SENIOR PROJECT
PRODUCTION OF TEMPEH FROM VARIOUS LEGUMES

BY

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Senior Project

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A special problem submitted to the Faculty of Biotechnology, Assumption University in part fulfillment of the requirement for the degree of Bachelor of Science in Biotechnology.

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..... **Advisor**

(A. Nootrudee Siriboon)

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Abstract

Most of Tempeh is made from a soybean. Tempeh is cultured cake of beans or grains. It is made from fermented dehulled bean with *Rhizopus oligosporus.* Although soy bean tempeh is delicious and digestible, *R. oligosporus* can grow well in other legume seeds too. So this project aiming to study how to prepare tempeh from different legumes; they are groundnut, mung bean, kidney bean and cowpea.

Rhizopus oligosporus is able to ferment and grow on all legumes.Producing, the thick white mycelium covering the bean. Tempehs were deep fried palm oil for 2-3 minutes, depending on type of the legumes. All tempehs were rated in comparison with soybean tempeh. The result showed that groundnut tempeh was not significantly different from soybean. But chemical analysis showed that it contained the highest amount of fat and the lowest amount of protein among the 5 legumes used.

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Introduction

Tempeh is one of the first soy product, it is fast becoming the most popular soy food on the market because it is highly nutritions, easy to digest, deliciously, and easily to prepare. Originally developed in Indonesia, it is a traditional fermented soybean product made from cooked soybean inoculated with beneficial mold to give a chewy and meaty consistency. In Indonesia, a household art varies from home to home. A starter-usually a piece of tempeh from a previous bacth is added to begin the fermentation process. In traditional home-based tempeh making, the mixture is wrapped in banana leaves and left to ferment for 18 to 24 hrs. In western tempeh factories, the comercial starters are used to produce tempeh and the fermentation process takes place under carefully controlled conditions. The result is a cake of soybean with a rich flavor sometimes described as smoky or nutty. The flavor also has been compared to mushrooms (www.soyfoods.com/).

Tempeh is usually sold in natural food stores, where it is found infrozen food case. In the frozen form it can be kept well for several months. Tempeh can be kept in the refrigerator for about 10 days. As other fermented products, like cheese, a little mold on the surface of tempeh is harmless. Tempeh is a nutritional super hero. It is high in protein, dietary fiber, iron potassium, calcium, and phytochemicals to chemicals. It has been shown to lower cholesterol, high blood pressure and the risk of heart attrack.. It also reduces the risk of some cancer.

One serving of tempeh which is (4ounces), provides an average of 19 grams of soy protein, 60 milligrams of isoflavones and 7 grams of dietary fiber. Tempeh is a great choice for people who have difficulty digesting legume foods as tofu. Because tempeh is a fermented soy product that enzymes lipase partially break down the lipid components, making theme easy to metabolize. It does not produce the unpleasant gas from gastrointestinal that many legumes do, expecting that some oligosaccharides must have been broken down and utilized by the mold (www.soyfoods.com/).

Tempeh has a firm texture that is easy to create into a fantastic meal. It does not need much preparation or cooking time. That can be derived into dishes and adding onions, mushroom, peppers some cooked pasta. Normally salt and peper are an excellent addition with tempeh (www.soyfoods.com/).

The objectives of this project are :

1. To replace soybean with other legumes in preparing tempeh.
2. To determine the sensory preference of the products.
3. To determine some chemical components of the finished products.

Literature reviews

Tempeh products are made with soybean (as a control), ground nut, mung bean, kidney bean, cowpea and culture; *Rhizopus oligosporus.*

Legumes Seed

Soybean

Species Information.

Scientific name : *Glycine max*

Common name : Soybean

Soybean is a crop which can produce high quality and highest quantity of protein per unit area. The oil content of soybean is around 20% which is of high quality, while all other pulses contain about 1-2% oil (Rahman 1992). An excellent crop, that can be grown extensively, may reduce the protein and fat deficiency. In terms of area and production, soybean is such a minor crop concentrated only in few distinct locations, so that in the regular national statistics, the reference of soybean does not appear. This crop has shown a spectacular increase in its increased to about 760 ha and now it is no less than 1000 ha (Anon. 1990a) with a total domestic production of over1000 t. This expansion in area was possible due to increases in processing and utilization of this crop. The major uses of local soybean production at present have been found to be home consumption (40%) and industrial use (60%) (Anon. 1990).

The marketable products are soybean grains and snack items such a *chanachur,* biscuit and soymilk. Its use in *chanachur,* biscuit or bread however, depends on its price in relation to the price of wheat, *Lathyrus* and chickpea.

Small quantities of soymilk are produced under subsidies by the government and supplied to local children hospitals and schools. Soybean is popular for its edible oil. Mostly urban people consume soybean oil in their daily dishes. But the supply of edible oil is met from import of soybean oilseed, crude oil and refined oil. Soybean oilseeds are crushed locally and solvent extracted and the crude oil is refined. (Narong and Paisan 1995)

A good number of recipes has been identified for village and home level utilization. These include preparation of flour, mixed preparation with potato/pulses/rice, etc. (Anon. 1990).

