)	2.71 (0.0001)	8.51 (0.0001)
Ammonia-like	13.87 (0.0001)	1.32 (NS)	34.00 (0.0001)	2.61 (0.0001)	4.21 (0.006)
Flavor-By-Mouth					
Nutty	68.90 (0.0001)	1.81 (0.0001)	240.07 (0.0001)	2.56 (0.0001)	2.48 (NS)
Mushroom-like	50.95 (0.0001)	1.03 (NS)	158.40 (0.0001)	1.94 (0.0001)	13.07 (0.0001)
Beany	36.56 (0.0001)	1.30 (NS)	137.17 (0.0001)	3.11 (0.0001)	6.69 (0.0002)
Cerealy	5.49 (0.0001)	0.90 (NS)	102.76 (0.0001)	1.70 (0.0001)	17.51 (0.0001)
Texture					
Firmness	53.01 (0.0001)	2.87 (0.0001)	41.54 (0.0001)	1.75 (0.0001)	5.34 (0.001)

[a]Probabilities are given in parentheses; NS = not significant.

Table 14. Mean values[a] for panelists' scores for the interaction betweeen inoculum and incubation time for odor attributes of raw and fried tempeh

Prepa-ration	Inoc.x (NRRL)	Incub. (hr)	MEAN VALUES Nutty	Mushroom	Yeasty	Beany	Ammonia
Raw	0	0	9.0gh	6.8e	5.2f	38.7a	1.4h
	1526	12	8.7gh	13.8d	14.1e	15.8bc	6.3cdef
	2710	12	9.6gh	20.8bc	20.2cd	18.3b	5.4def
	2549	12	9.8gh	24.2ab	20.9bcd	14.5c	5.1efg
	1526	18	6.6h	19.7c	18.0de	10.3d	7.0cdef
	2710	18	9.0gh	23.6ab	26.1a	10.0d	8.9bcd
	2549	18	10.9g	22.8abc	25.1abc	8.4def	7.1cdef
	1526	24	8.6gh	25.0a	23.0abcd	7.7defg	9.8abc
	2710	24	10.9g	24.3ab	24.3abc	10.0d	8.8bcde
	2549	24	10.4gh	23.8ab	22.9abcd	8.7def	11.1ab
	1526	30	9.3gh	23.3abc	24.1abc	9.0def	12.6ba
	2710	30	12.0g	26.3a	25.5ab	6.6efgh	8.8bcde
	2549	30	9.8gh	25.1a	25.3ab	9.9ed	11.6ab
Fried	0	0	29.6ef	3.0f	1.3f	6.1fghi	0.9h
	1526	12	31.8def	2.5f	2.8f	3.8hijk	0.9h
	2710	12	32.9cde	2.5f	2.4f	5.1hijk	1.0h
	2549	12	35.2bcd	3.1ef	2.1f	2.9ijk	1.3h
	1526	18	28.5f	4.5ef	4.5f	3.6hijk	3.5fgh
	2710	18	35.2bcd	4.1ef	3.6f	2.3jk	1.0h
	2549	18	40.5a	5.4ef	3.5f	2.6jk	1.1h
	1526	24	36.1bc	4.9ef	3.0f	2.3jk	0.8h
	2710	24	36.0bc	4.7ef	4.0f	2.4jk	1.0h
	2549	24	37.4ab	5.3ef	4.2f	1.8jk	1.6gh
	1526	30	34.6bcd	4.6ef	3.4f	2.5jk	1.4gh
	2710	30	35.1bcd	3.5ef	2.6f	1.3jk	1.1h
	2549	30	34.8bcd	3.8ef	2.6f	0.9jk	0.5h
LSD (0.05)			4.12	3.72	5.02	3.34	3.68

[a]Each value is a mean for four determinations; means with the same letter in a column are not significantly different ($p<0.05$).

inocula at 12 hr incubation had similar scores for nutty odor (Figure A5). The 18 hr incubated samples showed the most variation among inocula, especially for the fried samples. This could be because NRRL 2549 grew much faster than NRRL 1526, which affects the nutty odor development that is especially evident upon frying. NRRL 1526 consistently had lower scores than the other inocula for each incubation time. NRRL 2710 was lower in nutty odor than NRRL 2549, except during the 24 hr incubation for the raw and 30 hr incubation for the raw and fried tempeh. This could be because NRRL 2549 accelerated its growth in the last incubation periods and produced other odors that were more prevalent. Raw tempeh samples had no significant differences ($p<0.05$) for nutty odor, because nutty odor is not a predominant odor characterstic in raw tempeh or soybeans.

Mushroom-like Odor. The control had the lowest score for mushroom-like odor in the raw samples because no mycelia growth was present. Odor in raw samples generally increased as incubation time increased since the mold partially generates the mushroom-like odor. Steinkraus (1983) described freshly fermented tempeh as having "a clean, mushroom-like aroma". Hesseltine et al. (1963) also described "good" tempeh as having "a pleasant, fresh, slightly mushroom odor". NRRL 1526 had lower odor scores for the raw tempeh than the other two inocula, except during the 24 hr incubation period, but the differences were negligible and not statistically different. NRRL 2710 after 30 hr incubation had the strongest mushroom odor. There probably was the most fermentation after this time without sporulation. Frying caused the mushroom odor to diminish or to be masked by other odors. This is shown in Table 14

where no significant difference ($p<0.05$) is seen among the fried samples.

Yeasty Odor. Yeasty odor was examined because Wang and Hesssletine (1979) described raw tempeh having "a clean, fresh, and yeasty odor". No significant difference was found in yeasty odor ($p<0.05$) among fried samples (Table 14). This was because the yeasty odor was masked by frying the tempeh. The control maintained the lowest scores for yeasty odor for all the fried samples. The control for raw tempeh was not significantly different ($p<0.05$) from the fried control sample, but was significantly different ($p<0.05$) from the other raw samples. NRRL 1526 had a lower yeasty odor than the other two inocula in all incubation periods in the raw samples. This was especially evident after 12 and 18 hr incubation periods (see Figure A7). This result was produced because of NRRL 1526's slow growth,and, therefore, less mycelia. Raw tempeh from NRRL 2710 and NRRL 2549 after 30 hr incubation had the highest scores for yeasty odor, attributed to its prolific mycelia production.

