STUDY ON UTILIZATION OF SOY MILK RESIDUE TO MANUFACTURE "TEMPEH"

By

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DECLARATION

The work described in this project report was carried out at the Food Research Unit, Department of Agriculture, Gannoruwa, Peradeniya and Faculty of Applied Sciences under the supervision Mr. T.D.W.Siriwardana and Mr. M. A. J. Wansapala. A report on this has not been submitted to any other University for another degree.

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AFFECTIONATELY DEDICATED TO

MY EVER LOVING PARENTS AND SISTERS

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ABSTRACT

Tempe is a traditional fermented soybean food of Indonesia. It is rich much of its good flavor, sliceable meat-like texture, and excellent nutritional properties due to the process of fermentation. Consumption of Tempe is usually in the form of slices which have been seasoned and deep fat fried in oil. The product is especially suitable for nourishment of lactating and pregnant mothers, and malnourished children.

Homo fermentation is involved and it is a versatile process. Therefore, the aim of the study was to make Tempe by using versatility of the fermentation, that is the making of Tempe by substituting okara (soy milk residue) which is the by-product of the tofu industries for soybean substrate.

The two main steps involved in okara Tempe manufacturing are preparation of okara as a key ingredient, to produce good quality of Tempe and its fermentation. A two stage of fermentation process is involved in Tempe manufacturing. The product serves as a major source of high quality protein, calories, vitamins and dietary fiber in the diet. *Rhizopus oligosporus* is the principle mold species that carries out the homo fermentation process.

The temperature, moisture content and acidity of the okara substrate are critical if high quality Tempe is to be obtained. The moisture content of okara is in the range of 42-47%, acidity should be in the range of pH 4.5-5.5. A temperature of 30^0-37^0C over a one to two day period results in Tempe with optimal sensory characteristics. The fermenting okara should be covered to retard moisture losses however, diffusion of air is essential to promote proper growth and metabolic activity of *R. oligosporus*. It has been found that perforated polyethylene bags are the most suitable package for inoculated okara substrate.Tempeh should be eaten within one day of its preparation. Otherwise, the release of ammonia gas as a result of break down of mycelia and okara proteins causes the product to be inedible.

Variations of temperature, pH, acid value and formal values are important measurements which are usually used as a criteria of Tempe analysis All of these

measurements are intended to expose the mold activities during the second stage of okara Tempe fermentation.

Okara Tempe is an ideal food for use in developing countries as a source of tasty, inexpensive high-quality protein and its commercial production requires only the simplest, low-level technology.

During the study, it was found that there was no post fecal contamination in the product. Sensory evaluation proved that there is no significance different of consumer acceptability of the product when comparing with Tempe made from soybean that is the already existing product.

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ABBREVATIONS

PER	- Protein Efficiency Ratio
Hrs	- Hours
Conc	- concentrated
No	- Number
MT	- Metric ton
i.e.	- that is
1st	- first

CHAPTER 1

INTRODUCTION

Tempe is a popular traditional fermented soybean food that is a key protein source in the rich diet of the people in Indonesia. Today, it is increasingly becoming as meat replace in the vegetarian diet, cholesterol- free and low fat food product.

A well Tempe by definition, it is a compact cake completely covered and penetrated by the white mold mycelium of *Rhizopus*. *Rhizopus oligosporus* is the principle microbe, responsible for the Tempe fermentation, that has unexpected and beneficial properties which makes the food more valuable. The product has a unique meaty flavor and aroma. Tempe is usually fried or cooked with vegetables.

Tempe fermentation is an indigenous processing technique and it consists of two phases of fermentation, which transforms beans, grains and other substances into highly digestible and palatable food. The fermentation process enhances the nutritional value of the finished product by increasing with its vitamins.

Tempe is generally safe food and its supplement in the diet of malnourished children, pregnant and lactating mothers has been found to be beneficial. Tempe is ideal food for use in Sri-Lanka like developing countries as an inexpensive source of high quality protein and other nutrients and calories.

The principles of Tempe fermentation are such that a number of beans types and even cereals or mixture of cereal grains and beans can be substituted for soybean substrate. Therefore, it is a versatile process. According to that, versality of the Tempe fermentation, by-product of the food industry, such as the residue (okara) from soybean milk or soybean curd (tofu) manufacture can be substituted as a major substrate or as an ingredient in manufacture new type of "Tempe".

