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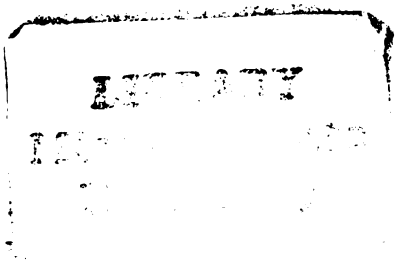

This is to certify that the

thesis entitled

EVALUATION OF TEMPEH PREPARED FROM GERMINATED SOYBEANS

presented by

Suparmo

has been accepted towards fulfillment
of the requirements for

M.S. degree in Food Science

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Date May 18, 1984

EVALUATION OF TEMPEH PREPARED FROM GERMINATED SOYBEANS

By

Suparmo

A THESIS

Submitted to

Michigan State University

in partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE

Department of Food Science and Human Nutrition

1984

ABSTRACT

EVALUATION OF TEMPEH PREPARED FROM GERMINATED SOYBEANS

By

Suparmo

Tempeh is a traditional Indonesian food made from soybeans fermented by a mixed culture of Rhizopus species. The tempeh fermentation improves the acceptance and the digestibility of the soybeans.

The objective of this research was to combine the tempeh fermentation with soybean germination in order to further improve the nutritive value of tempeh.

Germination for 24 hours reduced the oligosaccharide, phytic acid and crude fat content, and slightly increased the total and soluble protein content.

The combination of soybean germination and tempeh fermentation reduced more oligosaccharides and phytic acid than tempeh fermentation alone, while further decrease of fat content and further increase of total and soluble protein were also observed. Lectin activity was not observed in any of the tempeh samples.

The PER of tempeh prepared from germinated soybeans was 2.25, while that of regular tempeh was 2.18. Tempeh from germinated soybeans could not be differentiated in taste and appearance.

ACKNOWLEDGMENTS

The author would like to thank his major professor, Dr. P. Markakis, for his encouragement and excellent guidance throughout the course of this study and assistance in the preparation of this manuscript.

Appreciation is also expressed to Drs. R. Herner and D.R. Dilley of the Department of Horticulture, and Drs. C.M. Stine and M.A. Uebersax of the Department of Food Science and Human Nutrition, for their participation in my graduate committee.

The author wishes to express his gratitude to the Government of Indonesia and Gadjah Mada University for providing a leave of absence, and to The Rockefeller Foundation for the financial support for this program.

Finally, the author is deeply grateful to his parents, Padmomartono, his wife, Niniek, and his daughters Dian and Dani for their support, encouragement and understanding.

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INTRODUCTION

Soybeans are an inexpensive protein-rich food which has been widely consumed in the Orient for thousands of years. They are of special value for supplementary feeding in many countries where milk and meat cannot be produced sufficiently. According to Autret and van Veen (1955), soybeans can satisfy the nutritional needs of infants, children, pregnant and nursing women. Soy milk is popular in China. It was adopted by the FAO in its program to meet the protein needs for protein malnutrition in infants and young children in many developing countries. It is also useful as a milk substitute, especially for infants and children who are allergic to milk.

As in other countries in south-east Asia, rice is a staple food in Indonesia. It is eaten daily, two or three times a day. Since foods of animal origin are not sufficiently available, they are not consumed daily, and vegetables are the main supplement commonly eaten along with rice. Soybean products like tempeh (fermented soybeans) and tofu (soybean curd) are supplements frequently used. Nutritionally, these supplements add protein to the rice diet. Lysine and threonine are the first and second limiting amino acids in rice, while sulfur containing

amino acids are the limiting factors in soybeans. The supplementation of a small amount of soybeans to rice slightly improves the chemical score of the amino acids in rice by increasing the threonine content. Because of the sulfur amino acid deficiency in soy, sulfur amino acids become the first limiting as the proportion of soy protein increases (Jansen, 1972). Germination and fermentation of beans are two popular processes which can improve both the sensory values such as texture, flavor, aroma and color, and the nutritional quality of soybeans. Everson et al. (1943) reported that germinated soybeans were superior to the ungerminated ones when they were fed to laboratory animals. Germination reduced growth inhibiting factors and increased the protein efficiency ratio of the soybeans.

Fermentation of food, especially high-protein food, is commonly practiced in Asia. Whitaker (1978) mentioned the following advantages and reasons for fermenting food: improving flavor, aroma, texture, color, facilitate solubilization, improving digestibility, nutrition, less cooking and removing toxic substances. Another reason why fermentation is so popular in Asia is that this process is relatively simple and inexpensive, and it can be performed with simple household utensils available locally. It can be performed by people in rural areas who have no scientific background about the process of fermentation. The tempeh fermentation is traditionally performed in Indonesia. It

consists of two steps: the lactic acid fermentation and the mold fermentation. The lactic fermentation appears to facilitate the subsequent growth of the fungus, while the mold fermentation changes the soybeans to a cake-like solid, in which the soybeans are bound together by cottony mycelia. Tempeh has a pleasant odor and a relatively mild taste (Steinkraus et al., 1960).

The tempeh fermentation was studied extensively by Steinkraus et al. (1960), Hesseltine et al. (1963), and Hesseltine and Wang (1967). They described the mold species to be used and reformulated the process in order to have optimal yield in a laboratory scale. Progress in the development of larger scale tempeh processing has also been made (Martinelli et al., 1964). Steinkraus et al. (1965) developed a pilot plant process for the production of dehydrated tempeh. The production of Rhizopus oligosporus spores and their application in tempeh fermentation has been studied by Wang et al. (1975). Wang and Hesseltine (1966) tried to make wheat tempeh and Hesseltine et al. (1970) introduced a new fermented cereal product using mold isolated from tempeh. All of the tempeh-like products were reported to have a pleasant, mild taste and a desirable color.

Biochemical changes occurring during mold fermentation have been described by many researchers. There are changes in protein, soluble protein and amino acids composition

(Steinkraus et al., 1960; Hesseltine et al., 1963; Stilling and Hackler, 1965; and Murata et al., 1967), carbohydrates (Hesseltine et al., 1963), lipids (Steinkraus et al., 1960; Wagenknecht et al., 1961; Murata et al., 1967; and Sudarmadji and Markakis, 1978), vitamins (Roelofsen and Talens, 1964; Murata et al., 1967; Robinson and Kao, 1977; and Liem et al., 1977), phytate and phytase (Sudarmadji and Markakis, 1977).

There are some contradictory results regarding the nutritive value of tempeh fed to rats. Rats on tempeh diets ate more, gained more weight and had higher PERs than did the rats eating autoclaved-unfermented soybeans (Gyorgy et al., 1964; Kao and Robinson, 1978). However, Smith et al. (1964) and Hackler et al. (1964) reported that rats fed tempeh showed a small reduction in growth and protein efficiency compared with autoclaved and dehulled full-fat soybean meal. When methionine was supplemented to the diets, the rate of rat growth and protein efficiency values increased significantly (Smith et al., 1964). Hackler et al. (1964) reported that acceptance of tempeh containing diets by rats was decreased with each 12-hour increment in fermentation time. Either mold or something elaborated by the mold depressed the acceptance of the diets containing the fermented soybeans.

Germination has been reported to improve protein quality, digestibility and reduce certain anti-nutritional

factors. It was the purpose of this research to combine germination and tempeh fermentation in an effort to improve the overall quality of tempeh.

LITERATURE REVIEW

Soybeans (*Glycine max. L.*) are a rich source of protein, yet the direct utilization of the beans is limited because of the presence of anti-nutritional factors and undigestible constituents. The composition of soybeans is presented in Table 1.

Germination generally increases the nutritive value of seeds. Whyte (1973) pointed out that during germination, stored materials are converted into more usable forms for the plant and eventually for man (Wang and Fields, 1978). Chen *et al.* (1975) reported that the first stage of seed germination involves the breakdown of seed reserves for their utilization by the growing root and shoot.

During germination, some constituents were degraded, whereas others were synthesized. Protein and oil are major sources of energy for the developing embryo (Hsu *et al.*, 1973). Abrahamsen and Sudia (1966) reported that protein decreases and amino acids increase as seeds germinate. Oil is depleted rapidly and is broken down into glycerol and fatty acids which finally give rise to carbohydrates via the glyoxylate cycle followed by reversed glycolysis. Soluble carbohydrates are also an important

Table 1. Proximate composition of soybeans and seed parts (% dry basis).*

Fraction	Protein (% N x 6.25)	Fat	Carbohydrate	Ash
Whole bean	40	21	34	4.9
Cotyledon	43	23	29	5.0
Hull	8.0	1	86	4.3
Hypocotyl	41	11	43	4.4

*From Wolf and Cowan (1971).

energy source during the early stages of germination.

Everson et al. (1943) reported that germinated soybean protein is distinctively superior to that of raw beans. The nutritive value of both improved by heat treatment (autoclaved). The PER ranged from 0.5 for the mature raw seed to 1.4 for the germinated seeds, to 1.7 for the autoclaved seeds and to 1.9 for the autoclaved germinated seeds. Freed and Ryan (1978) reported that some proteinase inhibitors disappear completely during germination, while certain new inhibitors appear. Increases in lysine and tryptophan and decrease of prolamine occurred during germination of cereal grains (Tsai et al., 1975; Dalby and Tsai, 1976).

The interest in the changes of carbohydrates, especially the oligosaccharides, raffinose and stachyose, is due to the belief that they are primarily responsible for the flatulence often experienced by persons consuming soybean-based foods (East et al., 1972; Abrahamsen and Sudia, 1966; Hsu et al., 1973). Reddy et al. (1980) studied the flatulence in rats following ingestion of cooked and germinated black gram and a fermented product of black gram and rice blend. The maximum hydrogen production was obtained with 60% cooked black gram cotyledons in the diet. Germinated black gram seeds and fermented steamed product significantly produced lower flatus than the cooked black gram products. A positive significant correlation was found between oligosaccharides of the raffinose family

present in black gram and hydrogen production by rats. Rackis et al. (1970) reported that an in vitro assay using intestinal bacteria showed that toasted, dehulled, defatted soybean meal contains a gas-producing factor and a gas-inhibiting factor. The oligosaccharides - sucrose, raffinose and stachyose - are associated with the gas-producing factor when incubated in thioglycollate media with anaerobic bacteria of the intestinal tract of dogs. The phenolic acids of soybeans, syringic and ferulic acids, are effective gas inhibitors in vitro and in the intestinal tract of dogs. The lipids, proteins and water-insoluble polysaccharides of soybean meal have no gas activity.