Beany Odor. The raw control sample had more intense beany odor ($p<0.05$) than all the other raw samples (Table 14). Even though not much mycelia growth was noted in the raw 12 hr incubated samples, they were still scored much lower than the raw control. Samples from NRRL 2710 after 30 hr incubation had the lowest beany odor score for raw samples, although during the 12 hr incubation it had the highest score of the three inocula. NRRL 2549 had less intense beany odor during 12 and 18 hr incubation periods, but at 24 hr incubation it was not significantly different ($p<0.05$) from the other two inocula. After 30 hr incubation

period NRRL 2549 had the highest beany odor score of all the inocula. Wang and Hesseltine (1979a) indicated the beany flavor in soybeans that many people find unpleasant was not present in tempeh. Hesseltine (1985) said that the mold had interesting enzymes because the beany flavor was destroyed completely during tempeh production. In the fried samples, the control sample showed the greatest beany odor (see Figure A8). There was a slight decrease in beany odor as incubation time increased attributed to the enzymes produced by the mold. Differences among fried samples were not great, because the fried odor was dominant.

Ammonia-like Odor. All fried samples had low scores for ammonia-like odor (see Figure A9). Steinkraus (1983) stated frying would decrease development of ammonia-like odor because of the decrease in protein quality. In the raw samples the control showed almost no ammonia-like odor. This odor appeared to increase steadily as incubation time increased. NRRL 2549 produced the greatest ammonia-like odor. This probably is related to development of increased sporulation. Since this inoculum had the most sporulation according to the color data (see Figure A2), it was probably the fastest growing. Growing quickly would accelerate the proteolytic activity, resulting in the release of ammonia. Hesseltine and Wang (1979a) stated when tempeh fermentation is not stopped at a proper time, the proteases will form ammonia. Steinkraus (1983) indicated when the flavor of tempeh becomes stronger and the free ammonia is released, the initial white cake becomes black because of the spores produced by the mold. This was the case with NRRL 2549 which produced a stronger ammonia-like odor. This free ammonia is lethal to cultures of Rhizopus (Steinkraus et al., 1960). Perhaps, if

incubation time was increased to 48 hr, NRRL 2549 would have produced these results. NRRL 2710 after 30 hr incubation was significantly different ($p<0.05$) from the other two inocula at this incubation time, indicating less proteolytic activity (Table 14).

Flavor-By-Mouth Attributes

Nutty Flavor. Nutty flavor was similar to results found for nutty odor (see Figures A5 and A10) for all samples. The fried samples were scored much higher than the raw samples for nutty flavor. Frying develops a characteristic nutty flavor in tempeh. As incubation time was increased for fried tempeh, the nutty flavor also increased up to 24 hr incubation. After 24 hr incubation the nutty flavor remained relatively the same. The control sample had one of the lower scores for nutty flavor. Sample NRRL 1526 after 18 hr was lowest in nutty flavor. This parallel result is reported in a preceding section for nutty odor. The most variability among samples in a specific incubation time period was at the 18 hr incubation period. This is attributed to differences in growth rate of the three inocula. NRRL 2710 at 24 hr had the most nutty flavor, although not significantly different from NRRL 2549 samples at 18 hr ($p<0.05$)(Table 15). No single sample was prevalent in both nutty odor and nutty taste.

Mushroom-like Flavor. Although the mushroom characteristic for tempeh was described in the literature as an odor, the mushroom-like flavor and odor produced similar results (Steinkraus et al., 1960; Hesseltine et al., 1963b). For both attributes, raw tempeh had significantly higher scores than the fried samples. Fried samples had

Table 15. Mean values[a] for panelists' scores for the interaction between inoculum and incubation time for flavor-by-mouth and texture attributes of raw and fried tempeh

Prepa-ration	Inoc.x (NRRL)	Incub. (hr)	MEAN VALUES Nutty	Mushroom	Beany	Cerealy	Firmness
Raw	0	0	10.5e	8.1f	43.9a	12.4defg	2.3hi
	1526	12	8.9e	12.7e	23.3bc	16.8abc	3.7ghi
	2710	12	10.0e	17.2d	26.8b	13.6bcdef	8.0fgh
	2549	12	12.0e	19.8cd	21.8c	13.3cdef	3.1ghi
	1526	18	9.5e	21.6bc	16.7d	17.1abc	9.0fg
	2710	18	11.4e	23.1bc	15.9de	17.4ab	20.0cd
	2549	18	10.8e	21.8bc	13.0defg	15.0abcde	16.0de
	1526	24	12.2e	23.2bc	12.2efgh	17.2ab	22.8bc
	2710	24	12.4e	22.9bc	15.9de	14.6bcde	23.7abc
	2549	24	10.5e	27.4a	12.0efghi	17.3ab	27.2ab
	1526	30	10.0e	24.5ab	11.9efghi	19.0a	27.0ab
	2710	30	11.9e	26.9a	11.9efghi	16.7abc	28.5ab
	2549	30	9.2e	24.8ab	12.9defg	15.9abcd	28.8ab
Fried	0	0	26.7cd	3.9g	10.3fghijk	10.3fgh	0.4i
	1526	12	28.8bcd	3.9g	11.1fghij	9.7fgh	4.6ghi
	2710	12	28.3bcd	4.0g	14.5def	11.7efg	7.9fgh
	2549	12	32.7ab	4.9fg	11.1fghij	9.9fgh	13.1ef
	1526	18	25.3d	5.5fg	8.8hijkl	10.5fgh	11.5ef
	2710	18	31.4abc	5.4fg	6.5kl	9.2gh	23.3abc
	2549	18	35.1a	5.9fg	8.0hijkl	8.9gh	24.4abc
	1526	24	34.6a	6.4fg	5.7l	11.3efgh	22.4bcd
	2710	24	36.1a	5.9fg	7.7ijkl	9.7fgh	27.1ab
	2549	24	32.4ab	6.0fg	8.0hijkl	9.9fgh	29.0ab
	1526	30	31.4abc	6.3fg	6.4kl	9.3gh	26.9ab
	2710	30	34.5a	4.9fg	7.3jkl	8.9gh	29.9a
	2549	30	32.5a	5.7fg	8.2hijkl	7.6h	28.9ab
LSD (0.05)			5.00	3.57	4.32	3.95	6.70

[a]Each value is a mean value for four determinations; means with the same letter in a column are not significantly different ($p<0.05$).

no significant differences ($p<0.05$) among themselves for mushroom-like flavor frying masked this flavor note (see Table 15). The mushroom-like flavor in raw samples steadily increased as incubation time was increased. Exceptions were noted in tempeh from NRRL 2549, which slightly decreased from the 24 hr to the 30 hr incubation periods and NRRL 2710 which stayed relatively the same from 18 hr to 24 hr incubation periods. NRRL 2549 at 24 hr incubation and NRRL 2710 at 30 hr incubation were rated highest for mushroom-like flavor for raw samples ($p<0.05$) (see Figure A11). Therefore, NRRL 2710 after 30 hr incubation had the greatest mushroom-like flavor if odor and taste are considered together. Mushroom-like flavor in NRRL 2549 tempeh decreased from the 24 to 30 hr incubation periods, which was attributed to an increase in sporulation masking or altering the mushroom-like taste.