The soybean milk residue is removed as a worthless product in the manufacturing of tofu, but it is versatile, abundant and highly nutritious product. It is an excellent source of high quality protein, vitamins, minerals and rich source of dietary fiber.

Moisture content of the okara is critical factor and plays an important role in the conversion of soymilk residue into "Tempe". The fermentation of okara into Tempe is similar to that the Tempe is made from soybeans. pH adjustment and proper hygienic practices are also govern the making of good quality Tempe from okara.

OBJECTIVES

(1) Manufacturing of "Tempe" from okara (Soymilk residue) to study and extend the usefulness of okara.

(2) Establishment of product identities.

CHAPTER 2

LITERATURE REVIEW

2.1 Soybean

Soybean is one of the oldest crops of the world. It has been considered as a "miracle" crop due to its good quality oil and protein content and soil enriching properties. Soybean is a leguminous crop. It grows annually. Its plant is bushy with height ranging from 0.75 to 1.25 m, branching densely depending on cultivates and growing conditions.

2.1.1 Origin and Distribution

The soybean is closely associated with history of China, where it has been almost the sole source of proteins for generations (Ahmed, 1984). From China, soybean cultivation is spreaded to different countries such as Japan, Korea and throughout the South Asia, Europe, and America. Soybean was first introduced to Sri Lanka in 1947.

2.1.2 Classification

Family	: *Leguminosae*
Sub family	: *Papilionoidae*
Genus	: *Glysine*
Species	: *max*

2.1.3 Seed Morphology

Soybean seed is a typical legume seed. It is different in size, shape and color depending on the variety. They range from small round beans to large, oblong, flattened seeds. Yellow, brown, green and black or combination of these colored seeds can see.

Mature seeds are made of three basic parts: the seed coats, the embryo and one or more food storage structures.

2.1.4 Soybean : A Unique Food Crop

There are several major reasons why soybeans are an economical and valuable agricultural commodity. The reasons are the following ,

1. Soybeans have favorable agronomic characteristics and soil enriching properties through Nitrogen fixation.
2. Soybeans have a unique chemical composition. On an average dry-matter basis, soybeans contain about 40% protein and 20% oil (Liu, 1997).
3. The protein and oil components in soybeans are high not only in quantity but also in quality. Soybeans are low in saturated fats and free of cholesterol (Aoyagi, 1979).

Soy protein contains all the amino acids needed by the body. It is also low in calories, but rich in minerals (iron and calcium). It also contains many minor substances known as phyto-chemicals.

4. Soybeans have versatile end uses, including human food, animal feed, and industrial material.

2.1.5 Nutritional Value of Soybeans.

Table 2.1 Nutritional value of Soybeans (per 100g).

Nutritional components	Quantity
Energy	432.0
Protein (g)	43.2
Fat (g)	19.5
Carbohydrate (g)	20.9
Calcium (mg)	240.0
Iron (mg)	10.4
Beta-carotene (mcg)	426.0
Thiamin (mg)	0.73
Riboflavin (mg)	0.39
Niacin (mg)	1.00

Source : From Srilakshmi (1996)

2.1.6 Problems of upgrading Soybean as a Human Food

Although, soybean is versatile, it has little direct use Because of,

- a high satiety value caused by high oil content.
- bitterness and poor digestibility.

- green beany flavor,
- presence of anti- nutritional factors that include trypsin inhibitors, haemoglutanins and phytates,
- long cooking time,
- Tough and chewy in texture.

2.1.7 Processing of Whole Soybeans.

In order to obtain full complement of nutrients in soybeans, it is necessary to eliminate or inactivate the undesirable factors associated with it. These factors include trypsin inhibitors, haemoglutanins and phytates. Other undesirable factors include the enzyme lipoxidase which is responsible for the beany flavor, unacceptable to Sri Lankan palates. Therefore, when processing soybean, it is very essential to inactivate lipoxygenase enzyme before it is allowed to develop beany flavor and to destroy all anti-nutritional agents during processing of raw soybean products.