Total import value of soybean and its oil during these three years was 3,820 million annually (about $ 100 million). This is about 75% of the total import of edible oilseed and oil in the country. With the rising trend in population, soybean has a strong potential for the future. The market is expected to expand to partly substitute animal protein intake in human food and also to supplement fish meal in animal and human food. Increasing urbanization will also lead to an increased demand for snack food, which in turn will encourage expansion of soybean production in the country. (Narong and Paisan 1995)

2) Groundnut

Species Information

Scientific name : *Arachis hypogaea*

Common name : Groundnut

Groundnut is known as one of the most drought tolerant cultivated Crop far its preference of poor soils since it tends to produce too much leaf at the expense of the pods and seeds in rich soils. Thus, it is an ideal crop for the semi-arid areas with erratic rainfall having poor soils. Yet in which it is grown commercially the only two areas in Asia – West Java and southern Thailand– are located in the very high rainfall region (Narong 1993).

Groundnut is highly adaptive to wide range of ecological and climatic conditions. Thus, it could be grown in most areas where other food legumes cannot be grown well to serve local demand for nutritive food.(Narong 1993). It is an annual herb with short freely creeping branched stems. It has very short internodes. It forms pods and seeds on, or just beneath, the ground.

The pods are round and wrinkled. Each pod contains one, or rarely two, round seeds, 1.0-1.5 cm in diameter. Seed has smooth texture when dry with varying color such as white, cream, tan, brown, red or black ; of the dried seed. The leaves are trifoliateand are borne on long slender petioles. The flowers spread out at ground level on a hairy peduncle. Each peduncle may produce one to three flowers attach to the peduncle by pedicels. Flowers are mostly yellow but some have deep yellow color.

They are self compatible and largely self pollinated.After fertilization, the flower stalk elongates, and its tip penetrates the soil, burying the fertilized flowers in the soil to form pods (Narong 1993).

Similar to other legumes, Ground nut is consumed in a variety of ways. Young seeds and pods are used as vegetable in spicy soup. Immature seeds are boiled or roasted, and eaten as snack. Mature (dry) seed is boiled, roasted or fried in oil, and eaten as delicacy. Roasted ripe seeds, mixed with flour, can be made into many delicious foods; roasted seeds can be ground into flour and used in many native dishes. In Africa, its main use is as a pulse in a porridge: while in Southeast Asia its main used are as a vegetable in soup as delicacy, and as dessert. Agronomically, bambara groundnut is useful in crop rotations as it contributes nitrogen to the soil which benefits subsequent crops.

Both immature and mature seeds have sweet taste (after cooking) are pleasant to eat. Dry seed contains 6 – 7 %oil, 18 – 20 % protein and 55–72 % carbohydrate (Narong 1993).

Groundnut is the most neglected food legumes in terms of research and devolopment. Thus, improved cultivars are not available. The present cultivars are the result of selection by the farmers. They are of long duration, with low yield, and low oil content. The ripe seeds have hard seed coat which must be softened by boiling or crushing before consumption. It has limited utilization as well as limited market at present.

3) Mung bean

Species Information

Scienctific name : *Phaseolus aureus*

Common name : Mung bean

The mung bean is frequently much branched the few cultivars having a spreading habit. The stems normally branch from the base and are angular and usually green, but occasionally splashed with purple. The leaves are trifoliolate with large, ovate, entire, or rarely lobed, membranous leaflets, 2 – 4 in. long, with scattered hairs on both sides and light to dark- green in color, never yellowish. The yellow or greenish-yellow flowers are crowded in clusters, (10-20), on axillary or terminal racemes, usually the latter. The flowers are fully self-fertile and are normally self –pollinated.

The seed pods are sub cylindrical 2-4 in. (5-10 cm) long and 0.15-0.24 in. (4-6 mm) wide, straight or slightly curved and normally containing 10 to 20 small seeds. When unripe the pods are various shades of green, but when fully mature may be grey, greyish- olive green, brown . The seeds are globular or oblong, often green, but may be yellow, brown, or speckled with black. Two main types of mung bean are usually recognized: (i) *aureus* yellow or golden gram, which has yellow seeds, is generally low in seed production, has a tendency for the pods to shatter, and is often grown for forage or green manure; (ii) *typica*, green gram, which has dark or bright- green seeds

A sample of oil from Pakistani mung beans was reported to have following fatty acid composition: palmitic 28.1 %, stearic 7.8 %, arachidic 0.9 % , behenic 2.4 %, cerotic 6.3 %, oleic 6 %, linoleic 32.6 %, linolenic 14.4 %, The unsaponifiable matter contained 0.023 %, stigmasterol on an air- dry basis.

Flour – Mung bean flour may be produced by dry milling the seeds and sifting the resultant flour through a fine sieve. A superior product can be obtained by grinding the seeds in a pin mill with air separation of the seed- coat action.