Beany Flavor. Data for beany flavor had greater variability than data for beany odor, because the panelists felt they were more sensitive when tasting. Trends in the figures are similar (see Figure A8 and Figure A12). The fried control was slightly lower than the inocula after 12 hr incubation, but this finding was not significantly different ($p<0.05$). Obviously, by the 12 hr incubation period, the lipases from the mold had little activity. Kinsella and Damodaron (1980) reported beany flavor resulted from autoxidation or the activity of lipoxygenase on linoleic acid to form furans and aldehydes, which are compounds that contribute to beany flavor of soybeans. Results on beany flavor showed that increased fermentation time resulted in decreased beany flavor. This could be related to an increase in free fatty acids during the

first 30 hr of incubation. Gyorgy (1964) reported a decrease in hydrogen peroxides of tempeh, This is an intermediate step of lipoxygenase reacting on free fatty acids to create off flavors. When an expert panel tasted the hydrogen peroxides of linoleic and linolenic acid, they said these compounds gave the typical off flavors of soy products, especially grassy/beany (Kinsella and Damodaron, 1980). Gyorgy (1964) theorized that the antioxidant of tempeh (6,7,4-trihydroxyisoflavone) stopped either this reaction or autoxidation of free fatty acids. Data in this study for beany flavor indicate a decrease in beany flavor when soybeans were exposed to all three inocula. Even the short 12 hr incubation period produced lower beany flavor scores than the unfermented control ($p<0.05$) for the raw samples (see Table 16). There was a steady decrease in beany flavor for raw tempeh with an increase in incubation time. However NRRL 1526 and NRRL 2549 showed no significant change from the 24 hr to the 30 hr incubation periods and NRRL 2710 showed no significant change from the 18 hr to the 24 hr incubations (see Figure A12 and Table 15). This could be caused by a lag period where free fatty acids were not increased. Hesseltine and Wang (1967b) stated that tempeh fermentation destroys or masks the soybean's undesirable tastes and odors.

Cerealy Flavor. The attribute of cerealy was difficult for the panelists to distinguish and differentiate. No studies were found in the literature indicating the cerealy component as a predominant flavor in soybeans or tempeh. The raw fermented soybeans (tempeh) had more cerealy taste than the unfermented control (see Table 15). The fried

sample showed little change from the unfermented control to the fermented soybeans, except in the 30 hr incubation period, where the cerealy taste was less than the control. Frying appeared to mask the cerealy taste since not much variability was noted in the fried samples (see Figure 15). From this data one would conclude that cerealy taste is not a high intensity characteristic of tempeh and shows no relationship to the other data.

Texture

Firmness. Firmness increased with incubation time (see Figure A14). Since the panelists evaluated raw and fried tempeh in independent sessions, the scores for the two were similar, which was not the case for the instrumental data. Texture of tempeh is an important parameter. Steinkraus (1979) indicated the tempeh fermentation process provides a method to introduce "texture" into the soybeans. A firm cake that is cheese-like is often used to describe tempeh (Steinkraus et al., 1960; Platt, 1964). The control sample, the 12 hr incubated samples, and NRRl 1526 after 18 hr incubation were significantly different ($p<0.05$) from the other samples for firmness in raw and fried tempeh (see Table 15). These data parallel the instrumental data (see Table 9). NRRL 2710 after 30 hr incubation and frying was the firmest product according to sensory scores and instrumental data (see Figures A1 and Figure A14). Wang and Hesseltine (1979a) believed the tempeh process gives the soybeans a texture that is familiar and highly acceptable to people around the world.

Table 16 and Figure A15 contain individual panelist's sensory mean

values for each attribute of the raw and fried tempeh. Although some variability existed in scoring (Figure A15), trends were similar, except for cerealy flavor. Data in Figures A15 and A13 and Table 15 indicate that the cerealy attribute should not have been evaluated or it should have been better defined. One panelist rated nutty and mushroom-like attributes higher ($p<0.05$) than the other panelists.

Relationship Between Instrumental and Sensory Data

Table 17 contains correlation coefficients and probabilities for selected sensory and instrumental measurements. Data for instrumental and sensory firmness were highly correlated ($p<0.01$). Therefore, even with altering factors such as saliva flow or enzymes present in the mouth, the panelists' firmness data were similar to the instrumental results. L-values for tempeh as determined by HunterLab spectrophotometer also were correlated with sensory firmness ($p<0.01$). According to the conditions of this study, the firmer tempeh also was whiter.

Table 16. Sensory mean values[a] for individual panelists for each attribute of the raw and fried tempeh

ATTRIBUTE	LSD (0.05)	MEAN VALUES A	B	C	D	E	F	G
ODOR								
Nutty	1.91	14.0d	17.0c	15.1cd	20.3b	47.5a	20.0b	20.1b
Mushroom	1.88	12.1bc	5.0e	10.5cd	5.9e	32.3a	13.5b	9.7d
Yeasty	2.10	10.4cd	10.9c	11.5bc	13.0b	23.4a	6.8e	8.5de
Beany	1.77	4.2d	12.3a	5.9cd	4.3d	13.5a	6.3c	8.6b
Ammonia	1.58	2.3b	8.0a	1.0b	8.6a	2.3b	2.5b	7.8a
TASTE								
Nutty	1.89	14.7de	16.1d	18.3c	21.9b	44.0a	13.8e	18.8c
Mushroom	1.80	8.6d	5.0e	11.6bc	10.0cd	30.3a	13.4b	13.2b
Beany	1.94	9.1b	24.4a	9.0b	5.8c	25.6a	9.3b	9.6b
Cerealy	2.13	2.5f	16.7b	25.4a	5.3e	13.1c	16.6b	10.0d
TEXTURE								
Firmness	2.02	17.0d	17.8cd	19.7bc	13.8e	26.1a	19.9b	11.4f

[a]Each value is a mean for eight determinations; means with the same letter in a row are not significantly different ($p<0.05$).