Abrahamsen and Sudia (1966) studied the soluble carbohydrates in germinating soybean seeds. They found that the most rapid decline in total soluble carbohydrate in soybean cotyledons and embryo axis occurred during the first three days of germination. Sucrose, stachyose and raffinose were the predominant soluble carbohydrates in the cotyledon of ungerminated soybean seeds in a ratio of approximately 7:3:1, respectively. By day 1 there was approximately a 50% reduction in raffinose with only slight decrease in sucrose and stachyose. Between day 1 and day 2 there was a sharp decrease in stachyose; raffinose also continued to decrease during this interval. By day 2 stachyose and raffinose were almost completely depleted. Sucrose showed a moderate decrease between day 1 and day 2 with a marked decrease

between day 2 and day 3. The predominant sugar in the embryo axis of ungerminated soybean seed was stachyose followed by sucrose and raffinose. Between day 0 and day 1, both stachyose and raffinose decreased rapidly while the sucrose content increased during this interval. It showed that there was a much earlier utilization of stachyose in the embryo axis of the germinating soybean seedling than in the cotyledon. While stachyose was depleted rapidly, a synthesis of sucrose occurred. Since sucrose may be a hydrolysis product of raffinose and stachyose, the accumulation of sucrose may be due to partial hydrolysis of stachyose during this interval. Several unidentified oligosaccharides were present during the first two days in both embryo and cotyledon. Changes in total soluble carbohydrate is presented in Figure 1, while Figure 2 shows raffinose and stachyose during germination of soybeans.

East et al. (1972) confirmed previous observations and pointed out that, during normal germination, the content of monosaccharides is increased, sucrose shows an initial stationary period followed by a decrease, and raffinose and stachyose experience a general decrease.

Tempeh

Tempeh is a traditional Indonesian food, made mostly from soybeans, fermented by a mixed culture of several species of Rhizopus. Raw tempeh has the appearance of a

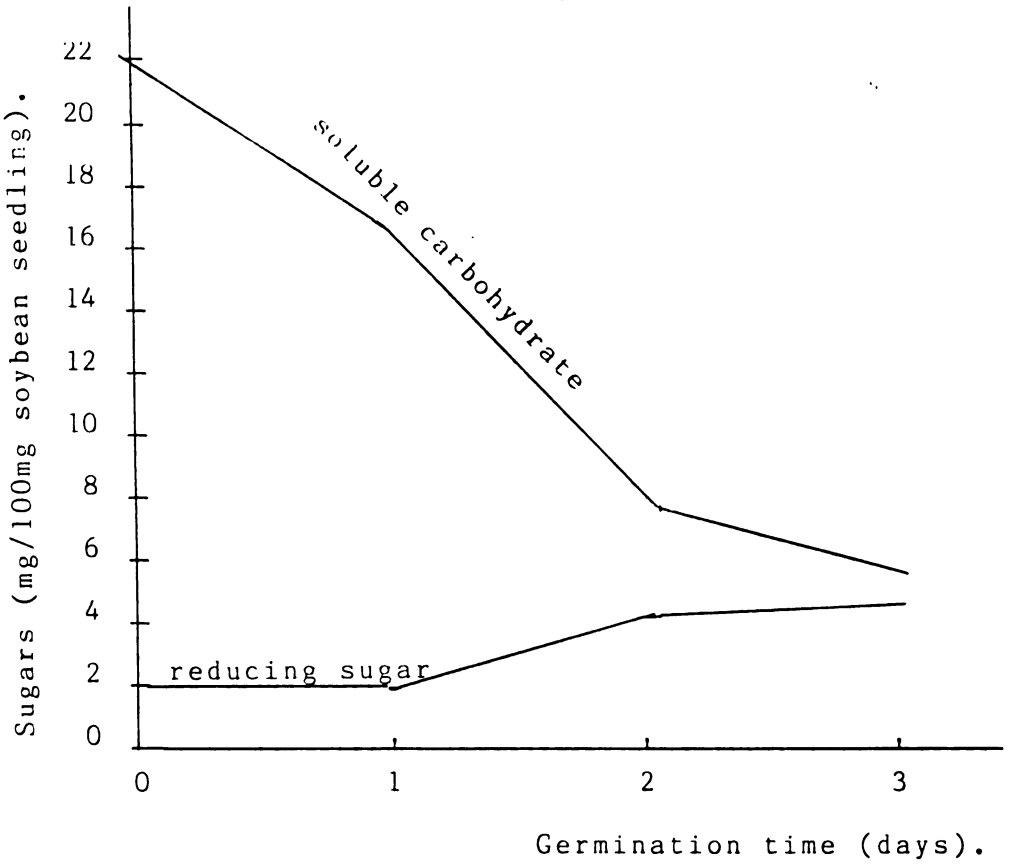

Figure 1. Changes in soluble carbohydrate and reducing sugar during soybean germination (Adapted from:Abrahamsen and Sudia, 1966).

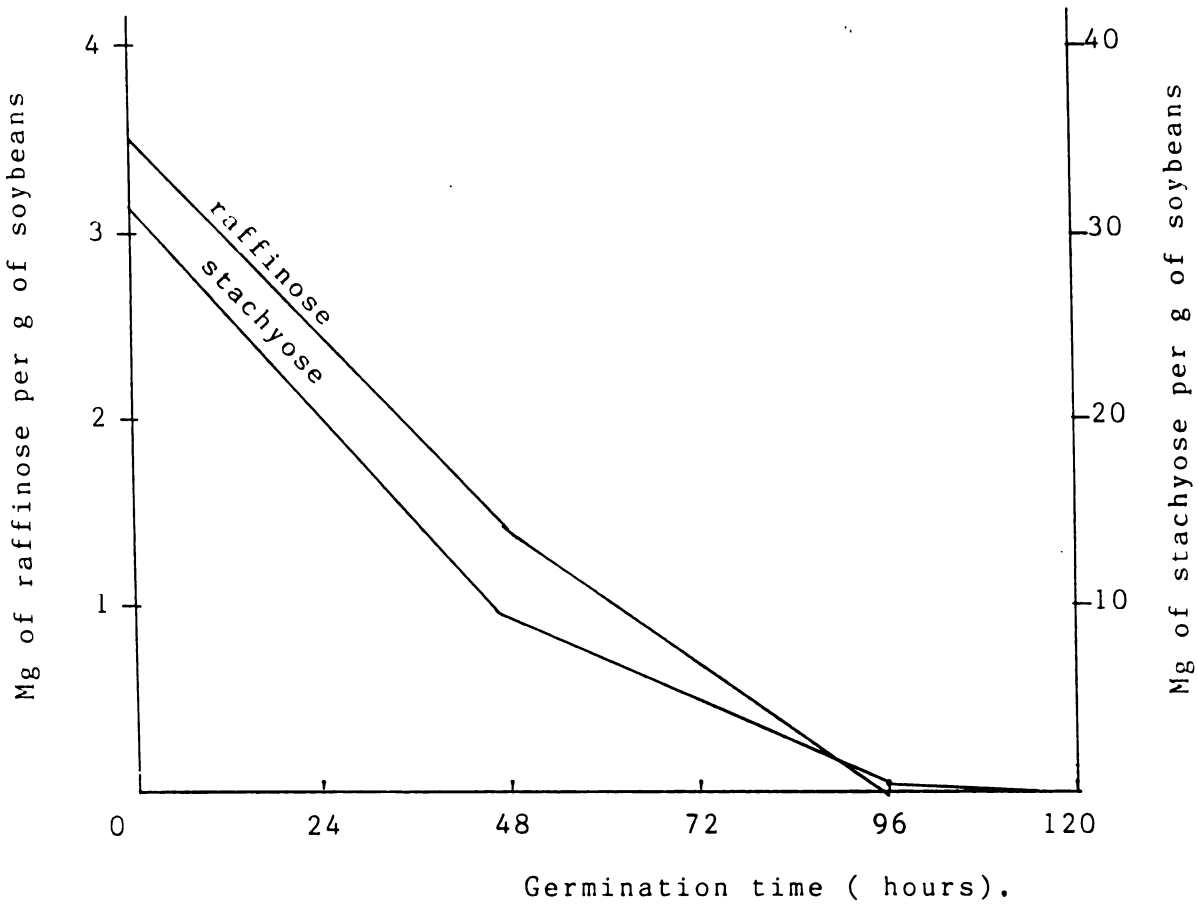

Figure 2. Changes of raffinose and stachyose content during germination of soybeans. (East et al., 1972)

cake-like solid mass of soybeans, held together by cottony mold mycelia. It is eaten, after cooking as a soup or frying, by millions of people in Indonesia and Surinam. Tempeh is also manufactured in Holland, Canada and the United States (Shurtlef and Aoyagi, 1978). The nutritional composition of tempeh is presented in Table 2.

Raw Material

The world tempeh (tempe) was originally applied to all cake-like products fermented by mold. There are many materials commonly used to make tempeh. Table 3 shows the raw materials used to prepare tempeh and the corresponding types produced from them. They are fermented using the same starter inoculum. The most popular type is tempe kedele, which is called tempe for simplicity and written tempeh in English.

Tempe bongkrek is not allowed to be produced since many deaths occurred as a result of eating tempe bongkrek contaminated with Pseudomonas cocovenenans.

In some places, soybeans are mixed with cheaper materials, such as cooked rice, rice bran, tofu by-products, or peanut press-cake in order to produce tempeh more economically.

A similar tempeh-type product prepared from insoluble fraction of ground soybeans fermented with Neurospora sitophila, oncom, is very popular in West Java. It has a

Table 2. Nutritional composition of tempeh before and after fermentation*.

Component	Before fermentation	After fermentation
Crude protein (%)	48.2	50.5
Crude fiber (%)	1.8	3.1
Crude fat (%)	23.6	19.3
Ash (%)	4.5	4.2
PER	2.17	2.27
Niacin (µg/g)	17.5	65.0
Riboflavin (µg/g)	2.6	8.6
Thiamin (µg/g)	7.8	5.8
Vitamin B-6 (mg %)	0.08	0.35
Vitamin B-12 (mg %)	0.15	5.00
Pantothenic acid (mg %)	4.6	3.30
Phytic acid (%)	1.23	0.96
Antitryptic act. (TIU/mg)	1.20	4.10
Flatus factor (raffinose family sugars)	16.5	2.10

*From: Reddy et al., 1981. Dry basis.

Table 3. Raw materials and the correspondent tempeh name.

Raw material	Name
Velvet beans	Tempe benguk
Peanut press-cake	Tempe bungkil
Soybeans	Tempe kedele
Water insoluble fraction of ground soybeans	Tempe gembus
Coconut press-cake	Tempe bongkrek

pinkish appearance rather than white.

Hesseltine and Wang (1967) developed new fermented tempeh-type product prepared from wheat, rye, oats, barley, rice and a combination of rice or wheat with soybeans. The products were reported to possess a very acceptable mild taste.

Organisms in Tempeh Fermentation

A traditional tempeh fermentation is prepared with impure cultures of mold. The inoculum is taken from pieces of a previous fermentation or its wrapper, which is usually leaves. A traditional inoculum is also available commercially as a dry spore preparation on dry hairy Hibiscus tiliaceous leaves. Tempeh starters are now available in powder form prepared from a pure culture of a mold, usually Rhizopus oligosporus.

Hesseltine et al. (1963) has isolated some strains of Rhizopus from Indonesian tempeh. They were identified as: Rhizopus stolonifer, R. oryzae, R. arrhizus, R. achlamydosporus, R. nigricans, R. formosaensis. It was concluded that R. oligosporus is the principal species used in Indonesia for making tempeh.