Canned bean sprouts –The bean sprouts are blanched, packed into cans by hand, a brine solution is added, the cans are then exhausted at 180- 190° F closed and processed at 240°F for 20 minutes, in the A2 (307 x 408) cans.

The dry seed is sometimes used for animal feeding, particularly poultry, when toasting or boiling is recommended to improve its nutritional value. The green immature pods are occasionally picked and eaten as vegetable. The mung bean makes quick, early growth and may be grown as a valuable green manure or cover crop. The flour is reported to be used occasionally in parts of SE. Asia as a soap substitute . It has also been suggested that the sprouted mung bean could be utilized as a source of L- aspargine (Daisy 1974).

The leaves and stalks left after the beans have been harvested can be utilized for animal feeding. An approximate analysis of mung bean hay has been given as follows: moisture 9.7 %, crude protein 9.8 %, fat 2.2 %, crude fibre

24.0 %, ash 7.7 %, N- free extract 46.6 % digestible crude protein 7.4 %,and total digestible nutrients 49.3% The residue left after starch extraction is rich in protein and can also be utilized for animal feeding. (Daisy 1974)

The mung bean has the following approximate composition: moisture 6.6- 11.6 %, protein 19.7- 24.2 %, total carbohydrate 60.3- 67.5 % crude fibre acid 865; proline 229; glycine 210; alanine 242; valine 259; methionine 33;isoleucine 223; leucine 441; tyrosine 156; phenylalanine 306; lysine 504; histidine 182; and arginine 345. (Daisy 1974)

Although the mung bean is a well known food legume, particularly in SE. Asia, and its nutritional value has long been recognized, it has not been a popular crop with many farmers, because of the relatively low yields and its susceptibility to attack from pests and diseases. Until comparatively recently it has received little attention from plant breeders, but the development by Indian workers of short- duration improved cultivars, with increased protein contents, indicates, that the crop has considerable potential for improvement. There is need for plant breeders to intensify their efforts to develop improved cultivars, particularly disease resistant strains capable of producing seeds with a high protein content.

The work currently being undertaken at Missouri studying: (i) the range of adaptation of the mung bean, (ii) the range of adaptation of specific cultivars, and (iii) the characteristics of the mung bean plant influencing adaptation, could result in the development of much improved cultivars with relatively high productivity levels. (Daisy 1974)

4) Kidney bean

Species Information

Scientific name : *Phaseolus vulgaris*

Common name : Kidney bean (Common bean)

Kidney bean, has several common name depending on stages of pods and seeds consumed. They are called French bean, frijoles garden bean, green bean, haricot bean, Italian bean, kidney bean, pole bean, romano bean, runner bean, snap bean, string bean, yellow bean and wax bean. Mature seeds are called dry bean, kidney bean, or navy bean. It is probably the most widely used species of the genus *Phaseolus*. Although originated in Central America, it is extensively grown in eastern Africa, North and Central America, South America, eastern Asia and western and southeastern Europe (Peerasak 1993).

Kidney bean is a warm season crop and cannot grow well under the temperature lower than 20°C. The seeds are planted 5- 10 cm deep with the row spacing of 60- 90 cm, and hill spacing 5- 10 cm. The seed rate varies from 20- 55 kg/ha. Common bean can be grown on many soil types; a light soil with good drainage is best. A replant fertiliser of 65- 100 kg N/ha Well – decomposed manure is also recommended (Peerasak 1993).

Kidney bean is very sensitive to soil moisture stresses. Yields decrease substantially when moisture stress occurs during flowering and subsequent pod development period. Green pods can be harvested within 45- 80 days,

depending on cultivars. Normally, the bush type takes less time than the climbing type. The pods should be harvested at about half- grown to three- fourths of the maximum length (Peerasak 1993).

Over 200 different insects attack common bean. The more devastating ones are aphids, thrips, white flies, leafhoppers, bruchids, cutworms and pod borers. The more important diseases are anthracnose, bacterial blight, powder mildew, curly top, and root rot (Peerasak 1993).

5) Cowpea

Species Information

Scientific name : *Vigna unguiculata*

Common name : cowpea

Cowpea, *Vigna unguiculata* (L.) , is one of the most important legume crops in Africa, some countries in Asia and South America. In fact, Africa produces 95 % of the world crop. It provides more than half of the plant protein in human diets and used as a staple food for the poorest sector of many countries of the dry zone. As dry seed has high protein content (23- 30 %) it can be substituted for animal protein for vegetarians to balance their protein diet. As a food, it is eaten in the forms of dry seeds, green pods, green seeds and tender green leaves. Cowpea flour is also used to make sweets, cakes and porridge. It is also utilized as feed, hay, pasture and green manure.

As it is a fast growing crop, it is grown as ground cover in rubber plantation in Sri Lanka, Philippines and Indonesia. In India, it is grown as green manure in tea and sugarcane estates.