Important Considerations in Soybean Processing

1. **Potential beany flavor development**

   Lipoxygenase enzyme + Lipid substrate + added water = Instant beany flavor

   Once this beany flavor is developed, it can not be eliminated

   In intact dry beans, these reactants are separated and no beany flavor is present. When bean tissue is broken or damaged, though enzyme and substrate in the tissue are exposed, still no beany flavor is developed as long as the tissue remains dry Addition of water, however, results in instantaneous beany flavor and off odor.

2. Anti – nutritional factors.

Most important factor is trypsin inhibitor which is the most heat resistant anti-nutritional factor. Destroying trypsin inhibitors through complete cooking, that indicate the destruction of all anti- nutritional factors in soybean during cooking.

2.1.8 Food Uses of Whole Soybeans.

Whole soybeans are processed and fermented into different kind of food items.

Fermented products are;

- Tempe
- Soy sauce
- Miso
- Natto

Processed products are;

- Soy beverage (milk)
- Cheese, curd, yogurt and ice cream
- Soy bean oil
- Soy protein products
- Weaning food
- Soy cereal snacks made by extrusion cooking

2.1.9 Current trend in Processing and Utilization of Soybean in Sri Lanka.

The main limitation in the use of soybean for human consumption is the lack of know how in processing methods. Cooking methods and products must be developed to suit the food habits and the local conditions.

Current researches are based on product development and extension in soybean processing. These two activities are carried out under two well defined areas of processing and utilization, namely, home and village level and commercial level (Gunasena and Herath, 1987). At present around 28.000 MT of soybean is required annually to service the major industries.

2.1.10 Occurrence of Aflatoxins in Soybeans.

Aflatoxins are one of main group of mycotoxin. It is highly toxic and carcinogenic metabolite. It is produced by strains of the fungus *Asprergilus flavus* and *A. parasiticus*. Occurrence of aflatoxin is very low in soybeans in comparison with other commodities (Bean *et al*,.1972).

Aflatoxins synthesis inhibition in soybeans was explained on the basis of zinc availability, which is low in amount and bound to phytic acid (Gupta *et al.* 1975). Zinc seems to play an important role in the biosynthesis of aflatoxin (Maygon *et. al.*, 1977). Stossel (1986) observed that seed coat integrity and low moisture content of harvest are responsible for the control of mold growth instead of zinc availability.

2.2 Tempe

Tempe is a popular Indonesian fermented (cultured) food consisting of tender-cooked soybeans bound together by a dense cottony mycelium of *Rhizopus* mold into compact white cakes. The product, which has a unique flavor, often described as "mealy" or "mushrooms".

2.2.1 History and Telecast of Tempe.

Tempe is originated hundreds of years ago in Central and East Java (Shurtleff *et. al.* 1979). It is produced widely in Indonesia and other countries in Southeast Asia. It is also manufactured commercially in the USA, Canada, the West Indies and the Netherlands and recently in the UK.

2.2.2 The Importance of Tempe

Tempeh is an important food use in developing countries as a source of tasty and inexpensive high quality protein. Because of;

1. Its commercial production requires only the simplest, decentralized, low-level technology.

2. The tropical climate characteristics of so many developing countries greatly facilitate the Tempeh fermentation.

3. The fermentation is short as compared with other fermented foods.

4. Tempe has a flavor, texture and appearance that are well suited to use in local cuisine throughout the world. It is an ideal meat substitute and extremely versatile.

2.2.3 Production and Utilization of Tempeh in Sri Lanka.

There are number of programs for utilization of soybeans. Among them, Large-scale food processing units, Small-scale food processing units and Nutritional intervention programs are important. These programs utilize soybeans to manufacture soy foods including Tempe, like fermented foods and other various types of non-fermented

products. With the help of "Soya Food Research Center" at Gannoruwa, researches on Tempe had more success and Tempe is gradually being accepted in wider scale.

2.3 Okara (Soy pulp).

Okara is an insoluble residue that remains after filtration of Soymilk. The okara has its own special uses and the soymilk is eventually made into Tofu. Therefore, it is considered a by-product of soy milk and tofu preparations.