Wang et al. (1975) successfully developed a method for mass production of Rhizopus oligosporus spores and its application.

Classification of Rhizopus (Frazier, 1957)

Division	: Thallophyta
Subdivision	: Eumycetes (true fungi)
Class	: Phycomycetes (non septate)
Subclass	: Zygomycetes
Order	: Mucorales
Genus	: Rhizopus

Genus Rhizopus has the following characteristics: non septate; has stolon and rhizoids; sporangiophores arises from the nodes where rhizoids are formed; large sporangia, usually black; hemispherical columella; thick cottony mycelia (Figure 3).

Growth Requirements

Hesseltine (1963) studied the growth requirements of 4 species of Rhizopus which were suitable for making tempeh. Carbon sources supporting the growth of the 4 species were: soybean oil, xylose, glucose, galactose, trehalose, cellobiose, but not raffinose, lactose and inuline, while the best nitrogen sources were asparagine and ammonium sulfate.

Steinkrause et al. (1960) reported that, in order to be complete, the tempeh fermentation required that two conditions be fulfilled. First, the soybeans had to be bound into a compact cake by the growth of mold mycelia. Second, the soybeans had to undergo a partial digestion by the mold enzymes, thus the tempeh fermentation was centered in the mold growth. Since tempeh mold would not grow well on the unskinned beans, skin removal is an essential step in tempeh fermentation.

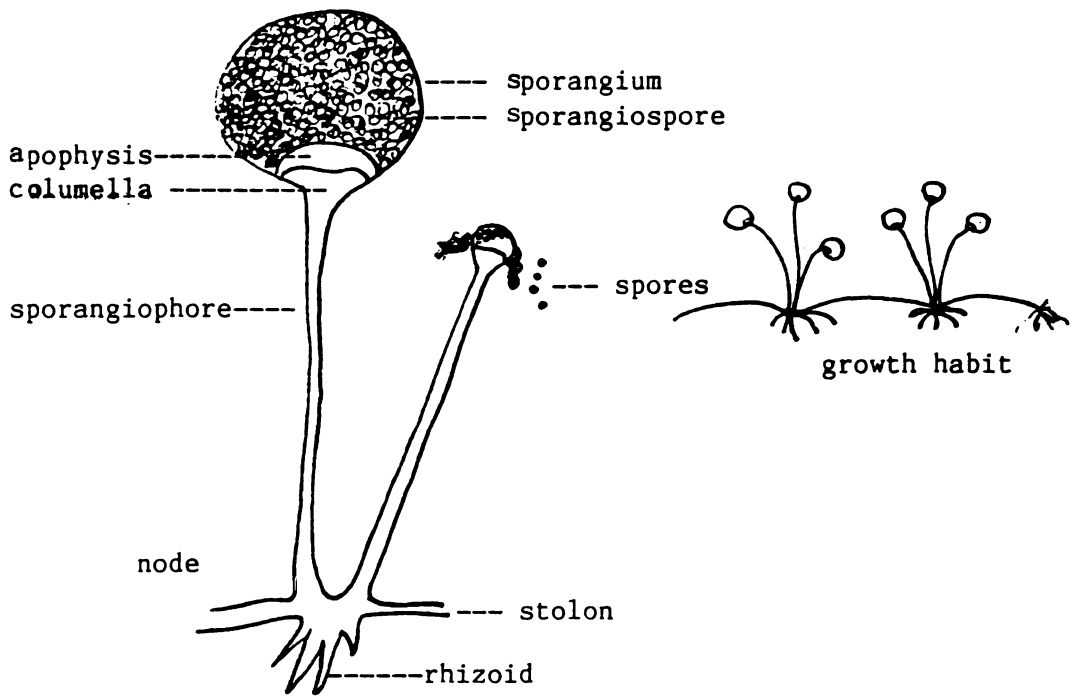

Figure 3. Rhizopus sp.

Oxygen is essential to mold. When the layer of the fermenting beans was thicker than 2 inches or about 5 cm, mold grew less heavy in the center than it was in the thinner layer. To make a good tempeh, the thickness should not be more than 3 cm (Steinkraus et al., 1960; Martinelly et al., 1964).

Robinson and Kao (1977) studied the mold fermentation on various sizes of soybean products. The mold did not grow on soybean flour and was unable to grow well on small grits, because there was too little oxygen below the surface of the mass to support mold growth. The best fermentation result was obtained when the diameter of the grits was between 0.2 to 0.4 cm.

In order to get the right amount of aeration, perforated metal trays or plastic bags can be used. However, if the amount of aeration is in excess, the soybeans at the surface will dry out and sporulation will start producing undesirable black spores and poor appearance. It is obvious that high relative humidity is absolutely needed (Steinkraus et al., 1960; Martinelli et al., 1964).

Temperature is a critical factor for microbial growth. Temperature slightly above room temperature is best for tempeh fermentation. Traditional tempeh makers in Indonesia start to mix the inoculum when the cooked soybeans cool down to about 50^{o}C. During mixing and wrapping the inoculated beans cool further to room temperature. For incubation, the

wrapped, inoculated soybeans are packed in baskets with the temperature about 35°C. The temperature will rise to about 40°C when the mold is growing overnight. The following morning, the tempeh should be taken out from the baskets and put in racks for further incubation at room temperature until the tempeh is ready for marketing. If the tempeh is not cooled when the temperature is high, the mold will stop growing and bacteria will grow very quickly producing a poor appearance and a bad odor.

Steinkraus et al. (1960) reported that the optimum temperature for tempeh mold is 37°C. Temperatures as low as 25°C were used to produce an acceptable tempeh. Such fermentation required as long as 5 days for completion while fermentation at 37°C required only 1 day.

Later investigations by Hesseltine et al. (1963) showed that Rhizopus strains that produce satisfactory tempeh can grow from 14°C to 44°C except R. stolonifer, which has a maximum temperature of no more than 35°C. Some of the R. oligosporus isolates grow poorly at 50°C. Temperature of 31°C was the best for making tempeh, except for R. stolonifer which required a temperature below 31°C. At optimum temperature, 22 to 24 hours was needed to complete the mold growth and produce good tempeh.

For good tempeh fermentation, it is essential to ensure that the beans are acidified to a sufficient degree at the start of fermentation. The pH of soaked and cooked soybeans

in acidified water (30 ml lactic acid/3000 ml water/1000 g beans) was about 5. This pH was sufficiently low to inhibit growth of most contaminating bacteria which would spoil the tempeh but did not interfere with the growth of the mold. The mold growth is inhibited when the pH drops below 3.5.

Changes Occurring During Tempeh Fermentation

General Changes

Steinkraus et al. (1960) studied general changes occurring during tempeh fermentation. By referring to Figure 4, the changes can be followed.

The increasing temperature of fermenting bean mass was indicative of the relative growth rate of the mold. The first 20 hr, during which time the germination of spores took place, the temperature rose gradually. After 4 to 5 hr of accelerated growth, the temperature reached 43-44^{o}C and then gradually decreased as the mold growth subsided. At this stage, the beans were already bound into compact mass by mold mycelia. Following the mold growth, sporulation and NH_3 production due to protein breakdown appeared.

During the period of most rapid mold growth, the soluble solids rose from 13 to 22% and continued to rise up to 27.5%. At this stage, the sporulation and NH_3 production would be considered too far advanced.

The soluble nitrogen rose from 0.5% to nearly 2% while the total nitrogen remained relatively constant, about 7.5%.

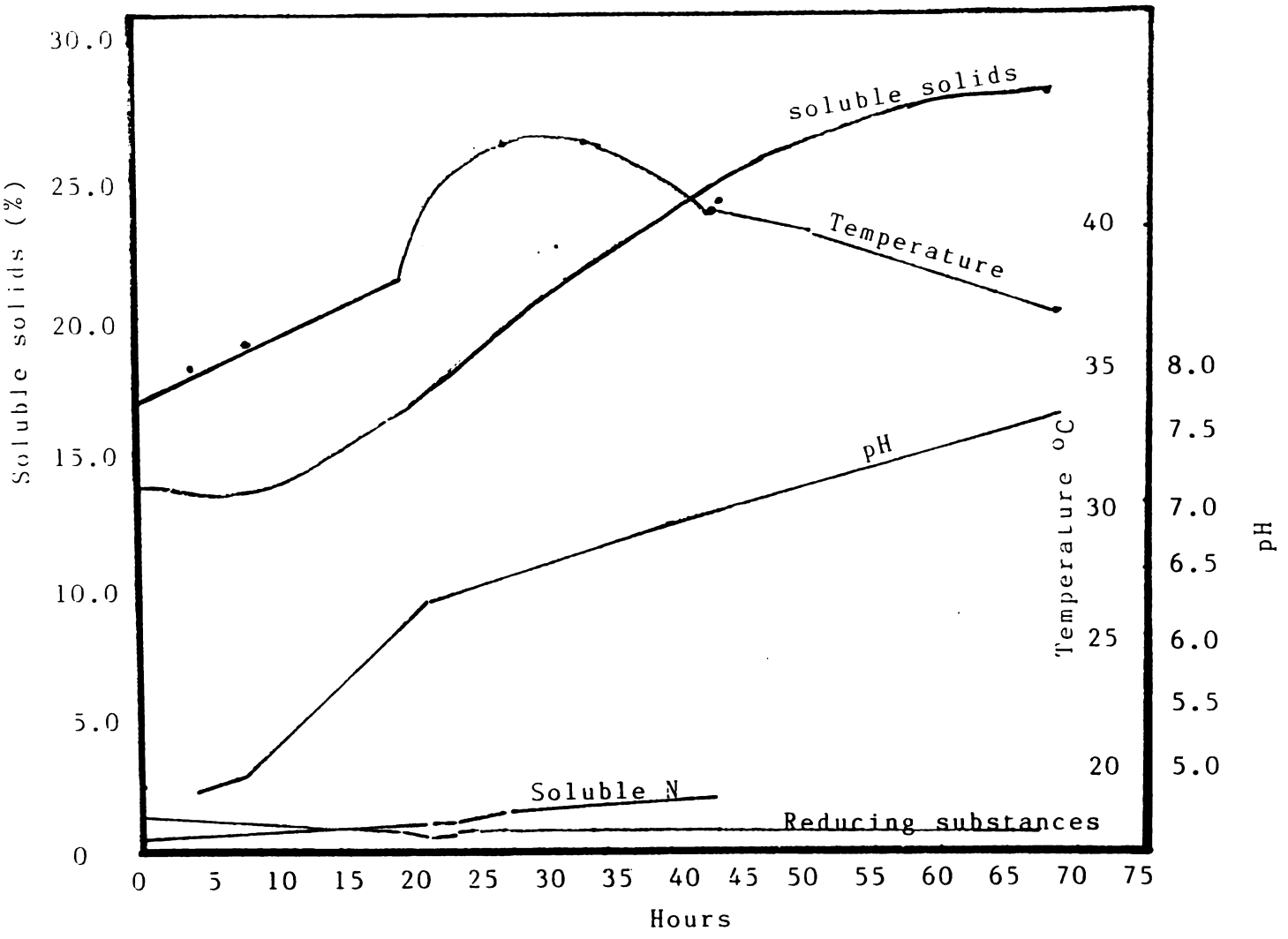

Figure 4. Changes occurring during the tempeh fermentation (Steinkraus et al., 1960).