On account of its ability to fix nitrogen, the crop besides meeting a high proportion of its own nitrogen requirements, leaves a fixed- N deposit in the soil up to 60- 70 kg/ha for the succeeding crops. For this quality and also for being a short duration crop, it is a also highly compatible as a companion crop of a wide range of food and fibre crop (Sanit 1993).

Cowpea is protein rich crop and widely adapted to marginal soils and varying agro - ecological situations. The prospects of this crop are very good through crop improvement and better management practices. The crop fits in well in multiple cropping and/or relay- cropping systems and thus has added advantage especially for poor soil condition. Efforts to gather genetic resource and use in breeding are the keys for future improvement. As the demand for vegetable will grow in southeast Asia, the crop has future in the region.

Cowpea is an annual crop and can be grown very well in hot and dry regions of tropical, sub- tropical, semi- arid and arid zones of Asia, Africa, South and North America. It is a short- day plant. It is also sensitive to day and night temperatures. The optimum temperatures for growth and development is between 25° – 30°C. Temperatures below 15°C may kill because of frost and temperatures above 35°C are reporte to increase flower and pod shedding. It is relatively drought tolerant and can tolerate moderate shade.

However, some of the new cultivars are day neutral. When its flowers were destroyed by insects or other animals, the new flower buds would regenerate and produce the flowers and pods (Sanit 1993).

Leaf and pod of cowpea are used as fresh vegetable. Immature seed is boiled and eaten as snack, while mature (dry) seed is boiled with sugar and coconut milk as a sweet dish. The stem is used as animal feed, green manure and compost. In America, cowpea is planted for immature seeds for canning and the stem is used as hay. In Africa, cowpea with rough or wrinkle seed coat is soaked to remove the seed coats and ground in order to make dough (Sanit 1993).

The amounts of nutritional value and crude proteins in the dry seeds are highly variable among genotypes, management and environment. On an average, 100 g of dryseed contain:11.0 g of water; 23.4 g of protein; 56.8 g of carbohydrate; 1.3 g of fat; 3.9 g of fibre and 3.6 of ash.

Amino acid contents (expressed as average percentage of total protein) are : lysine 6.6 %, cysteine 0.9 %, methionine 0.9% histidine 3.3%, threonine 4.1%, and tryphan 0.9%. In general, such a composition considered ideal although methionine and cysteine (Sanit 1993).

Table 1: Nutritive value from diffent legumes (per 100g. edible portion)

Common Name	Calories (g)	Water(g)	Protein(g)	Fat (g)	Fiber (g)	Ash (g)
* Soy bean	335	8	38.0	18.0	4.8	4.7
** Groundnut	343	5	25.6	43.4	3.3	2.5
*** Mung bean	340	11	23.9	1.3	4.2	3.4
** Kidney bean	341	11	22.1	1.7	4.2	3.8
** Cowpea	342	11	23.4	1.8	4.3	3.5

Sources : *Food and Agriculture Organization of the United Nations, 1949.

**Food and Agriculture Organization of the United Nations, 1954.

***U.K. Medical Research Council, 1962. Tables of representative value of foods commonly used in tropical countries, by B.S platt.

Table 2 : Price in bath/km of legume seeds obtained from the retailed market during 15/11 to 1/12 2001.

Legume seed	Amount	Cost (Bath)
Soy bean	1 kg.	24
Groudnut	1 kg.	54
Kidney bean	1 kg.	28
Cow pea	1 kg.	30
Mung bean	1 kg.	28

Culture

Rhizopus oligosporus

The fermentation of tempeh can be achieved by a mixed moulds, yeasts and bacteria but the most important component appears to be from *Rhizopus oligosporus.* Although other *Rhizopus* and *Mucor* species are often isolated. Over two days incubation at temperature 30°- 35°C is required to develop mycelliume througout the mass of beans, knitting them together.
During fermentation the pH rise to around 7, fungal proteases activate the reaction and subsequently convert more than 1/3 of the neutral fat present to free fatty acids.

The optimum growth of *R.. oligosporus* was observed at RH 75- 85 %, 66 % of nonnitrogenous compounds, 50% of crude proteins,and 50% of fat are solubilized during tempeh fermentation. The most critical issue appears to be the loss of protein during tempeh preparation, which is generally considered to be more than 25%.

In addition to improve the nutritional quality of soy bean by reduction or removal of various anti- nutritional factors at different stages in the processing. The abitity of bean to produce flatulence is also regarded as an anti- nutritional property and flatulence- inducing oligosaccharide such as stachyose and raffinose are leached out of the bean during the soaking stage.