Okara is beige in color. It has a crumbly, fine-texture, somewhat resemble freshly grated coconut. It is tasty and nutritious food, which serves as an important ingredient in traditional Japanese cuisine.

2.3.1 The proximate composition of okara

Proximate composition of okara is shown in Table 2.2

Table 2.2 Proximate composition of okara

Component	Quantity
Moisture	87.0
Protein	2.6
Fat	0.3
Soluble carbohydrate	7.6
Fiber	1.8
Ash	0.7

Source: From Liu (1999).

2.3.2 Nutritional Value of Okara.

The most important constituent of okara is "dietary fiber" and now it is considered to be an essential part of every well balanced diet. Not only, okara is a rich source of dietary fiber, it also contains a high quality of protein on dry-matter basis, contains 23.6- 24.0% protein and 8.1 – 15.2 % fat (Liu,1999).

According to the Liu (1999), the protein quality of okara is higher than any fractions in the tofu making process; the protein efficiency ratio (PER) value for okara was 2.71, dehulled soybeans had a PER of 2.51. The PER of okara was attributed to its higher content of cysteine, a limiting Sulfur- contain amino acid in the whole soybeans.

2.3.3 Food Application of Okara.

Basically, okara can be made into various types of foods, including salads, soups, sauces, baked goods and desserts. Some novel uses of okara by tofu manufactures in North America including soysages and okara burgers, all of which use okara as a key ingredient. Okara served as a pickle, or simply made into a dish with meat or vegetables. Also, okara can be made into a dry powder that use as a flavor ingredient.

With the growing awareness of the improvement of dietary fiber in human health, there is increasing interest in using okara as a food ingredient.

2.4 Okara Tempe

The Tempe, which is made from okara (soy milk residue) as major ingredient instead of whole soybeans known as "Okara Tempe" that is also a compact white cake, fermenting by the mold *Rhizopus* species with its dense cottony mycelium.

2.4.1 Role of Ingredients.

2.4.1.1 Okara - Okara which is the key ingredient of okara Tempe. It provides both substrate and nutritional components for proper growth of Tempe inoculum.

2.4.1.2 Tempeh starter or Inoculum – Inoculum is another major ingredient used in Tempe. *Rhozopus oligoporus* was tentatively identified as the principal mold species used for the Tempe fermentation.

The main external function of *R. oligosporus* is to bind together the particles of the substrate into compact cake and cover the cake with its attractive, white and velvety mycelium in not more than 20 hours. The growth is also very dominant and does not give too much chance for other microorganisms to grow properly. So far, there are no reports concerning the toxicity or infectivity of Tempe made with *R. oligosporus*

(Hermana *et al.* 1990). This means that the mold makes Tempe, which is very safe for the consumers.

The main biological function is to convert the components of the *raw* material into a more palatable and digestible food. For that *R. oligosporus* produces extracellular enzymes such as amylase, protease and lipase and increase soluble substances during Tempe fermentation.

2.4.1.3 Acidulants – Acid ingredients that used in food processing create an acidic condition to reduce the pH of a given product and subsequently inhibit the microbial growth. In Tempe making, either Citric acid or Acetic acid (vinegar) can be used as microbial inhibitors.

Generally, all microorganisms have a range of pH over which growth may occur, and an optimal pH for high growth rate. Usually it is considered that bacteria will grow best at pH of 5.5 to 7.0. Yeast and mold will grow well at low pH values.

Acidification has a number of important role on the process (Gurbertt, 1997):

reduce the number of potentially harmful Enterobacteriaceae that may contaminate the original beans;

prevent the growth of *Bacillus* species that may cause spoilage problems;

produce an environmental pH that is ideal for rapid growth of the fungus in fermentation process.

2.4.1.4 Rice Flour – Rice flour is added as an additional ingredient to enhance the nutritional quality and improve the texture of the final product.