The pH, which initially was 5.0, rose to 7.6. The change from 6.0 to 6.7 occurred during the period of most rapid mold growth. Optimum quality tempeh has pH 6.3 to 6.5.

Reducing substances decreased very slowly during the entire fermentation. They did not regain their initial level even though breakdown of higher carbohydrates continued.

There was an increase of fiber from 3.7% dry basis in hydrated, peeled, cooked soybeans to 5.8% in the tempeh. Fermented soybeans freed from their superficial mold layer, showed a reduction in total fiber to 2.8%. The mold mycelium, removed from the tempeh, contained 7.1% fiber, which was responsible for the increase in fiber content.

Later investigations by Murata et al. (1967) showed the following general changes:

- There were no large differences in protein and ash content between tempeh and unfermented soybeans.
- During fermentation the fiber was slightly increased.
- Fat content decreased but the acid value increased noticeably.
- Free amino acids increased.
- Riboflavin, vitamin B_6, nicotinic acid and pantothenic acid increased during fermentation, although thiamin was altered slightly.

Carbohydrate Changes

The carbohydrate content of soybeans is about one third of their dry weight. It consists of polysaccharides, stachyose (3.8%), raffinose (1.1%), and sucrose (5%). About 7% of the total carbohydrate is located in the hull, 26% in the cotyledon, and 1% in hypocotyl. Reducing sugars in dormant seeds are very low (Gould and Greenshields, 1964; Abrahamsan and Sudia, 1966; East *et al.*, 1972 and Hsu *et al.*, 1973).

Soluble carbohydrate increases, while reducing sugars decline during fermentation. The level of reducing sugars remains low even though complex carbohydrates continue to breakdown, presumably because the sugars are used by the mold.

The oligosaccharides, stachyose and raffinose are of particular interest because they are considered responsible for the flatulence often experienced by people who eat soybeans (East *et al.*, 1972; Abrahamsen and Sudia, 1966).

Stachyose as shown in Figure 5 is a tetrasaccharide, made up of three simple sugars: galactose, glucose, and fructose, which are joined in such a manner that upon hydrolysis, raffinose, manninotriose, galactobiose, melibiose, sucrose, glucose, galactose and fructose may be formed. The structure of stachyose and raffinose can be cleaved by acid or enzymes. α-Galactosidase liberates

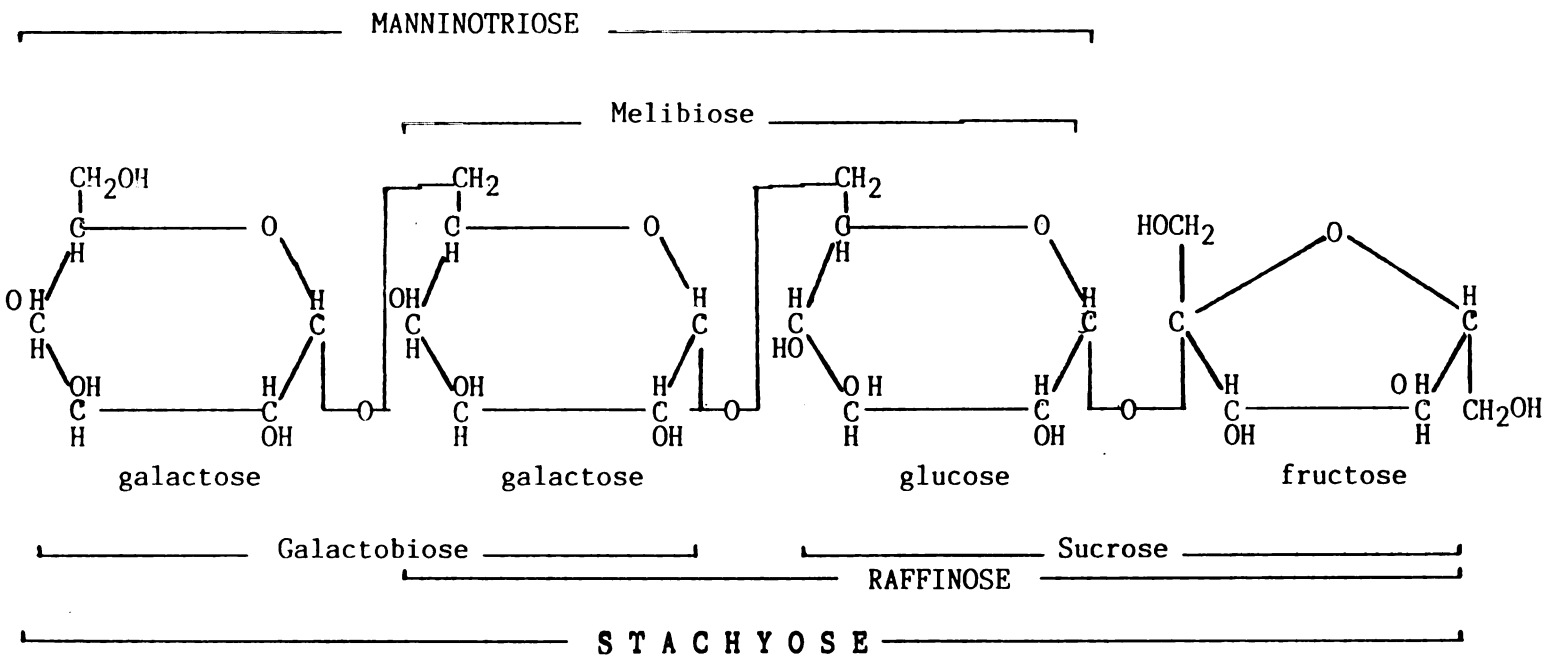

Figure 5. Structure of stachyose.

galactose and invertase cleaves the glucose-fructose bond (Pigman, 1957).

Due to lack of α-galactosidase in mammals' intestinal tract, stachyose and raffinose cannot be digested and finally these sugars are fermented by anaerobic bacteria in the colon, producing gas and causing flatulence.

Shallenberger et al. (1967) reported that soaking and cooking the soybeans reduced the sugar content about 50%, largely at the expense of sucrose (from 4.53% to 1.85%). Raffinose decreased from .73% to .35% and stachyose from 2.73% to 1.4%. During tempeh fermentation sucrose decreased slightly, while raffinose appeared to be in a steady concentration state throughout the study. The stachyose disappearance was about twice as quick as that of sucrose, and it was nearly absent after 72 hours.

Lipid Changes

The most noticeable change in lipids during tempeh fermentation is the accumulation of free fatty acids, resulting from the hydrolysis of soybean triglycerides by mold lipase (Wagenknecht et al., 1961; Murata et al., 1967; and Sudarmadji and Markakis, 1978).

Wagenknecht et al. (1961) studied changes in soybean lipids during tempeh fermentation. Total ether-extractable lipids at the time of most active mold growth (20-30 hr of fermentation) slightly increased and then diminished, while

the acid number increased from 1.7 to 78.3 after 69 hr at 37°C. Despite the relatively large amount of acids being liberated, the pH of the fermenting beans showed a steady increase probably because of ammonia formation. Because the soybeans had been boiled during tempeh preparation, which inactivated the enzymes originally present in the beans, liberation of fatty acids during fermentation must have been caused by action of the lipase of the mold. Lipase activity was also readily demonstrable through all stages of fermentation.

Murata et al. (1967) confirmed previous findings stating that there is a noticeable increase of the acid value and some increase of oleic acid and decrease of linoleic acid after fermentation. This result confirms the Wagenknecht et al. (1961) study that only linolenic acid is used by the mold and about 40% of the linolenic acid is used during the fermentation time. Murata et al. (1967) characterized the tempeh's lipid as shown in Table 4.

The changes of free fatty acids during the entire fermentation, along with changes of bacterial plate count and temperature were studied by Sudarmadji and Markakis (1978). Those changes are shown in Figure 6. The fermentation was divided in three phases. The first phase was the fermentation which occurred during the first 30 hr of incubation at 32°C during which microbial growth, lipolysis and temperature increased and the result was a product of

Table 4. Chemical characteristics of oils from tempeh and unfermented soybeans.

	Unfermented (control)	Tempeh
Acid value	1.02	50.95
Refractive index (n_D25)	1.4730	1.4711
Iodine number	128.5	126.1
Saponification number	191.1	189.2
Fatty acids composition including free fatty acids	%	%
C 14 (F_0)	trace	trace
C 16 (F_0)	10.4	10.9
C 16 (F_1)	trace	trace
C 18 (F_0)	5.1	4.9
C 18 (F_1)	26.8	28.1
C 18 (F_2)	50.0	49.4
C 18 (F_3)	7.8	6.8

From: Murata *et al*. (1967).

Tempeh fermented for 48 hr at 37°C.

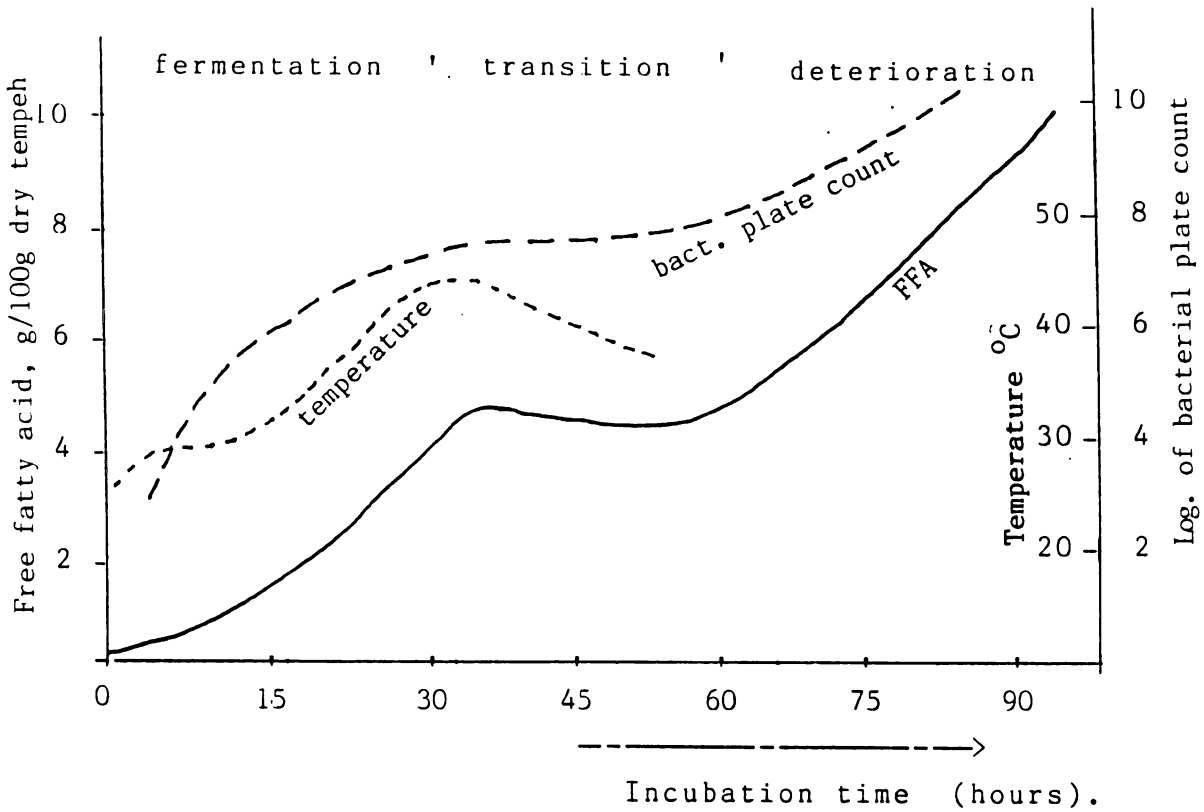

Figure 6. Free fatty acid (FFA) content, temperature and total bacterial plate count of soybeans inoculated with R.oligosporus at 32°C. (Sudarmadji and Markakis, 1978).