Table 17. Correlation coefficients and probabilities for instrumental and selected sensory measurements for tempeh

Measurements	Correlation coefficient	t-value	P-value
Instrumental firmness and Sensory firmness	-0.72989	3.5414	0.01
HunterLab L-value and Sensory firmness	0.76781	3.9748	0.01

CONCLUSIONS

Based on the conditions of this study, the following conclusions can be made:

1. Type of inoculum (NRRL 1526, 2710, or 2549), incubation time (0, 12, 18, 24, or 30 hr), and preparation method (raw or fried) affected tempeh firmness, whiteness, and sensory characteristics.

2. Firmness of tempeh increased as incubation time was increased from 0 to 30 hr, as determined by instrumental and sensory scores. NRRL 2710 produced tempeh that was incubated for 30 hr was firmest, and the control was least firm.

3. Tempeh whiteness increased as incubation time was increased up to 24 hr, after which sporulation occurred.

4. Beany odor and taste were highest in control samples that were not inoculated or incubated.

5. Beany odor and flavor decreased as incubation time increased.

6. Nutty odor and flavor were more intense in fried samples rather than in raw tempeh.

7. Mushroom-like odor and flavor, and yeasty odor were more intense in raw tempeh rather than in fried samples.

8. Ammonia-like odor was low until after the 24 hr incubation period.

REFERENCES

Autret, M., and VanVeen, A.G. 1955. Possible sources of proteins for child feeding in underdeveloped countries. Am. J. Clin. Nutr. 3: 234.

Banwart, G.J. 1981. Microorganisms associated with foods. In "Basic Food Microbiology," 1st ed. AVI Publishing Co., Inc. Westport, CT.

David, I.M. and Verma, J. 1981. Modification of tempeh with the addition of bakla. J. Food Technol. 16(1): 39.

Dijen, K.S. 1982. Indigenous fermented food. In "Microbial Biomass," 1st ed., vol. 4 of Economic Microbiology, A.H. Rose (Ed.), p. 115. Academic Press, Inc., London.

Dijen, K.S. and Hesseltine, C.W. 1961. Indonesion fermented foods. Soybean Digest 22: 14.

Djurtoft, R. and Nielsen, J.P. 1983. Increase in some Vitamin B during fermentation of tempeh from cowpeas or soybeans. J. Plant Food 5: 135.

Ellis, J.J., and Hesseltine, C.W. 1983. Maintaining stock cultures of Rhisopus oligosporus for tempeh making. Soyfood World: 29

Gyorgy, P. 1961. The nutritional value of tempeh. Meeting Protein Needs of Infant and Children, Pub. 843, National Academy of Science--National Research Council, Washington, D.C., p. 281.

Gyorgy, P., Murata, K., and Ikehata, H. 1964. Antioxidents isolated from fermented soybeans (tempeh). Nature 203: 870.

Gyorgy, P., Murata, K., and Sugimoto, Y. 1974. Studies on antioxidant activity of tempeh oil. J. Am. Oil Chem. Soc. 51: 377.

Hackler, L.R., Steinkraus, K.H., VanBuren, J.P., and Handy, D.B. 1964. Studies on the utilization of tempeh protein by weanling rats. J. Nutr. 82: 452.

Hesseltine, C.W. 1985. Fungi, people, and soybeans. Mycologia 77(4): 505.

Hesseltine, C.W. 1965. A millennium of fungi, food, and fermentation. Mycologia 57: 149.

Hesseltine, C.W. and Wang, H.L. 1979. Fermented foods. Chem. Indust. 16: 303.

Hesseltine, C.W., DeCarmargo, R., and Rackis, J.J. 1963a. A mould-inhibitor in soybeans. Nature 200: 1226.

Hesseltine, C.W., Smith, M., Bradle, B., and Dijen, K.S. 1963b. Investigations of tempeh, an Indonesion food. Dev. Indus. Microbiol. 4: 275.

Hesseltine, C.W., Smith, A.K., and Wang, H.L. 1967a. New fermented cereal products. Dev. Indus. Microbiol. 8: 179.

Hesseltine, C.W. and Wang, H.L. 1967b. Traditional fermented foods. Biotechnol. and Bioeng. 9: 275.

Ikehata, H., Wakaizumi, M., and Murata, K. 1968. Antioxidant and antihemolytic activity of a new isoflavone, "Factor 2" isolated from tempeh. Agric. Biol. Chem. 32: 740.

Jurus, A.M. and Sundberg, W.J. 1976. Penetration of R. oligosporus into soybeans in tempeh. Appl. Env. Microbiol. 32(2): 284.

Kakade, M.L., Rackis, J.J., McGhee, J.E., and Pusk, G. 1974. Determination of trypsin inhibitor activity of soy products: a collaborative analysis of an improved procedure. Cereal Chem. 51: 376.

Kinsella, J.E. and Damodaron, S. 1980. Flavor problems in soy proteins: Origin, nature, control and binding phenomena. In "The Analysis and Control of Less Desirable Flavors in Foods and Beverages," p. 95. Academic Press, New York, NY.

Liem, I.T.H., Steinkraus, K.H., and Cronk. T.C. 1977. Production of vitamin B-12 in tempeh, a fermented soybean food. Appl. Env. Microbiol. 34(6): 773.

Martinelli, A.F., and Hesseltine, C.W. 1964. Tempeh fermentation: package and tray fermentations. Food Technol. 18(5): 167.

Murakami, H., Asakawa, T., Terao, J., and Matsushita, S. 1984. Antioxidant stability of tempeh and liberation of isoflavones by fermentation. Agric. Biol. Chem. 48(12): 2971.

Murata, K., Ikehata, H., Edan, Y., and Koyanagi, K. 1971. Studies on the nutritional value of temkpeh. Part II. Rat feeding test with tempeh, unfermented soybeans, and tempeh supplemented with amino acids. Agric. Biol. Chem. 35(2): 233.

Murata, K., Ikehata, H., and Miyamoto, T. 1967. Studies on the nutritional values of tempeh. J. Food Sci. 32: 580.

Platt, B.S. 1964. Biological ennoblement of foods. Food Technol. 18(5): 667.