And the most important aspect is the ability *R. oligosporus* to produce antibacterial compound during tempeh fermentation, which is very active against Gram-positive bacteria, including *Clostridium butulinum* , *C. sporogenes* and *Staphylococcus aureus.*

Materials and Methods

Raw materials

1. Soy bean
2. Groundnut
3. Kidney bean
4. Cowpea
5. Mung bean

Culture

Rhizopus oligosporus

Chemicals

1. Catalyst mixture - anhydrous sodium sulfate 96%, copper sulfate 4%
2. Sulfuric acid (nitrogen free)
3. 2% Boric acid solution
4. Methyl red indicator
5. 50% Sodium hydroxide solution
6. 0.05M Standard sulfuric acid solution.
7. Petroleum ether

Equipment

1. Macro-Kjeldahl digestion apparatus
2. Macro-Kjeldhal distallation apparatus
3. Soxhlet apparatus
4. Analytical Balance, OHAUS model AP 210S
5. Hot air oven, memmert model 600
6. Aluminum can
7. Glassware
8. Plastic bag

Method

(1.) Preparation of Tempeh from different legumes.

Whole beans

↓

Clean to remove foreign matters

↓

Boil for 30 minsutes to partially hydrate the bean and removing of the hull

Dehull by rubbing the hull off

↓

Wash the dehulled bean

↓

Soak overnight

↓

Drain excess water

↓

Steam for 40-60 minutes to pasteurize the bean

↓

Remove from heat and cool to room temperature

↓

Let dry

↓

Inoculate with *R.oligosporus* (one slant / 0.5 kg bean)

↓

Wrap in a clean plastic bag

↓

Incubate at room temperature for 48 hours

↓

Raw tempeh containing thick white myce lium covering the legume

2) Frying method

Deep fry in palm oil at 180°C for 1-3 minutes until the product is cooked. Frying time will vary depending on the kind of bean. The fried tempeh has chewy consistency with mushroom like flavor.

3) Sensory Evaluation

Preference test using 9 poin-hedonic scale is used in 4 samples with soybean as control to determine five attributes are judged based on preference of 20 taste-panelists.

4) Chemical Analysis

(4.1) Moisture content (A.O.A.C 1984)

(4.2) Fat content (A.O.A.C 1984)

(4.3) Protein content (A.O.A.C 1984)

5) Statistical Analysis

Compare the mean of each quality factors and the total mark of each tempeh by using SPSS program.

Results and Discussions

(1.) Preparation tempeh from different legumes.