high sensory quality. The second phase was a transition phase lasting 24 hr after the first phase, when microbial growth and lipolysis subsided the temperature decreased and the product was still in good condition. The third phase was a deterioration phase. In this phase, the bacterial growth and lipolysis reappeared and the tempeh quality deteriorated rapidly.

Protein Changes

Steinkraus et al. (1960) showed that during tempeh fermentation, soluble nitrogen including NH_3 increased due to protein breakdown.

In general, most amino acids either remained unchanged or declined according to Stilling and Hackler (1965). A notable exception was tryptophan, which was significantly higher in tempeh fermentation for 24 hr at 37°C, but declined thereafter. During fermentation ammonia increased significantly, possibly from amino acids deamination.

A comparison of amino acids composition between soybeans and tempeh made in Indonesia, and soybeans and tempeh prepared in the laboratory was made by Murata et al. (1967). The amino acids composition is shown in Table 5. In general most amino acids were not changed by fermentation. It was observed that the tryptophan content of the samples from Indonesia increased about 20% and alanine in tempeh prepared in the laboratory also increased about 20%.

Table 5. Amino acid composition of tempeh and soybeans (mg/gN)*

Amino acids	Indonesian Soybeans		Harosoy Soybeans**	
	Soybeans	Tempeh	Soybeans	Tempeh
Aspartic acid	744	756	704	673
Threonine	278	282	241	218
Serine	270	268	323	273
Glutamic acid	1050	1000	1110	974
Proline	342	309	342	307
Glycine	292	275	263	257
Alanine	250	228	280	338
Cystine	113	121	98	80
Valine	328	345	336	319
Methionine	77	81	61	61
Isoleucine	338	356	301	310
Leucine	525	565	518	492
Tyrosine	171	161	188	178
Phenylalanine	302	302	384	307
Tryptophan	67	87	63	66
Lysine	392	410	384	330
Histidine	160	167	187	171
Arginine	491	440	392	373

* Adapted from Murata et al. (1967).

**Prepared in Food and Nutritional Laboratory, Osaka City University. Fermentation was done according to Steinkraus method, 48 hr.

Changes in the amino acids of tempeh from Indonesia and of tempeh prepared in the laboratory were not always parallel.

During the fermentation, the amount of free amino acids increased progressively. After 48 hr of fermentation, the amount of individual free acids increased 85 times over those of the unfermented soybeans.

Robinson and Kao (1977) reported that the water soluble protein increased two to three times after tempeh fermentation. The increase resulted from the proteolytic activity of the mold, which partially hydrolyzed the insoluble protein to soluble protein. From the amino acids study, it was found that the essential amino acids remained unchanged after fermentation.

Vitamin Changes

The nutritional value of tempeh and utilization of protein from tempeh might be affected by its content of B vitamins. A sharp increase of riboflavin, nicotinic acid, vitamin B_6 and pantothenic acids during tempeh fermentation were reported (Steinkraus et al., 1960; Roelofsen and Talens, 1964; Murata et al., 1967; Liem et al., 1977). Robinson and Kao (1978) reported that water soluble vitamins increased during tempeh fermentation.

Phytic Acid Change

Phytic acid (inositol hexaphosphate, with the proposed structure shown in Figure 7), is one of many anti-nutritional factors present in plant origin foods. It has been demonstrated that the presence of phytic acid in diets may reduce the availability of some essential, di- and trivalent metals (Welch and House, 1982; House *et al.*, 1982). The interaction between phytic acid and mineral ions to form insoluble complexes appears to be the major factor responsible for the adverse nutritional effects observed in high phytate diets.

During tempeh fermentation, the *Rhizopus oligosporus* used in this fermentation produced phytase which reduced about one-third of the phytic acid content (Sudarmadji and Markakis, 1977). Other molds, such as *Neurospora sitophila*, which is used to prepare oncom, a tempeh-like product from peanut press cake (Fardiaz and Markakis, 1981), *Mucor dispersus* and *Actinomucor elegans* (Wang *et al.*, 1980) were also reported to produce phytase.

Changes in Aflatoxin Content

No aflatoxin presence in tempeh prepared from soybeans were reported. However, cases have been reported of tempeh and oncom prepared from peanut press-cake which contained aflatoxin at a significant level. Van Veen *et al.* (1968)

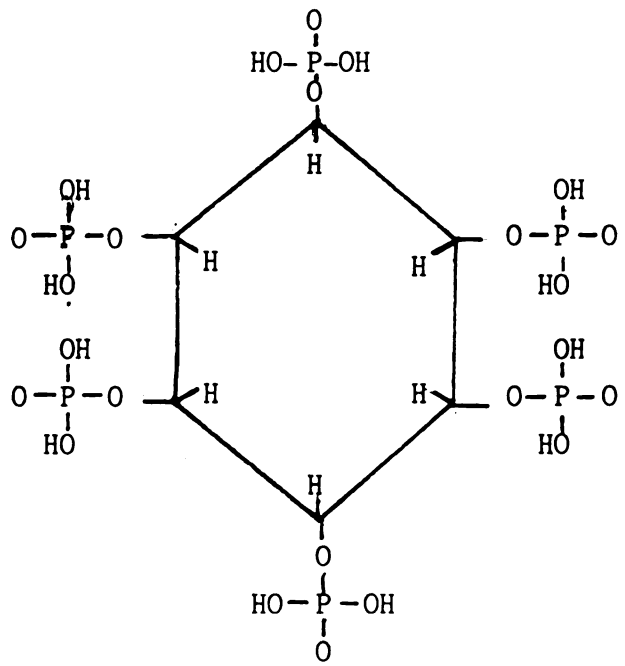

Figure 7. Proposed structure for phytic acid (Wheeler and Ferrel, 1971).

reported that Neurospora sitophila reduced the aflatoxin B_1 of peanut press cake by about 50%, while Rhizopus oligosporus reduced it by about 70%.

Soybean Lectin

Early investigators in nutrition were fully aware that certain legumes were very poor sources of protein unless subjected to heat treatment. Heat labile substances can interfere with the normal growth of animals, and can even cause death after prolonged ingestion. Crude trypsin was not effective in counteracting this inhibitor of growth (Liener, 1953; Honavar et al., 1962). Liener (1953) reported that a protein other than trypsin inhibitor and having hemagglutinating properties was lethal when injected into rabbits. The name "Soyin" was proposed for this protein which was characterized by marked hemagglutinating action and was devoid of either urease or antitryptic activity. Since then the names agglutinin, hemagglutinin, phytohemagglutinin were also proposed for this factor. Later, this substance was known to be able to distinguish among blood groups, and for this reason the term lectin (Latin, legere, to select or pick out) was suggested. Lectins from plant, as well as from other sources as snails and fishes, have become the subject of intense activity since several groups of investigators showed certain lectins not only have high specifity in agglutinating erythrocytes, but also

preferentially agglutinate malignant cells (Lis and Sharon, 1973).

Chemically, lectin is a glycoprotein with molecular weight about 96,000. A unique feature of its composition is the high mannose and glucosamine content, which are up to 10 percent (Wada et al., 1966). Lis et al. (1966) reported four distinct soybean hemagglutinins chromatographically separable. Catsimpoolas and Meyer (1969) reported that at least four different forms of lectins were separated from soybean and their isoelectric points were 5.85, 6.00, 6.10, and 6.20. Lis and Sharon (1973) later reported that soybean lectin is comprised of four apparently identical subunits, each of molecular weight 30,000 ± 500, and is therefore a tetramer.

Biologically, lectin was thought to be active in sugar transport. Most of lectin in seeds was found in the cotyledons, but lectin was also detected in the embryo axis and the seed coat. Soybean lectin was detected to be present in all of the tissue of young seedlings, but decreased as the plant matured and was not detectable in plants older than two to three weeks (Pueppke et al., 1978). Bhuvaneswari et al. (1977) linked lectin with the symbiosis between legumes and Rhizobia. Soybean lectin was found to bind to living cells 15 of the 22 Rhizobium japonicum strains tested. The lectin did not bind to Rhizobia strains which do not nodulate soybean. The binding of

lectins to the bacteria was shown to be specific and reversible.

Soybean lectin can be inactivated by heating. Maximum stability toward thermal inactivation was obtained in the region of pH 6 to 7. It is also inactivated by some chemicals such as urea, guanidine and quadrivalent and trivalent metallic ions. Pepsin addition readily inactivated soybean lectin, but trypsin was found not to be effective for this purpose (Liener, 1958).

The method in quantitative assay for measuring cell agglutination seems to be in the improvement process. Visual estimation of the degree of agglutination either in the test tube or with microtitrator were used. The agglutinating activity is measured by serial dilution of the lectin, with visual estimation of the end point where no agglutination is observed (Liener, 1953). This method was reported as very precise, but does not permit the detection of small differences in hemagglutinating activity. Liener (1955) developed a quantitative procedure in which the degree of agglutination is evaluated photometrically by measuring the absorbance of unsedimented cells.

Protein Quality Evaluation

Protein quality in human nutrition is mainly related to the efficiency with which food proteins are used for the synthesis and maintenance of tissue protein. This quality

is a function of the essential amino acid (EAA) and the digestibility of the proteins. The closer the EAA pattern to a reference EAA pattern (Table 6) required for human growth and maintenance, the better the quality of the protein. Usually proteins from animal origin have better digestibility than plant proteins.

There are two types of tests used to determine the quality of protein, in vitro tests and in vivo tests. The in vitro tests are designed to provide a rapid estimate of the nutritive value of protein. The in vivo tests involve either animals or humans. Protein efficiency ratio (PER) is the most common in vivo test used for the nutritional evaluation of proteins or foods.