Rackis, J.J. 1974. Biological and physiological factors in soybeans. J. Am. Oil Chem. Soc. 51: 961.

Rackis, J.J., Honig, D.H., Sessa, D.J., and Steggerda, F.R. 1970. Flavor and flatulence factors in soybean protein product. J. Agric. Food Chem. 18(6): 977.

Rathburn, B.L. and Shuler, M.L. 1983. Heat and mass transfer effects in static solid-substrate fermention chambers. Biotechnol. and Bioeng. 25: 929.

Robinson, R.J. and Kao, C. 1977. Tempeh and miso from chickpea, horse bean, and soybean. Cereal Chem. 54(6): 1192.

Roelofsen, R.A. and Talens, A. 1964. Changes in some B vitamins during molding of soybeans by Rhizopus oryzae in the production on tempeh kedelee. J. Food Sci. 29: 224.

Roozen, J.P. and De Groot, J. 1985. Electrophoresis and assay of trypsin inhibitors in different stages of tempeh production. J. Food Biochem. 9: 37.

Rusmin, S. and Dijen, K.S. 1974. Rice-grown R. oligosporus inoculum for tempeh fermentation. Appl. Microbiol. 28(3): 347.

SAS Institute. 1985. "SAS User's Guide." Statistical Analysis System Institute, Inc. Cary, N.C.

Shurtleff, W. and Aoyagi, A. 1979. "The Book of Tempeh," 2nd ed. Harper and Row Publishing, New York, NY.

Smith, A.K., Rackis, J.J., and Hesseltine, C.W. 1963. Processing losses and nutritive value of tempeh, a fermented soybean food. Cereal Sci. Today 8: 129.

Smith, A.K., Rackis, J.J., Hesseltine, C.W., Smith, M., Robbins, D.J., and Booth, A.N. 1964. Tempeh: Nutritional values in relation to processing. Cereal Chem. 41: 173.

Sorenson, W.G. and Hesseltine, C.W. 1966. Carbon and nitrogen utilization by R. oligosporus. Mycologia 58: 681.

Stahl, H.D. and Sims, R.J. 1986. Tempeh oil -- antioxidant(?). J. Am. Oil Chem. Soc. 63: 555.

Stanier, R.Y., Doudoroff, M., and Adelberg, E. 1963. The primitive fungi: Phycomycetes. In "The Microbial World," 2nd ed. p. 119. Prentice-Hall, Inc. Englewood Cliffs, N.J.

Steinkraus, K.H. 1978. Tempeh -- An Asian example of appropriate/intermediate foods. Food Technol. 32(4): 79.

Steinkraus, K.H. (Ed.) 1983. "Handbook of Indigenous Fermented Foods," 1st ed. vol. 9 of Microbiology Series, Marcel Dekker, Inc., New York, NY.

Steinkraus, K.H. 1985. Bioenrichment: production of vitamins in fermented foods. In "Fermented Foods," Vol. 1, B.J.B. Wood (Ed.), p. 337. Elsevier Applied Science Pub. Ltd., London and New York.

Steinkraus, K.H., Hwa, Y.B., VanBuren, J.P., Provvidenti, M.I., and Hand, D.B. 1960. Studies on tempeh -- an Indonesian fermented soybean food. Food Res. 25: 777.

Steinkraus, K.H., Lee, C.Y., and Buck, P.A. 1965a. Soybean fermentation by the Ontjom mold Neurospora. Food Technol. 19(8): 119.

Steinkraus, K.H., Van Buren, J.P., Hackler, L.R., and Hand, D.B. 1965b. A pilot-plant process for the production of dehydrated tempeh. Food Technol. 19(1): 63.

Stillings, B.R. and Hackler, L.R. 1965. Amino acid studies on the effects of fermentation time and heat-processing of tempeh. J. Food Sci. 30: 1043.

Stone, H. and Sidel, J.L. 1985. Descriptive Analysis. In "Sensory Practices," 1st ed. p. 194. Academic Press, Inc., Orlando, FL.

Sudarmadji, S. 1975. Certain chemicals and nutritional aspects of soybean tempeh. Doctoral Dissertation, Michigan State University. East Lansing, MI.

Sudarmadji, S. and Markakis, P. 1977. The phytate and phytase of soybean tempeh. J. Sci. Food Agric. 28: 381.

Sudarmadji, S. and Markakis, P. 1978. Lipid and other changes occurring during the fermentation and frying on tempeh. Food. Chem. 3: 165.

Sutardi and Buckle, K.A. 1985. Reduction in phytic acid levels in soybeans during tempeh production, storage and frying. J. Food Sci. 50: 260.

Tanaka, N., Kovats, S.K., Guggisberg, J.A., Meske, L.M., and Doyle, M.P. 1985. Evaluation of the microbiological safety of tempeh made from unacidified soybeans. J. Food Prot. 48: 438.

Teutonico, R.A. and Knorc, D. 1985. Impact of biotechnology on nutritional quality. Food Technol. 39(10): 127.

Van Buren, J.P., Hackler, L.R., and Steinkraus, K.H. 1972. Solubilization of soybean tempeh constituents during fermentation. Cereal Chem. 42: 208.

VanVeen, A.G., Graham, D.C.W., and Steinkraus, K.H. 1968. Fermented peanut press cake. Cereal Sci. Today 13(3): 97.

Wagenknecht, A.C., Mattick, L.R., and Lewin, L.M. 1961. Changes in soybean lipids during tempeh fermentation. J. Food Sci. 26: 373.

Wang, H.L. 1984. Tofu and tempeh as potential protein sources in Western diet. J. Am. Oil Chem. Soc. 61: 528.

Wang, H.L., Ellis, J.J., and Hesseltine, C.W. 1972a. Antibacterial activity produced by mold commonly used in oriental food fermentations. Mycologia 64(1): 218.

Wang, H.L. and Hesseltine, C.W. 1965. Studies on the extracellular proteolytic enzymes of Rhizopus oligosporus. Can. J. Microbiol. 11: 727.

Wang, H.L. and Hesseltine, C.W. 1966. Wheat tempeh. Cereal Chem. 43(5); 563.

Wang, H.L. and Hesseltine, C.W. 1974. Acid protease production by fungi used in soybean food fermentation. Appl. Microbiol. 27: 906.