Figure 1 : Tempeh produced from soybean

Figure 2 : Tempeh produce from groundnut

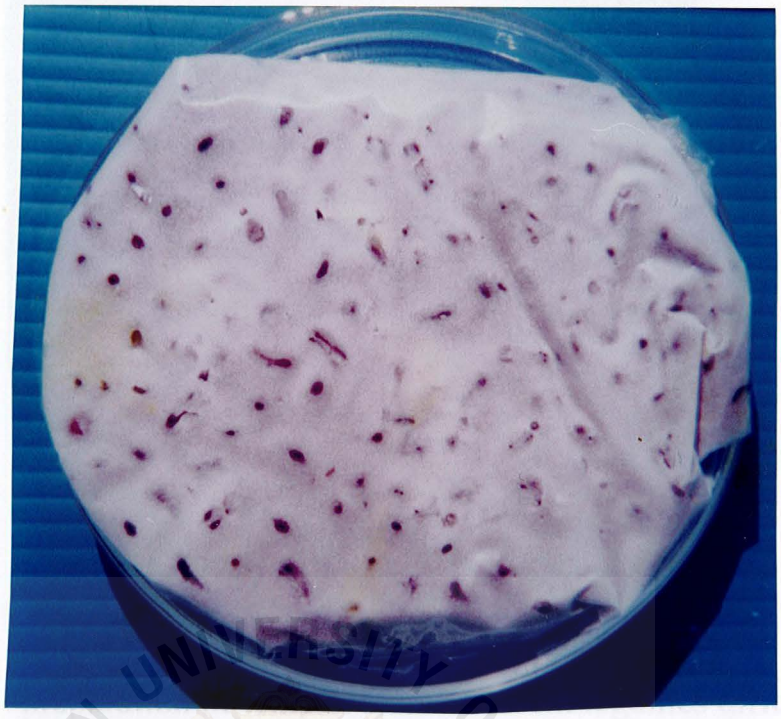

Figure 3 : Tempeh produced from kidney bean

Figure 4 : Tempeh produced from cowpea

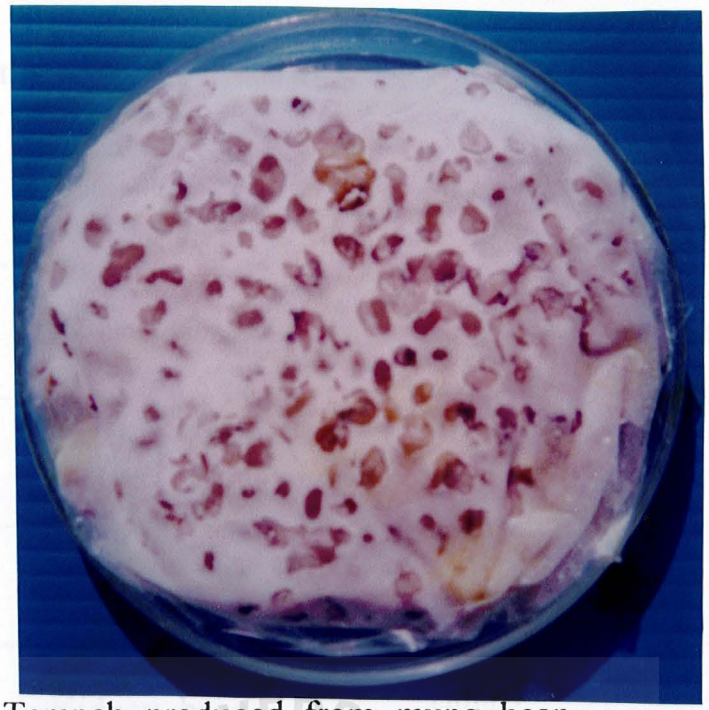

Figure 5 : Tempeh produced from mung bean

The preparation of tempeh from different legumes differed in some step. Because Some legumes as kidney bean and cowpea, having hull attaching tightly to the cotyledons, the removing of the hull is impossible. Inoculation and fermentation is done with the hull beans.

Other bean as soybean and groundnut, removal of the hull is possible. they are inoculated and fermented as naked seeds. Unlike other beans, mung bean is too soft to rub off its hull. Therefore, it is inoculated and fermented with the hull. However after fermentation, they are covered with thick white knitting myce lium of the mold as shown in figure 1 to 5. The knitted mat of mycelium helps binding them together as one singular item. *R.oligosporus* grow quickly at 37°C producing white mycelium connecting the bean together.

Table 3 : Frying time

Tempeh product	Temperature	Time (min)
Soy bean	180°C	1.45
Groundnut	180°C	1.40
Kidney bean	180°C	2.30
Cowpea	180°C	2.50
Mung bean	180°C	2.10

From the table 3, it was demonstrated that the frying time from different legumes. The legumes with hull required longer frying time , about 2 minutes , than those without hull about 1 minutes. Moreover, large seeds also require longer frying time than small seed as in kidney bean and cowpea.