Table 6. Amino acid patterns required for growth and maintenance of rat and man*

	Rat		Man	
	Growth	Maintenance	Growth	Maintenance
Histidine	1.9	2.2	1.6	--
Isoleusine	5.0	4.7	4.1	2.8
Leusine	6.3	4.3	9.4	3.8
Lysine	8.2	3.4	6.1	3.4
Phenylalanine + Tyrosine	6.6	6.3	7.4	3.8
Methionine + Cystine	4.6	4.4	3.4	3.7
Threonine	4.6	4.6	5.2	2.0
Valine	5.1	4.7	5.5	2.8
Tryptophan	1.0	1.0	1.0	1.0

*From Jansen (1978).

MATERIALS AND METHODS

Germination of Soybeans

The soybeans variety Corsoy 79C 82 were supplied by the Michigan Crop Improvement Association, East Lansing. Unbroken seeds were washed and soaked in five times the volume of freshly boiled and cooled water overnight. Petri dishes, 9 cm in diameter, lined with water saturated filter paper were used for sprouting the beans. Soaked soybeans were put in the Petri dishes making one layer on the filter paper, covered and incubated in the dark at room temperature (about 23^{o}C). To avoid mold growth during germination, all samples were washed with water at intervals of 12 hr.

Samples were harvested at 6 hr intervals for 24 hr, washed with water, steamed (100^{o}C) for 10 minutes and then frozen until they were used for tempeh fermentation or analyzed.

Tempeh Preparation

The tempeh used in this experiment were prepared by a method that is practiced in many Indonesian villages.

Soybeans or germinated soybeans were soaked overnight in tap water at room temperature. About 100 g portions of

soybeans or germ, soybeans were soaked overnight in 300 ml tap water containing about 10 ml of the liquor of a previous fresh soybean soaking. This treatment favored some lactic acid fermentation in the soybeans. The hull was removed in running water and the dehulled soybeans were boiled for 90 minutes. The purpose of the boiling is to partially cook and sterilize the beans. The boiled soybeans were drained and cooled. After the temperature reached about 60°C, the hot mass was inoculated with mold spores.

Culture of Rhizopus oligosporus NRRL 2710 was used in this experiment. Mold culture was grown and stored on Sabouraud Dextrose Agar which provides good mycelia growth and grown on Potato Dextrose Agar to produce spores for inoculation. After one week on Potato Dextrose Agar, the mycelia and spores were harvested with 3 ml sterile water for each slant. The suspension contained about 10^6 spores per ml, and 3 ml of it was used to inoculate 100 g of germinated soybean mass.

The inoculated mass was packed in disposable Petri dishes and incubated at 30-31°C for 36 hr. The tempeh produced was then steamed for 10 min and frozen until used for analysis. For analysis, the tempeh was dried in a vacuum oven at 70°C and reduced to fine powder using an Arthur Thomas mill.

Protein and Soluble Protein

Protein determination was performed by micro-Kjeldahl according to AOAC (1970) and soluble protein determination was done according to the method of Rhee et al. (1981) as follows: 0.1 g of finely-ground sample (No. 60 sieve) was extracted with 9.9 ml of 0.1 M phosphate buffer, pH 7.2 for 1 hr, at room temperature followed by centrifugation. An aliquot of the supernatant was used for the nitrogen determination. A factor of 6.25 was used to convert nitrogen content to protein.

Extraction of Crude Fat

About 2 g of finely ground dried sample was extracted with diethyl ether in a Goldfisch extraction apparatus (Labconco.) for 4 hr. The extract was dried at 100°C for 30 min, and weighed.

Oligosaccharides Determination

Oligosaccharides were determined utilizing HPLC according to the method of Conrad and Palmer (1976). The high pressure liquid chromatograph consisted of a Model M-45 solvent delivery system, 4.2 mm i.d. X 30 cm long μBondapak/Carbohydrate analytical column, and a model R401 differential Refractometer detector, all from Waters Associates, Inc., Milford, MA. The responses were recorded on a Kontes recorder 100.

Four extraction solvents were tried, namely hot alcohol 80%, hot alcohol 60%, hot water and cold water. The best extraction was obtained with 60% hot alcohol. Extraction with hot water resulted in a very viscous solution.

Two grams of sample were first extracted with 15 ml of hexane twice. The clear supernatant was discarded and the residue was allowed to dry under the hood overnight. To the residue 20 ml of 60% alcohol was added, mixed for 2 hr in a 80°C waterbath, and centrifuged for 15 min. The supernatant was saved and the precipitate was extracted again, twice with 10 ml of 60% alcohol. The three supernatants were combined and heated in a boiling waterbath for 30 min, cooled and a few drops of 10% lead acetate was added to precipitate the protein which was not coagulated by the heat treatment. After the contents of the tube were mixed well they were allowed to stand for a few minutes and then centrifuged. The supernatnat was saved and a few drops of 10% oxalic acid was added to remove the excess of lead. The tube was centrifuged again and the supernatant was saved. To remove the color in the supernatant, 1 g of activated carbon was added and let stand for 30 min. The tube was centrifuged, the supernatant was saved and the precipitate was washed with 10 ml of alcohol 60%. After centrifugation, the supernatant was combined with the previous supernatant and the mixture was concentrated in a flash-evaporator at 40°C until about 2 ml of the solution was obtained. The

concentrated solution was transferred into a 5 ml volumetric flask, and brought to volume with water.

Prior to HPLC analysis, the concentrated extract was filtered through a .22 μ-pore diameter membrane filter utilizing a Swinnex syringe filter (Millipore Corp.). A 20 μl of water-clear extract from the filtration was injected into the HPLC. A degassed mixture of acetonitrile and water (80:20, v/v) was used as solvent at a flow rate of 2.3 ml per minute. The detector attenuation was 8x for sucrose and 2x for raffinose and stachyose, and the recorder chart speed was 1 cm per minute.

Identification and quantification of oligosaccharides were based on a comparison of retention times and area of those obtained with a standard mixture containing known amounts of sucrose, raffinose, stachyose and inositol. Standard curves were also made from known concentration mixtures.

Phytic Acid Determination

The determination of phytic acid consisted of the extraction of phytic acid, iron chelation and determination of the chelated iron according to the method of Wheeler and Ferrel (1971) with a slight modification.

One gram of finely ground sample (50 mesh) was extracted with 50 ml 3% Trichloroacetic acid (TCA) in 125 ml Erlenmeyer flask for 90 minutes using a mechanical shaker. For better

extraction, the flask was occasionally swirled by hand. The suspension was centrifuged and 10 ml of the supernatant was transferred into a 40 ml conical bottom centrifuge tube. Four ml of $FeCl_3$ solution (2 mg Fe^{3+} per ml of 3% TCA) was added to the aliquot by blowing rapidly from the pipet. The tube and content were heated in a boiling water for 45 minutes. The tube was centrifuged for 15 minutes and the clear supernatant was decanted carefully. The precipitate was washed twice by dispersing well in 25 ml 3% TCA, heated in boiling water bath for 5 minutes and centrifuged. The washing was repeated once more with water. The precipitate was dispersed in 0.5 ml H_2O and mixed with 3 ml 1.5 N NaOH. After mixing well, the volume was brought to approximately 30 ml with water and heated in a boiling water bath for 30 minutes. The tubes were centrifuged for 15 minutes and the supernatant was decanted carefully. The precipitate was washed twice by dispersing well in 20 ml of water and then centrifuged. The $Fe(OH)_3$ precipitate was dissolved in 40 ml of hot HNO_3 and transferred into 100 ml volumetric flask. The flask was cooled to room temperature and the volume was brought to mark with water. The iron was then determined according to AOAC (1975).

Ten ml of aliquot from the previous step was transferred into a 25 ml volumetric flask and then 1 ml of 10% hydroxylamine solution was added. The flask was rotated and let it stand a few minutes, and 9.5 ml 2 M NaOAc solution and

1 ml o-phenanthroline solution (0.1 g/100 ml) was added. The volume was then brought to mark, mixed a few minutes and its absorbance was measured at 510 nm against a water blank with a Beckman DU Spectrophotometer (Beckman Instruments, Inc., Fullerman, CA).

A standard curve was previously prepared using a series of solution containing 0 to 2.4 µg Fe per ml. The following relationship was obtained.

$$A_{510} = 0.224\ C - 0.001675$$
$$(r = 0.9995)$$

where C is the concentration of Fe in g/ml and A_{510} is the absorbance at wave length of 510 nm. This equation was plotted and the curve in Figure 8 was obtained.

The phytate phosphorus content was calculated based on the assumption that the molecular ratio of iron:phosphorus is 4:6.

Hemagglutination Assay

A quantitative determination of lectin is based on the agglutination of 50% of countable red blood cells (RBC) according to the method of Coffey (personal communication). This method consists of the extraction of lectin, the preparation of a RBC suspension, the agglutination of the cells and the counting of the remaining suspended cells, utilizing the Coulter Counter (Coulter Electronics).

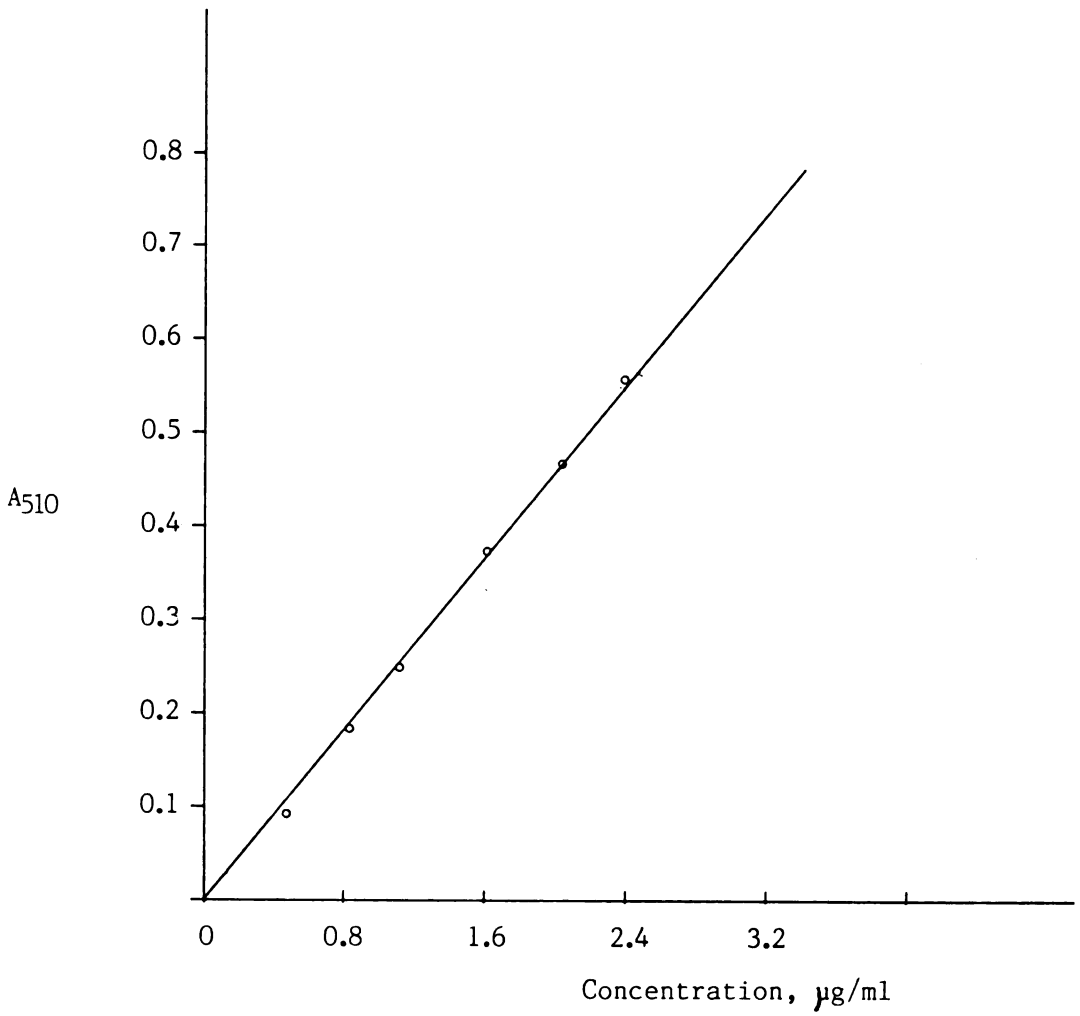

Figure 8. Standard curve for Fe determination.