2.4.2 Different Strains of Culture available for Tempeh Fermentation.

Different mold species have been reported in the literature as the microorganisms responsible for Tempe fermentation. The essential microorganisms in theTempe fungal fermentation are a mold belonging to the genus *Rhizopus*. There are at least four species of *Rhizopus* capable of producing Tempe, namely *R. oligosporus, R. stolanifer, R. oryzae, and R. arrhizus.*

2.4.2.1 ***Rhizopus oligosporus*** – It has unique characteristics, which make the food more valuable than it already is. *R. oligosporus* shows the following unique features;

1. Ability to grow rapidly at temperatures from 30^0 to 42^0 C. Many molds will not grow at such high temperatures and thus growth of *R. oligosporus* is favored.
2. Inability to ferment sucrose.
3. High lipolitic activity.
4. High proteolitic activity.
5. Production of strong anti-oxidants.
6. Ability to grow on wheat or other starch cereal substrate without producing noticeable amounts of organic acids that would cause sour product.
7. Ability to produce the typical Tempe cake with its characteristic aroma and flavor.
8. Produces an antibacterial compounds which are especially active against some Gram positive bacteria species.
9. Does not produce aflatoxin.

2.4.3 Fermentation of Okara Tempeh.

Production of okara Tempe involves a two – stage of fermentation. The first, which occurs during hydration (soaking) of soybeans and results in acidification of the beans. It is a bacterial fermentation. The second fermentation is fungal and results in overgrowth of the okara by the mold mycelium.

Stage 1 - During the period in which the beans are soaked in water, an acid fermentation takes place. Organisms that naturally contaminating the soybeans ferment sugar, extracted from the beans during soaking to produce acid. The pH of the beans falls to a range of 4.5 to 5.3. Lactic acid bacteria, Enterobacteriaceae, *Bacillus* species and yeast have been isolated from the soak liquid (Gurbertt, 1997).

Stage 2 – When preparation of Tempe, Okara is inoculated with the Tempe starter, then pressed into a thin cake and incubated in suitable containers. During incubation, fungal mycelium grows rapidly, invading the compressed okara and binding them into compact cake by white mycelium.

Mold grows has a major influence on the texture and the nutritional characteristics of the okara. There is considerable protein hydrolysis and break down of fats with the production of free fatty acids. Mold growth also causes a rise pH from 5.0 to 6.5 – 7.0.

2.4.4 Factors affecting for the Okara Tempeh Fermentation.

For a good Tempe fermentation, two conditions should be fulfilled. These are:

The raw material (okara) need to be bound together into compact cakes by mold mycelia, and

The substrate (okara must undergo a partial digestion by mold enzyme (Liu, 1999)

Therefore, the Tempe is centered in the mold growth. Any factors,which are affecting the mold growth also affect Tempe making.

The affecting factors are;

1. **Tempeh Starter (Inoculum)** – The availability of an appropriate starter is essential for producing a good quality Tempe. The amount of inoculum required to make a satisfactory Tempe is important. Fermentation time becomes too critical if the amount of inoculum is larger. How ever, too small amount of inoculum may provide a change for growth of contaminating bacteria.

2. **Aeration** – *R. oligosporus* does not require much aeration, as do many other molds. Too much aeration should be avoided, because excessive aeration causes dehydration of okara surface and sporulation.

When making Tempe from okara, other than soybeans, providing of satisfactory aeration is critical. Because, the spaces between okara mass are considerably less than soybeans. Therefore, packing of okara into containers after inoculation with Tempe mold should be done gently to facilitate optimum aeration.

3. **Moisture** – Tempeh fermentation is an example of solid substrate fermentation in the absence of free water. Therefore, relative humidity (R.H) as well as absorbency of the substrate determine the moisture content of the substrate. In general, high R.H and good absorbency of the substrate are absolutely needed for the proper fermentation.

4. **Temperature** – The temperature slightly above room temperature is best for Tempe fermentation. Incubation takes place at temperatures from 25° C to 37° C. The higher the incubation temperature stimulates the more rapid growth of Tempe mold.

5. Acidity – Final pH of 4.5 – 5.5 prevents development of undesirable bacteria that might spoil the

2.4.5 Changes Occurring in Okara Substrate during Fermentation.

Many aspects of changes take place during fermentation, including temperature, pH and chemical composition of the substrate. All of these are brought about by microbial growth and enzyme actions.

Changes in Protein – Although, the total protein content as well as the amino acid pattern remains relatively unchanged (Murata *et al.* 1967). There is a distinct increase in the amount of free amino acids during Tempe fermentation.