2) Fried tempeh

Figure 6 : Tempeh produced from soybean

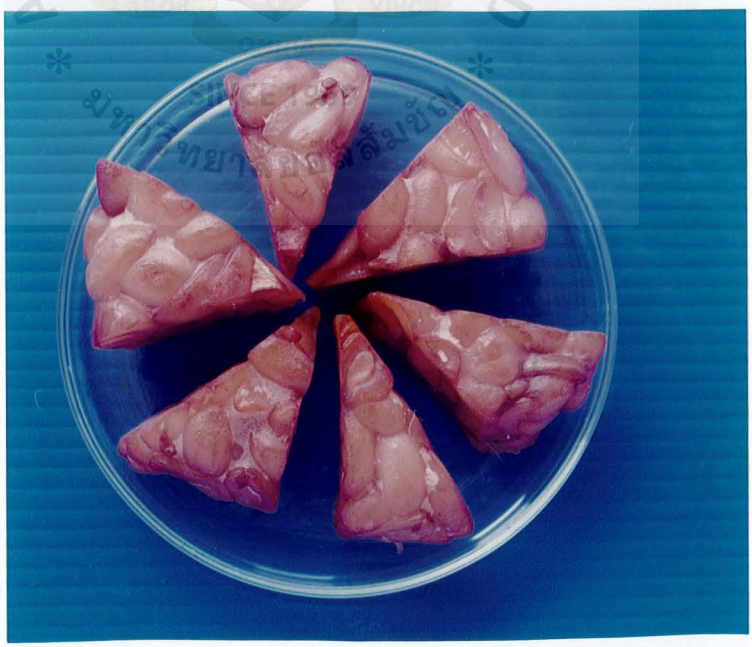

Figure 7 : Tempeh produced from goundnut

Figure 8 : Tempeh produced from kidney bean

Figure 9: Tempeh produce from cowpea

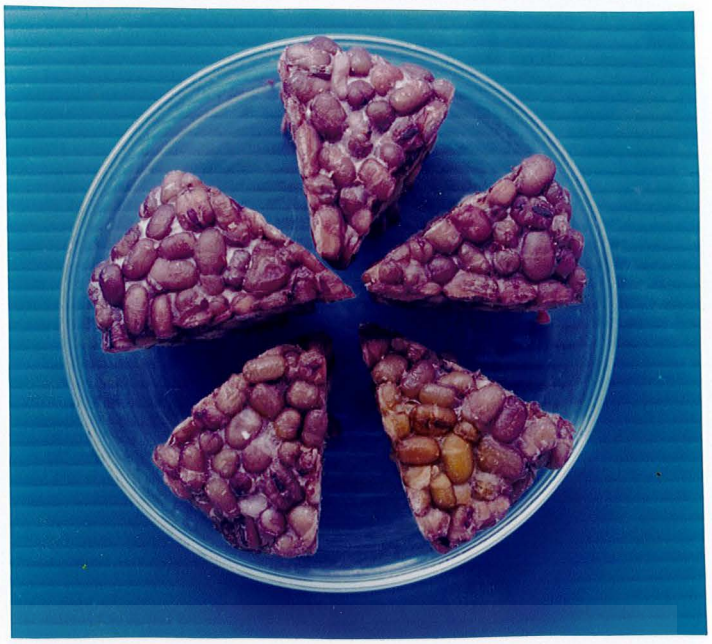

Figure 10: Tempeh produce from mung bean

From figure 6 to 10 show the fried tempeh made from soybean, groundnut, kidney bean, cowpea, and mung bean respectively. The soybean tempeh and groundnut tempeh are prepared from dehulled seed. After frying the products became beautifully golden brown. Kidney bean and cowpea still contained their hull produced dark brown to black color. Mung bean was softer than other legumes, its tempeh was also soft with green hull embedded in the finished product.

Frying causes partially dehydration of the product so that the fried tempehs develop the firm, solid cake texture and leave no trace of mold myce lium on them, as shown in figure 6 to 10.

(3.) Sensory Evaluation

Table 4 : Sensory evaluation of 4 tempehs prepared from 4 different legumes in comparison with the control (soybean).

Attributes	Average score*				
	Control	Groundnut	Kidney bean	Cowpea	Mung bean
Color	7.75^{a}	7.80^{a}	5.45^{b}	5.30^{b}	4.40^{c}
Flavor	7.40^{a}	7.80^{a}	6.05^{bc}	6.35^{b}	5.35^{c}
Odor	7.45^{a}	7.85^{a}	6.25^{b}	6.00^{b}	4.65^{c}
Texture	7.35^{a}	7.20^{ab}	6.50^{b}	6.45^{b}	4.65^{c}
Overall Acceptance	7.70^{a}	7.65^{a}	6.95^{b}	6.25^{c}	4.65^{d}

* Sample with the same superscribed litter are not significantly at $\alpha = 0.05$

In a comparison between 4 sample tempeh and the soybean , the result indicated that only groundnut were not significantly different from the control. Both groundnut and control were given score between 7 to 8 indicated moderately like to very much like of the products from the taste panelists in all attributes. On the contrary, kidney bean, cowpea, and mung bean obtained score from 6 to 4 that reflected slightly like to slightly dislike of the product. Overall acceptance shown that groundnut could be uesed to prepare tempeh with almost equally prefered the same as the soybean tempeh.

(4) Chemical Analysis

Table 5 : Percent moisture, fat and protein content of the legume tempehs.

Tempeh Sample	Percentage of Moisture content	Percentage of Fat content	Percentage of Protein content
Soy beam	26.4	10.4	25.3
Groundnut	12.6	35.9	9.2
Kidney bean	25.7	2.4	16.3
Cowpea	28.4	2.2	20.0
Mung bean	33.7	2.1	22.6

It was found that mung bean tempeh contained the highest percent of moisture content of 33.7% causing the tempeh to be softer texture than other legumes and received the lowest preference score. However its protein content, 22.6%, is considered high almost equal to soybean 25.3%, while its contained the lowest fat content, 2.1%. Other beans , the groundnut tempeh had 12.6% moisture content, the highest amount of fat ,35.9 %, and the lowest of protein,9.2% but was second most prefered tempeh.

For kidney bean and cowpea tempeh, both contained higher percent of protein than groundnut but lower than mung bean and soybean. However they are contained low amount of fat content ~2%.

Conclusion

1) Preparation of the tempeh from 4 legume seeds. All legume can be fermented by *R.oligosporus* and develop to raw tempeh. The preparation is differed in soybean and groundnut , the naked seeds are used while the kidney bean, cowpea, and mung bean have to use the whole seeds.

2) The sensory evaluation of 4 samples and control on 5 attributes and statistic analysis demonstrated that the tempeh produced from kidney bean , cow pea and mung bean were significantly different in the color,flavor, odour, texture and overall acceptance from the control at α=0.05.
By preference score they were slightly dislike to slightly like. Groundnut tempeh was rated with no significantly different scores with the control in all attributes.

3) The chemical analysis showed that protein content of tempeh from groundnut was the lowest, while other tempehs had protein content higher than those groundnut. Moreover, the fat content of groundnut was the highest 35%. But it were received equally preference score as those of soybean.

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Appendix

Table 6 : Questionaire for the preference test.

Name of the test panelist .....

Date.....Time.....

Product : Tempeh

Please test the sample by comparing with the"control" for the given attributes on your preference of the sample. Rinse your mount every time your change the sample.

Given score

9. Extremely like
8. Very much like
7. Moderately like
6. Slightly like
5. Neither like or dislike
4. Slightly dislike
3. Moderately dislike
2. Very much dislike
1. Extremely dislike

Table

Attributes	Sample code					
	control					
Color						
Flavor						
Ordour						
Texture						
Overall Acceptance						

Table 7 : Variance analysis of 9 point-hedonic scale preference test comparing the color acceptable of 4 tempeh sample with the control .