Extraction

A 5 gram of fresh sample (germinated soybeans or tempeh) was extracted with 25 ml of phosphate buffered saline (PBS) in a Waring blender. The PBS contained sodium 180 meq/L; potassium 5.1 meq/L; chlorine 153 meq/L; EDTA 1 mmol/L and was free from sodium azide. To avoid heat denaturation, the mixing was stopped at one minute intervals, and cooled in ice. The aliquot was centrifuged at 40,000 x g for 30 minutes, and the supernatant was saved for the assay. For quantitation purpose, the amount of nitrogen in the extract was determined using the micro Kjeldahl method.

Preparation of Red Blood Cells (RBC)

Fresh blood was collected from healthy pigs. The RBC was separated from the serum by centrifugation. The serum was decanted and the precipitate (RBC) was mixed with PBS, centrifuged and the supernatant was removed. The precipitate was washed again twice with PBS, using the same procedure. To have a better cell suspension for the assay, the RBC were diluted in 10 volumes of PBS and 9 parts of this suspension was mixed with 1 part of 10% trypsin solution. The mixture was incubated at $37^{o}C$ in a water bath for 1 hr. After incubation, the mixture was centrifuged, the supernatant was discarded and the RBC was washed 3 times with PBS. For the assay, the RBC was then diluted to give a Coulter count 4 x 10^{7} cells with millipore-filtered PBS.

Agglutination Assay

Serial two-fold dilution of an aliquot of bean extract were made starting with 25 µl of each sample. Each of the dilutions was added to 2 ml of RBC suspension and incubated for 1 hr. Samples were spinned at 400 x g for 45 seconds and resuspended by shaking. Samples were allowed to stand for 15 min. A 25 µl sample was drawn off from the midpoint of the tube and added to 10 ml PBS. Single erythrocytes were counted using a Coulter Counter at settings of 1/amplification and 1/aperture current = 1/2 with matching switch 20K and gain = 7. Duplicate samples were used and 2 counts were made for each duplicate. The agglutination strength was expressed as the inverse of the amount of µg protein in the extract per ml required to agglutinate 50% of the countable single cells.

Protein Efficiency Ratio (PER) Test

Diet

The PER test was performed according to the AOAC method (1970). Three diets were prepared using the following protein sources:

1. Casein (as control)
2. Regular tempeh
3. Tempeh prepared from germinated soybeans

The nitrogen content of samples was determined by the Micro-Kjeldahl method, and the fat content by the Goldfisch

diethyl ether extraction method. Protein = 6.25xN. All feeds were standardized to meet the following composition:

Protein	= 10%	Vitamin mix	= 1%
Fat/oil	= 8%	Cellulose	= 1%
Salt Mix USP	= 5%		

Corn starch + sucrose (1+1) to make 100%

The vitamin mix AOAC was from Teklad Test Diets and the salt mix and vitamin free casein from United States Biochemical Corporation, Cleveland, Ohio.

Experimental Animals

Thirty one male rats, age 21 days and average weight 43 g from Harlan Sprague Dawley, Inc. were supplied by the Spartan Research Animal Inc., Haslett, Michigan.

The animals were allowed to acclimate for 4 days on rat chow diet. At the beginning of the test, ten rats were assigned into one group of treatment. The average weight of the rats was 64 g.

Assay Period and Calculation of PER

The rats were kept in individual cages and both diets and water were given ad libitum. Body weight and diet consumed were recorded every 4 days. The experiment was terminated after 28 days from the beginning of the assay.

Weight gain and protein (N x 6.25) intake per rat for each group were calculated and the PER for each group were calculated as the ratio of weight gain per protein intake.

Corrected PER were also calculated by multiplying the PER of treatments with 2.50/PER of casein.

RESULTS AND DISCUSSION

Germination

Six hours of germination produced only a little elongation of radicle inside the testa which was hardly noticeable but the rupture of the testa occurred after 12 hours of germination. All radicles appeared after 18 hours of germination. Germination for 24 hours resulted in a 1 cm rootlets and the cotyledon became softer.

Tempeh Fermentation

The first step in tempeh preparation is soaking the soybeans overnight (or longer) to let lactic acid fermentation occur by microorganisms which naturally occur in soybeans. Because of steaming after each sample drawing from germination, these microorganisms were destroyed and soaking the samples in tap water did not result in a good lactic acid fermentation. Good lactic acid fermentation is indicated by the formation of foam on the surface, and was obtained by adding soaking water from fresh soybeans. Dehulling soaked germinated soybeans by hand was easier than soaked ungerminated ones.

There was no difference in mold growth on germinated or ungerminated soybeans. Mold growth was noticeable after 10 to 12 hours incubation, and after 20 hours mold appeared throughout the soybeans, at which time the temperature of the beans was higher than the incubator temperature. Steinkraus *et al*. (1960, 1965) and Sudarmadji and Markakis (1978) reported that during the tempeh fermentation by *R. oligosporus* the temperature rose to a peak of 40 to 45°C.

A satisfactory tempeh, with all of the soybeans completely covered with thick mycelium of *R. oligosporus*, was obtained after 32 to 36 hours of incubation. Tempeh from germinated soybeans can not be differentiated visually from tempeh of ungerminated soybeans.

Fermentation in small (9 cm diameter) Petri dishes was better than in 14 cm diameter Petri dishes. In 14 cm diameter Petri dishes, mycelial growth was very slow in the middle. Lack of air may be the cause of the slow growth. Poor mycelial growth was reported by former researchers.

Total Nitrogen and Soluble Nitrogen in Germinated Soybeans and Tempeh Prepared from Germinated Soybeans

The results of total and soluble nitrogen analysis from germinated soybeans and tempeh prepared from germinated soybeans are presented in Table 7. The shorter time of germination did not seem to significantly alter the total

Table 7. Total and soluble nitrogen in germinated soybeans and tempeh.

	Hours of germination	Total N (g/100 g sample)	Soluble N (g/100 g sample)
Germinated	0	6.9	1.1
soybeans	6	6.9	1.1
	12	7.0	1.1
	18	7.0	1.1
	24	7.0	1.2
Tempeh from	0	7.5	2.2
germinated	6	7.6	2.3
soybeans	12	7.6	2.5
	18	7.6	2.5
	24	7.6	2.6

nitrogen in germinated soybeans, but as the time of germination increased, the germinated soybeans showed an increase in total nitrogen content.

Hsu et al. (1973) reported that during germination, protein, oil and carbohydrates are sources of energy for the developing embryo. Soluble carbohydrates are important energy sources during early stages of germination, while protein nitrogen decreases and amino nitrogen increases as seeds germinate with the latter form being transported to the growing parts. The total nitrogen in the whole growing seed should remain the same as long as the growing parts are not removed.

The soaking and boiling steps of tempeh preparation result in some loss of water soluble compounds, such as sugars and amino acids. Steinkraus et al. (1960) reported that the total nitrogen during tempeh fermentation remained relatively constant, about 7.5%. Murata et al. (1967) reported a slightly higher protein content in tempeh than in soybeans for both tempeh produced in Indonesia and tempeh prepared in Osaka University. The data from this research showed an increase in total nitrogen during tempeh fermentation of ungerminated and germinated soybeans, confirming the Japanese report. The loss of non-nitrogen compounds during tempeh preparation seems to be much higher than the loss of nitrogen compounds. Non-nitrogen compounds are utilized by the mold more than nitrogen

compounds during the tempeh fermentation.

The soluble nitrogen content did not increase significantly during germination, but it was double after the tempeh fermentation. It seems that the increase of amino nitrogen during germination, as reported by Hsu et al. (1973) has not taken place during the short period of germination. The increase of soluble protein during tempeh fermentation has been previously observed by Steinkraus et al. (1960).

Crude Fat in Germinated Soybeans and Tempeh Prepared from Germinated Soybeans

The crude fat content of germinated soybeans and tempeh prepared from germinated soybeans is presented in Table 8. In general, the crude fat content decreased with increasing germination time and underwent further decrease during the tempeh preparation of the germinated soybeans. Both germination and mold growth seem to utilize fat for energy need. The tempeh preparation appeared to result in higher fat decrease (12-15%) than the germination of the soybeans (4%). Soaking and boiling may have contributed to the fat decrease in preparing tempeh. These data agree with those presented by Murata et al. (1967).

Table 8. Crude fat in germinated soybeans and tempeh prepared from germinated soybeans (% dry basis).

Samples	Germination time (hrs)	Crude fat
Germinated	0	22.3
soybeans	6	21.9
	12	21.9
	18	21.7
	24	21.5
Tempeh from	0	19.6
germinated	6	18.7
soybeans	12	18.7
	18	18.4
	24	18.1

Oligosaccharides

The HPLC separation of (a) a standard mixture containing sucrose, raffinose, stachyose and inositol; (b) the oligosaccharides of germinated soybeans; and (c) the oligosaccharides of tempeh, are shown in Figure 9. It may be noted that the higher the retention time of the sugar the broader the corresponding peak. A big difference in peak height between sucrose and both raffinose and stachyose was observed among samples, especially when germinated soybeans were analyzed.

Sucrose and inositol which had retention times of 3.2 and 3.9 min, respectively, do not separate very well using this column. The peak of inositol appeared in the extract of tempeh, but it was very close to that of sucrose. Because of the unsatisfactory separation and the poor replication of the inositol peaks, the quantity of inositol is not reported. No inositol peak appeared in the chromatogram from germinated soybean samples.