Wang, H.L. and Hesseltine, C.W. 1979a. Mold-modified foods. In "Microbial Technology," 2nd ed. H.J. Peppler and D. Perlman (Ed.) Vol. II, p. 95. Academic Press, Inc., New York, NY.

Wang, H.L. and Hesseltine, C.W. 1979b. Oriental fermented foods. In "Prescott and Dunn's Industrial Microbiology," 4th ed. G. Reed (Ed.) AVI Publishing Co. Westport, CT.

Wang, H.L. and Hesseltine, C.W. 1981. Use of microbial cultures: legume and cereal products. Food Technol. 35(1): 79.

Wang, H.L., Ruttle, D.I., and Hesseltine, C.W. 1968. Protein quality of wheat and soybeans after Rhizopus oligosporus fermentation. J. Nutr. 96: 109.

Wang, H.L., Ruttle, D.I., and Hesseltine, C.W. 1969. Antibacterial compound from a soybean product fermented from Rhizopus oligosporus. Proceed. Soc. Experiment. Biol. Med. 131: 579.

Wang, H.L., Swain, E.W., and Hesseltine, C.W. 1975a. Mass production of R. oligosporus spores and their application tempeh fermentation. J. Food Sci. 40: 168.

Wang, H.L., Swain, E.W., and Hesseltine, C.W. 1980. Phytase of molds used in Oriental food fermentation. J. Food Sci. 45: 1262.

Wang, H.L., Swain, E.W., Wallen, L.L., and Hesseltine, C.W. 1975b. Free fatty acids identified as antitryptic factor in soybeans fermented by Rhizopus oligosporus. J. Nutr. 105: 1351.

Wang, H.L., Vespa, J.B., and Hesseltine, C.W. 1972b. Release of bound trypsin inhibitor in soybeans by Rhizopus oligosporus. J. Nutr. 102: 1495.

Wilkins, W.F. and Lin, F.M. 1970. Gas chromatographic and mass spectral analysis of soybean milk volatiles. J. Agric. Food Chem. 18: 333.

Zamora, R.G. and Veum, T.L. 1979. The nutritive value of dehulled soybeans fermented with Aspergillus oryzae or Rhizopus oligosporus as evaluated by rats. J. Nutr. 109: 1333.

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to my Major Professor, Dr. Martha Stone, for her long hours, endearing patience, and much needed encouragement. I also wish to extend my thanks to Dr. Daniel Y.C. Fung of the Department of Animal and Industry, for his encouragement and enthusiasm, and for serving as a committee member.

I also appreciate all the hard work and dependability of my seven panelists. Thanks to my statistician, Dr. James Higgins, for his helpfulness, especially "at the last minute". An appreciation goes out to my third committee member, Dr. Carole Setser, for editing my manuscript.

I would like to thank Deb Ocken and Kevin Johnson for keeping me off the street and giving me food and shelter. A great appreciation goes to my very good friend, Tim Bickhaus, who helped me on all my graphs and always beat me in gin rummy for points. Thanks to Steve Specter, who shared my lab space, for his great sense of humor and good advice. My sincere gratification to my dear friend, Mark Endicott, for his help, patience, and encouragement.

Thanks also to my family for their love and support and to my "moms away from home," Nila Hines and Angie Hageman, who took care of me in Manhattan. To all the graduate students in Foods and Nutrition and Food Science, thanks for your friendship. Lastly, thanks to Cafe Royale for the many cups of coffee and great conversations.

APPENDIX

Table A2. Reference standards for attribute scaling of tempeh

Attribute	Standard[a]
Odor	
nutty	Planters dry roasted peanuts
mushroom-like	Freshly cut mushrooms
yeasty	Peppridge Farm sourdough bread
beany	Freshly made soy milk
ammonia-like	Tempeh incubated for 72 hr
Taste	
nutty	Planters dry roasted peanuts
mushroom-like	Freshly cut mushrooms
beany	Freshly made soy milk
cerealy	Quaker Instant oatmeal prepared according to directions
Texture	
firmness (low)	Philadelphia Brand cream cheese (1.25 cm cubes)
firmness (high)	Hersheys milk chocolate bar (1.25 cm cubes)

[a]all samples served at room temperature

Table A1. Score card to Attribute Scaling of tempeh

NAME_____ DAY_____
DATE_____ SAMPLE#_____

-TEMPEH-
ATTRIBUTE SCALING

ODOR

Nutty

not nutty _____ very nutty

Mushroom-like

not mushroom-like _____ very mushroom-like

Yeasty

not yeasty _____ very yeasty

Beany

not beany _____ very beany

Ammonia-like

not ammonia-like _____ very ammonia-like

FLAVOR-BY-MOUTH

Nutty

not nutty _____ very nutty

Mushroom-like

not mushroom-like _____ very mushroom-like

Beany

not beany _____ very beany

Cerealy

not cerealy _____ very cerealy

TEXTURE
Firmness

not firm _____ very firm

COMMENTS:

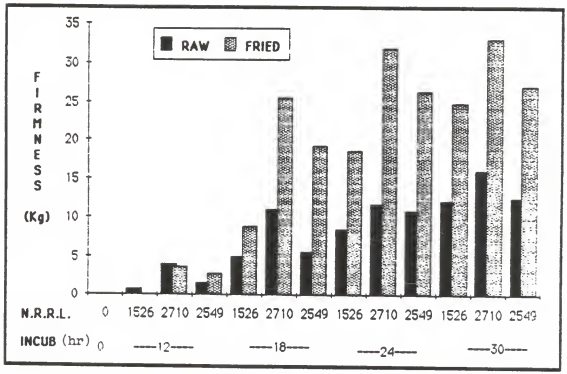


Figure A1. Instron mean values for the interaction between inoculum and incubation time for raw and fried tempeh

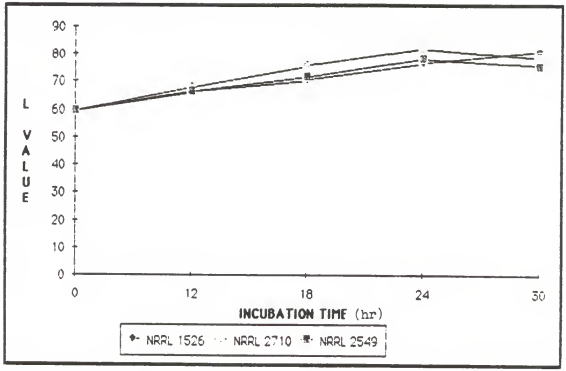


Figure A2. HunterLab spectrophotometer mean values for interaction between inoculum and incubation time for raw tempeh .....