Changes in Lipids – The mold possesses a strong lipolitic activity. The fatty acids are liberated during fermentation and there is an increase in free fatty acids.

Changes in Carbohydrates – During Tempe fermentation, *R. oligosporus* produced polysaccharidases, and water activity played an important role in the production of these enzymes (Liu, 1999).The principal changes in carbohydrates are the rapid removal of hexoses (soluble carbohydrates without sucrose) and the slow hydrolysis of stachyose.

2.4.6 Nutritional Quality of Okara Tempeh.

During short fermentation of Tempe, *R. oligosporus* brings about a total transformation of the substrate. It transforms okara or soymilk residue into highly digestible and palatable food. The fermentation process enhances the nutritional value of the finish product by increasing the digestibility of protein, reducing the anti-nutritional factors and enriching it with vitamins.The protein content of fresh Tempe is about 4- 5 %.

Tempeh has low fat content and no cholesterol. It is very low in saturated fatty acids and high in unsaturated fatty acids that are linolenic and linoleic acids. Hermana *et al.* (1990) reported that Tempe is a good source of vitamins (especially B vitamins,

which the Tempe mold can synthesize) and minerals. Fresh okara Tempe contains 225 g of Calcium and 1.4 g of Iron (Aoyagi *et al.* 1979).

.It is a rich source of dietary fiber. According to modern nutritional theory, dietary fiber promotes good health and cleaning the digestive tract. High- fiber diets also lower the serum cholesterol.

2.4.7 Medicinal and Antibiotic Effects of Okara Tempeh.

Recent scientific experiments have shown that Tempe has medicinal and antibiotic properties. *Rhizopus* mold produces natural heat stable antibacterial agents that act as antibiotics against some disease causing organisms. Tempeh' anti-bacterial agents inhibit the growth of some bacteria, especially gram-positive types such as *Stapylococcus aureus*, a food-poisoning bacterium; hence, it may be possible to increase the body's resistance to infection by eating Tempe.

2.4.8 Vegetarian Source of Essential Vitamin B 12

A non-pathogenic strains of the bacterium *Klebsiella pneumoniae* has a ability to produce the vitamin B12. This organism is found as a predominate bacterium in Tempe and which grows alongside with the Tempe mold. The growth of the bacterium does not apparently interfere with growth of Tempe mold. Therefore, Tempe is important to serve as an essential source of vitamin B12 in a vegetarian diet.

2.4.9 Physico-Chemical Properties of Okara Tempeh.

Okara Tempe has higher water holding capacity and oil retention value than okara powder and wheat flour. Emulsifying capacity of okara Tempe was higher than that of other powders. This may be caused by a high capacity of water and oil retention. Cellulose, xylan and lignin, which are high in oil retention value and emulsifying capacity, consist of hydrophobic polysporus substances. Thus, high value of oil

retention and emulsifying capacity of Okara Tempe is considered to be common to substances, which are hydrophobic, and polysporus. These properties of okara Tempe are applicable as a food stuff.

2.5 Comparison in Composition between Okara and Okara Tempeh.

There are remarkable differences between okara and okara Tempe. Contents of crude protein, crude fat, and carbohydrate were not different between okara and okara Tempe, but total fiber content was slightly lower in okara Tempe than okara. Six- fold increase in acid soluble Nitrogen/ total Nitrogen ratio, 3.5 fold increase in free fatty acids/ crude fat ratio, 1.5 fold increase in free sugars/ carbohydrate ratio and 25 fold increase in inorganic phosphate/ total phosphate ratio were observed in okara Tempe compared with okara (Hermana, 1999).

An increase in inorganic phosphate/ total phosphate ratio seems to be resulted from degradation of phytin that inhibits the absorption of protein, calcium, iron, copper etc., through forming a chelator. According to these results, availability of these essential nutrients is increased by ingesting okara Tempe.

2.6 Getting a Good Tempeh.

In a finish product of okara Tempe that the okara are bound together into a firm, compact cake by a dense, uniform mycelium of white mold. It should have a pleasant, clean aroma, somewhat like that of fresh mushrooms. The cake should be firm enough so, that it can be held horizontally by one end without bending or breaking, and thinly sliced pieces should together well without crumbling.