SOV	df	SS	MS	F-cal	F-table
Treatments	4	191.40	47.785	30.487**	2.483
Errors	95	148.900	1.567		
Total	99	340.040			

** = highly significantly different

Table 8 : Variance analysis of 9point-hedonic scale preference test comparing the flavor acceptable of 4 tempeh sample with the control .

SOV	df	SS	MS	F-cal	F-table
Treatments	4	80.140	20.035	11.748**	2.483
Errors	95	162.050	1.076		
Total	99	247.190			

** = highly significantly different

Table 9 : Variance analysis of 9point-hedonic scale preference test comparing the odor acceptable of 4 tempeh sample with the control .

SOV	df	SS	MS	F-cal	F-table
Treatments	4	128.840	32.210	25.123**	2.483
Errors	95	121.800	1.282		
Total	99	250.640			

** = highly significantly different

Table 10 : Variance analysis of 9point-hedonic scale preference test comparing the texture acceptable of 4 tempeh sample with the control .

SOV	df	SS	MS	F-cal	F-table
Treatments	4	92.260	23.065	15.404**	2.483
Erors	95	142.250	1.497		
Total	99	234.510			

** = highly significantly different

Table 11 : Variance analysis of 9point-hedonic scale preference test comparing the overall acceptable of 4 tempeh sample with the control .

SOV	df	SS	MS	F-cal	F-table
Treatments	4	127.040	31.760	25.569**	2.483
Errors	95	118.000	1.242		
Total	99	245.040			

** = highly significantly different

Chemical Analysis

Moisture content

Drying in a hot air oven is the method to find the percentage weight Loss of water that calculated after removal by heating under standard condition. The most common method of estimation the moisture content in the food. The containers are dried to a constant weight in the hot air oven for 20-30 minutes and cooled down in desiccator before weighing and recording their dry weigh. Weigh about 5 grams of sample to 4 decimal sin. The known weigh is added. Dry both Sample and container in the hot air oven at temperature ranging from 100-105°c for 3 hours. Remove from the oven and place in the disiccator to cool down , then weigh the cooled sample record the result. Repeat drying until the different in the result is less than 0.05 grams.

Calculate the weight loss and determine the moisture content as :-

% moisture content = weight loss*100/weight of sample.

Fat content

Direct extraction method by weight soxhlet appartus :

Fat or oil are extracted directly with organic solvents. Lipids in food are usually extracted by Ether and Pretroleum ether is common used .

- Dry a sample in a hot air oven and cool in a dissiccator .
- Weigh the dried sample on a dried known weight filter paper No. 1 .
- Record the weight of a sample
- Place the packed sample in a Soxhlet apparatus .
- Connect the Soxhlet to a known weigh round bottom flask with a condenser.
- Pour the petroleum ether until a solvent level reaching to the siphon. the solvent is then pull down into the flask. Repeat the step with the same amount of petroleum ether.
- Gently reflux the sample for 2-3 hours, depending on the sample to extract lipid from the sample .
- Evaporate the solvent in the Soxhlet by continuing heating until the solvent level almost reaching the siphon and then turn off the heater and remove the-Soxhlet from the flask and the condenser and pour the solvent to a discharging bottom.
- Remove the solvent from the sample as much as possible.
- Evaporate the residue solvent in the sample in a water bath

- Dry the flask and weigh the weight of the flask together with the extracted lipid

- Calculate percentage of lipid from the below equation :-

$$\% \text{ Lipid} = \frac{\text{Weight of the extraction lipid} * 100}{\text{Weight of original sample}}$$

Protein content

Macro – Kjeldahl Method is used in determine the total organic nitrogen content of the sample .This is including protein , and non – protein nitrogen (amino acids and peptide)

- Weigh about 2 grams of tempeh sample containing and Transfer to the Kjeldahl digestion fask .
- Add 8 grams of catalyst mixture and 20 ml conc. sulfunc acid , slowly pour along the neck
- Digest the mixture by slowly bring to boiling slightly decline the flask , and continue boiling till no bubble appears .
- Digest is completed in approximately 20 hours for legume seed , the blue color solution is gained and cool down .

- Slowly add distilled water to the solution . Make up the volume to 400 ml.

- Add 2-3 pieces of glass beads to pervent bumping .
- Slowly pour 75 ml of 50% sodium hydroxide solution .
- Immediately connect the flask to condenser which already has one opening submerging in 50 ml of 4% boric acid solution and contain in 500 ml conical flask .
- Add a few drops of methyl red indicator in the boric solution .
- Distill the digested solution until gaining about200-250 ml distillate in the receiving flask.
- Wash the condenser with distilled water .
- Titrate the distillate solution with standard sulfuric Acid, 0.05M
- Determine percentage of totel nitrogen from Tempeh sample. by using conversion factor 5.71for soybean, 5.41 for groundnut 5.83 for kidney bean , cow pea and mung bean .