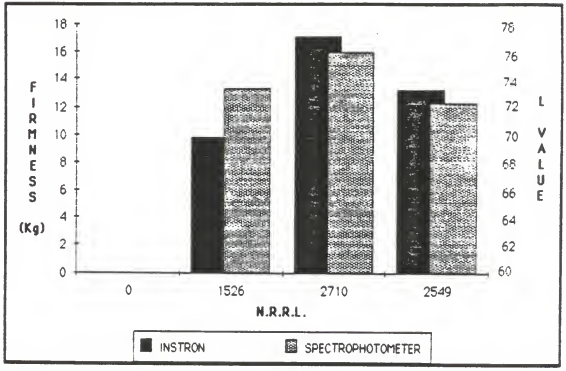


Figure A3. Comparison of Instron and HunterLab spectrophotometer mean values for type of inoculum of tempeh

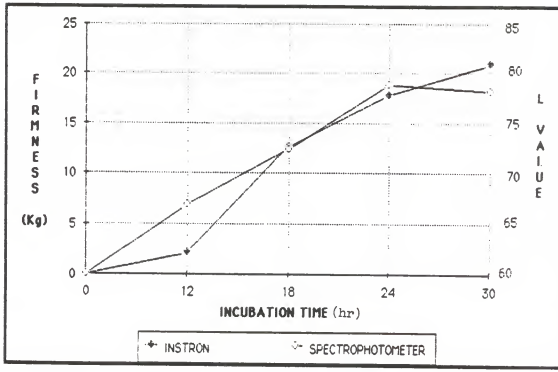


Figure A4. Comparison of Instron and HunterLab spectrophotometer mean values for incubation time of tempeh

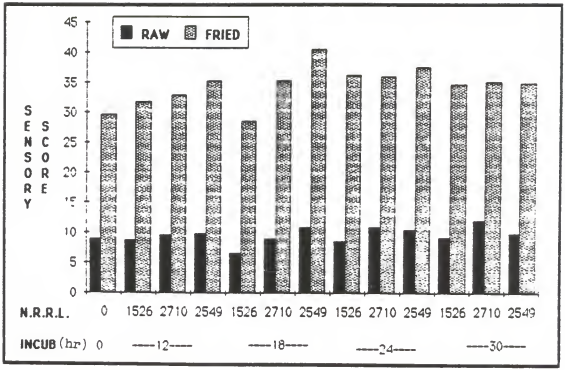


Figure A5. Mean values of panelists' scores for nutty odor of raw and fried tempeh

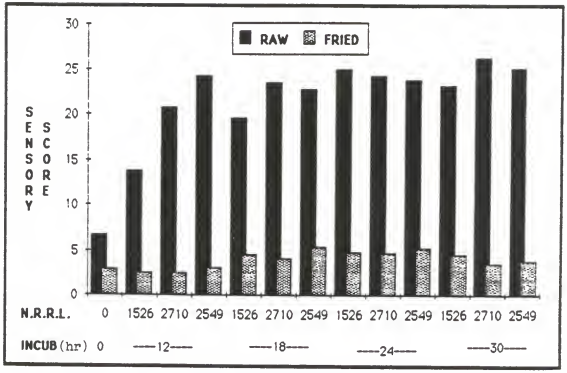


Figure A6. Mean values of panelists' scores for mushroom-like odor of raw and fried tempeh

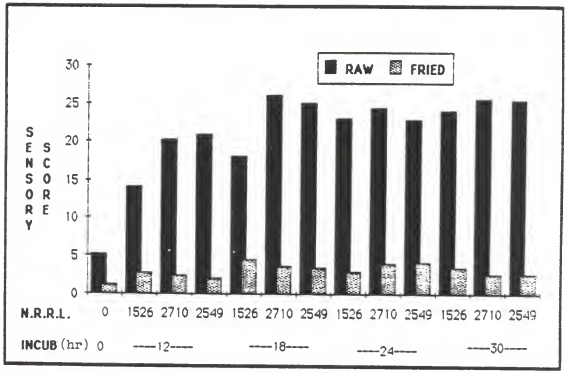


Figure A7. Mean values of panelists' scores for yeasty odor of raw and fried tempeh

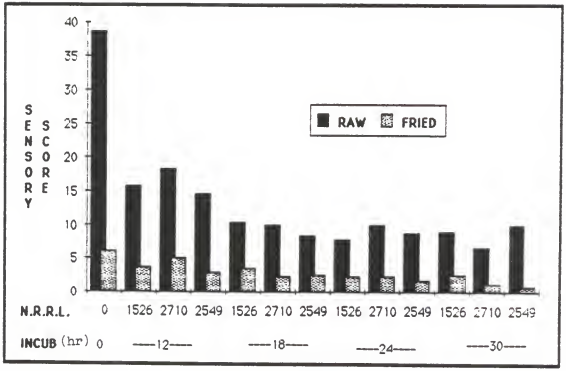


Figure A8. Mean values of panelists' scores for beany odor of raw and fried tempeh

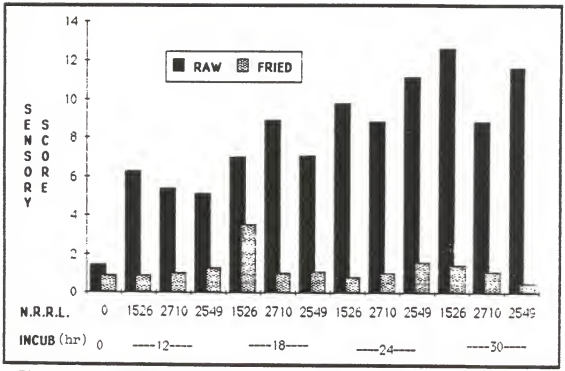


Figure A9. Mean values of panelists' scores for ammonia-like odor of raw and fried tempeh

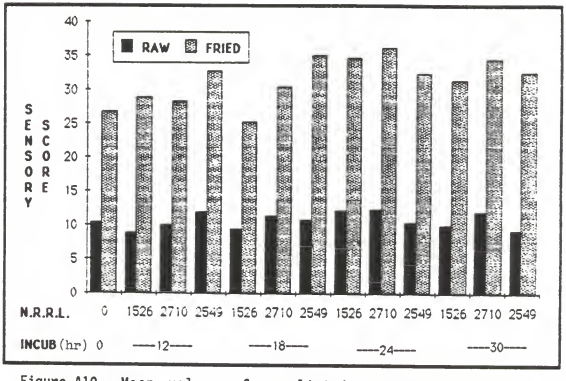


Figure A10. Mean values of panelists' scores for nutty taste of raw and fried tempeh