One peak with retention time of 5.2 min appeared in the extract of tempeh. Shallenberger *et al*. (1967) reported that during the tempeh fermentation, a reducing disaccharide appeared which attained maximum concentration after about 35 hr of fermentation and was absent after 60 hr. They identified it as melibiose.

Stachyose was reported to fully disappear after three days of germination (Abrahamsen and Sudia, 1966; and East

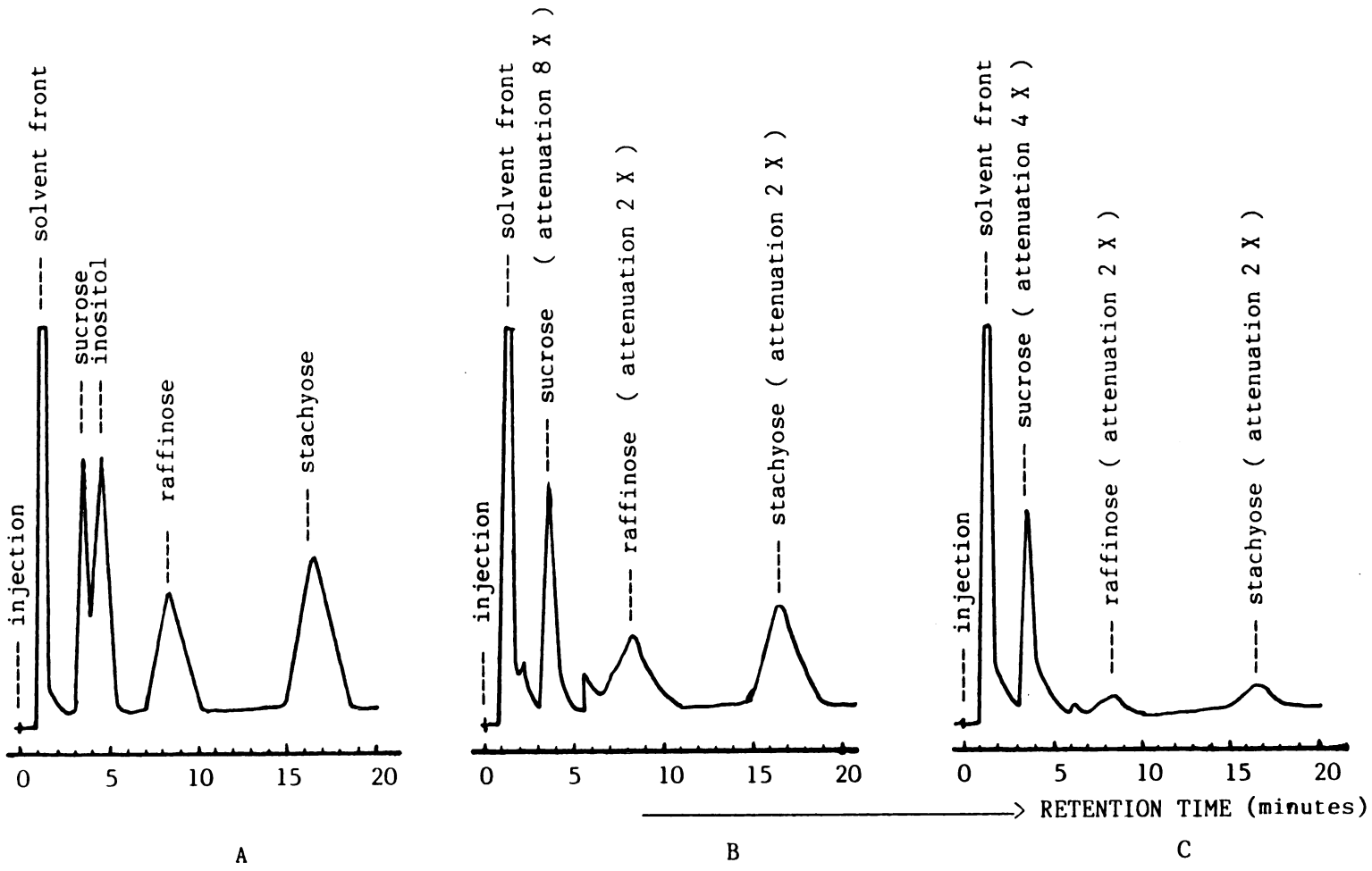

Figure 9. The HPLC separation of oligosaccharides. A = standard mixture containing sucrose, inositol, raffinose and stachyose. B = extract of germinated soybeans. C = extract of tempeh.

et al., 1972). Germination for short periods (up to 24 hr) reduced the stachyose content by less than 15%. However, this limited germination may be more valuable if it is used in the preparation of tempeh. Combination of short periods of germination and tempeh fermentation reduced stachyose by almost 90%, far more than the tempeh fermentation alone (Table 9).

Compared to stachyose, sucrose and raffinose disappeared more gradually, especially during the tempeh fermentation. This is probably due to the formation of raffinose and sucrose from the hydrolysis of stachyose.

Phytic Acid

The effect of germination and tempeh fermentation on the phytic acid content of soybeans is presented in Table 10. Phytic acid was not altered during germination up to 12 hr. A 6.4% decrease in phytic acid occurred after the seeds were germinated for 18 hr; a further reduction of 13.2% was observed after 24 hr germination.

Phytates are considered the main storage form of phosphorus in almost all seeds (Asada et al., 1969; Mandal et al., 1972; and Erdman, 1979). In the ripening process, phosphorus is transported to the seeds. Most of the transported phosphorus is deposited in the form of phytic acid (Asada et al., 1969). Mandal et al. (1972) studied germination of mung beans and reported that phytase was

Table 9. Oligosaccharides in germinated soybeans and tempeh prepared from germinated soybeans (% dry basis, average of triplicates).

Samples	Germination time (hr)	Sucrose	Raffinose	Stachyose
Germinated soybeans	0	5.61	0.21	3.82
	6	4.73	0.20	3.61
	12	4.42	0.15	3.36
	18	3.55	0.13	3.48
	24	3.46	0.11	3.30
Tempeh from germinated soybeans	0	2.99	0.09	1.83
	6	2.76	0.08	1.14
	12	1.90	0.06	0.72
	18	1.74	0.05	0.50
	24	1.39	0.05	0.49

Table 10. Phytic acid content in germinated soybeans and tempeh prepared from germinated soybeans (% dry basis).

Samples	Germination time (hr)	Phytic acid		
		I	II	Average
Germinated soybeans	0	1.3	1.3	1.3
	6	1.3	1.3	1.3
	12	1.3	1.3	1.3
	18	1.2	1.2	1.2
	24	1.1	1.2	1.2
Tempeh from germinated soybeans	0	0.8	0.8	0.8
	6	0.8	0.8	0.8
	12	0.8	0.8	0.8
	18	0.6	0.7	0.7
	24	0.5	0.6	0.6

not detected in the cotyledon of ungerminated beans but appeared in germination.

Tempeh fermentation appeared to be effective in reducing the phytic acid present in soybeans. About 40% of the phytic acid disappeared as a result of the 36 hr tempeh fermentation of ungerminated soybeans and an additional 20% was lost as a result of germinating the soybeans for 24 hr.

It was found that some microorganisms produce enzymes which have phytase properties. Sudarmadji and Markakis (1977) reported that the phytic acid content of soybeans was reduced by about one-third during tempeh fermentation. The reduction of the phytic acid was due to phytase elaborated by the Rhizopus oligosporus used in the tempeh fermentation. Wang et al. (1980) reported that two strains of R. oligosporus, one strain of R. Chinensis and eight strains of Aspergillus oryzae produced both extra and intracellular phytase. Neurospora sitophila and R. oligosporus were also reported to reduce phytic acid during oncom preparation (Fardiaz and Markakis, 1981).

Protein Efficiency Ratio of the Tempeh

The summary of the utilization of proteins from regular tempeh and tempeh from germinated soybeans by weanling rats is shown in Table 11. The PER of tempeh prepared from germinated soybeans (24 hr germination) were slightly higher

Table 11. Protein efficiency ratio of regular tempeh and tempeh prepared from germinated soybeans by weanling rats[1].

	Casein	Regular tempeh	Tempeh prepared from germinated soybeans
Average daily gain, g	3.51±0.34	2.94±0.35	2.88±0.34
Average feed intake, g	14.0±1.06	13.4±0.91	12.73±0.86
PER[2]	2.51±0.25	2.19±0.15	2.26±0.14
Corrected PER	2.50	2.18	2.25
Protein Quality (Sample PER/ Casein PER x 100)	100	87.3	90.0

[1]Each datum represents an average of 10 rats. Protein (NX6.25) content of the diets was 10%.

[2]PER ± standard deviation.

than the PER from regular tempeh (0 hr germination). However, these differences were not significant statistically. Rats on tempeh prepared from germinated soybeans diet ate and gain less than did rats on regular tempeh diet. Either the germination process or the combination of germination and tempeh fermentation may have depressed the acceptance of the diets by rats. Hackler et al. (1964) reported that the depressing effect may be caused by the mold or something elaborated by the mold used in tempeh fermentation.

Lectin Activity

The summary of the agglutination assay is presented in Table 12. There was no agglutination activity observed in any of the tempeh samples. The tempeh preparation steps, boiling and steaming, must have denatured the protein and inactivated the lectin of soybeans.

The amount of extract needed to agglutinate 50% of RBC decreased with germination. However, since the protein solubility increased with germination, the amount of protein in the extract also increased, a fact explaining the higher agglutinating power of germinated soybeans. The agglutination titer of the extracts were the same.

Table 12. Lectin agglutination titer[1]

Germination time (hr)	Extract needed for 50% RBC agglutination (μl)	Protein in the extract (mg/ml)	Protein needed for 50% RBC agglutination (μg)	Agglutination titer
0	230	0.38	87	0.011
6	228	0.39	89	0.011
12	210	0.45	95	0.011
18	180	0.50	90	0.011
24	165	0.57	94	0.011

[1]Agglutination titer = the inverse of the protein concentration in the extract at 50% RBC agglutination. The initial RBC count = 10^7.

CONCLUSION

Germination up to 24 hours markedly reduced the amount of oligosaccharides (sucrose, raffinose and stachyose) in soybeans; it also slightly reduced the crude fat content. The protein content slightly increased during germination. This increase can be explained by the decrease in the percentage concentration of carbohydrate and fat. Phytic acid content slightly decreased, but the strength of lectin remained constant. The changes occurring during germination are considered to be desirable.

The tempeh fermentation caused further decrease in oligosaccharides, crude fat and phytic acid with a corresponding increase in protein content. The soybean lectins were inactivated by either the boiling or the fermentation used in the preparation of tempeh.

Although the combination of soybean germination and tempeh fermentation resulted in better removal of some anti-nutritive factors (phytic acid and oligosaccharides) than the tempeh fermentation alone, it did not significantly increase the utilization of the protein by growing rats. The PER of regular tempeh and tempeh prepared from germinated soybeans were almost the same.

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