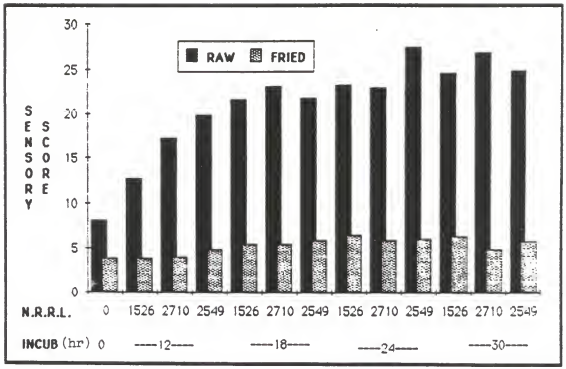


Figure A11. Mean values of panelists' scores for mushroom-like taste of raw and fried tempeh

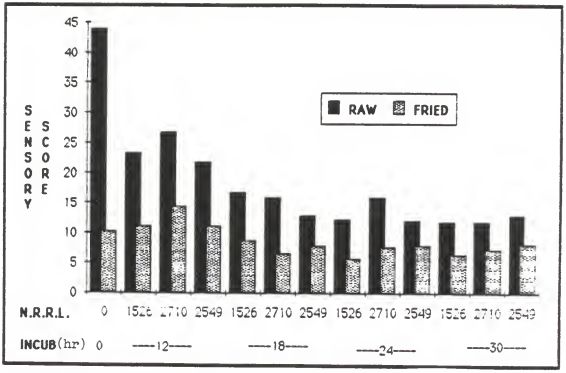


Figure A12. Mean values of panelists' scores for beany taste of raw and fried tempeh

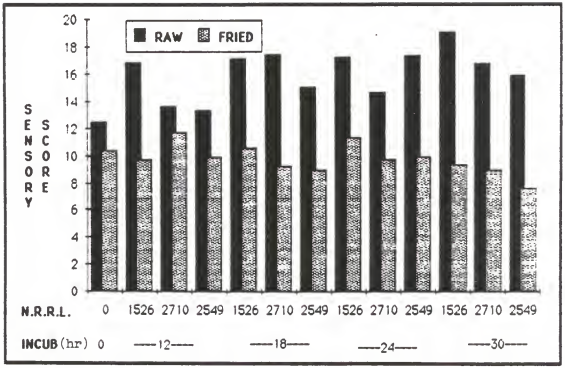


Figure A13. Mean values of panelists' scores for cerealy taste of raw and fried tempeh

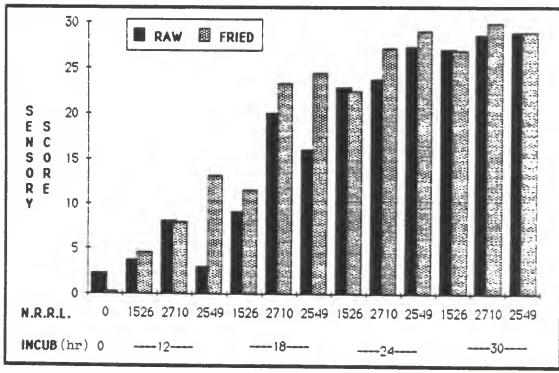


Figure A14. Mean values of panelists' scores for firmness of raw and fried tempeh

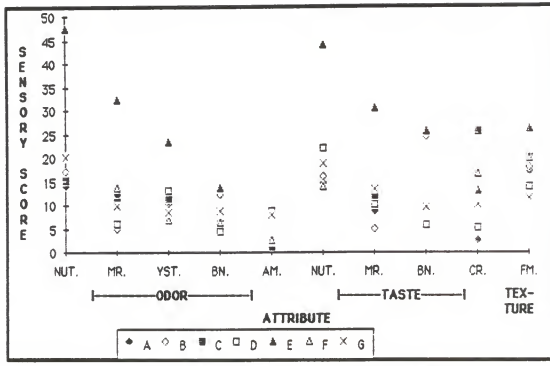


Figure A15. Sensory mean values of individual panelists for each attribute of raw and fried tempeh

EFFECTS OF INOCULA AND INCUBATION TIMES ON SELECTED SENSORY AND PHYSICAL CHARACTERISTICS OF TEMPEH

by

CYNTHIA M. LUND

B.S., Oregon State University, 1985

AN ABSTRACT FOR MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

in

Food Science

Department of Foods and Nutrition

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1988

Soybeans are an inexpensive source of quality protein, but they have off-flavors that are not acceptable to Westerners. Tempeh, a fermented soybean product, undergoes a process which alters the flavor components of soybeans. Little information on the flavor changes in tempeh production is available.

This study investigated flavor and textural changes which occurred during the fermentation of soybeans in tempeh production. Different incubation times (0, 12, 18, 24, 30 hr), types of Rhizopus inoculum (NRRL 1526, NRRL 2710, NRRL 2549), and preparation methods (raw and fried) were studied by instrumental and sensory methods. Data were analyzed by analysis of variance and least significant differences at the 5% level were calculated when F-values for effects of type of inoculum, incubation time, or preparation method were significant.

The type of inoculum (NRRL 1526, 2710, or 2549), incubation time (0, 12, 18, 24, or 30 hr), and preparation method (raw or fried) affected tempeh firmness and whiteness ($p<0.05$). Firmness of tempeh increased as incubation time was increased from 0 to 30 hr as determined by instrumental values and sensory scores. NRRL 2710 produced tempeh, incubated for 30 hr was firmest, and the control (no fermentation) was least firm. Tempeh whiteness increased as incubation time was increased up to 24 hr, after which sporulation occurred.

All treatments affected the sensory characteristics evaluated. Beany odor and flavor were highest in control samples that were not inoculated or incubated ($p<0.05$). Beany odor and flavor were lowest in tempeh NRRL 2710 after 30 hr incubation. Generally beany odor and flavor decreased as incubation time was increased. This supports the

theory that the tempeh fermentation process reduces beany flavor development.