2.7 Storing and Preserving of Okara Tempeh.

Tempeh is a perishable product that has its best flavor and texture when served fresh. A number of traditional and modern methods have been developed to store it with only small losses in quality.

Heat or cold is used to retard or inactivate the enzymes and kill the mold. The flavor and texture change slightly during storage, but it is not yet clearly understood. Improvements of these methods are important for the development of large – scale production and marketing. During storage, the anti- oxidants in dried Tempe help prevent it from becoming rancid.

Refrigeration, freezing, parboiling and freezing, storing at air temperature, deep-freezing, sun drying, canning and freeze- drying (lyophilization) are the available methods for storing and preservation of okara Tempe.

2.8 Microbiological Problems Associated with Okara Tempe Processing.

If acidulants act as successful microbial inhibitors and follow the proper hygienic practices, that will lower the spoilage problems. Sporulations of the mold takes place when too much of oxygen gains that access the blackening of the product surface with poor appearance. Therefore, sporulation affects the consumer acceptability. Yellowing of the product could be experienced in some situations. It may be a result of production of pigments by some other mold species that contaminate with rice flour. That problems can overcome, using sterilized flour.

When the product becomes over ripe, ammonia gas is released as a result of protein breaks down. It is also undesirable condition. Tempe mold produces an antibiotic effect against some bacteria that successfully contributes to minimize the microbial problems in okara Tempe.

2.9 Basic Principals of Cooking of Okara Tempeh.

Like meat, fish, chicken, etc. there are several principals of cooking Tempe.

Frying – Frying is a most popular cooking method of Tempe. Either shallow or deep frying can be practiced. Fat is used as a medium for cooking. Deep frying is very commonly practiced in cooking of Tempe, because it imparts a delicious rich and savory flavor. In deep-frying, oil is heated to 175^0 C (350^0 C) in a deep fryer, then sliced Tempe is dropped into and fry for about 3 minuets.

Baking or Grill – Hot air is used as a cooking medium. In here, food stuff is put on the fire.

Steaming – A method of cooking. Steam is used as a cooking medium. This process is used in many recipes that do not call for baking or frying. Tempeh slices place on a rack over the water in pre-heated steamer and steam for several minutes.

Boiling – A lot of water is used as a cooking medium, similar to that of making soup.

Stewing – Small quantity of vegetable oil is used, heat slowly and mix with water or coconut milk.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Production of okara Tempe

1. Okara (Soy milk residue)
2. Temp starter(*Rizophus oligosporus*)
3. Rice flour
4. Food grade Citric acid or Acetic acid (Vinegar)
5. Polyethylene
6. Sealer
7. vessels
8. Steamer
9. Electrical oven at 60^{0}C
10. Stainless steel trays
11. Muslin cloth
12. Spoons

3.1.2 proximate Analysis

3.1.2.1 Determination of moisture

3.1.2.1.1 Apparatus

1. Metal dish with a lid
2. Electrical oven at 105^{0}C
3. Desiccator
4. Electrical balance

3.1.2.2 Determination of Total Fat

3.1.2.2.1 Apparatus

1. Beaker
2. Hot water bath
3. Bottom flatted round flask
4. Electrical balance
5. Flask (Separating Funnel)

3.1.2.2.2 Reagents

1. 95% Ethanol
2. HCl Solution
3. Ether
4. Petroleum ether

3.1.2.3 Determination of Protein

3.1.2.3.1 Apparatus

1. Kjeldhal tube
2. Kjeldhal tablet
3. Kjeldhal apparatus
4. Distillation unit
5. Titration flask
6. Electrical balance

3.1.2.3.2 Reagents

1. Distilled water
2. Conc. Sulfuric acid

3. 0.04M HCl
4. 40% NaOH solution
5. $Na_2S_2O_3$
6. 5% Boric acid
7. Methylein blue and methylein red

3.1.2.4 Determination of Crude fiber

3.1.2.4.1 Apparatus

1. Electrical oven at 105°C
2. Reflux condenser
3. Buchner funnel.
4. Muffle furnace at 500°C
5. Crucible
6. Electrical balance

3.1.2.4.2 Reagents

1. Conc. Sulfuric acid (0.0128 mol/l solution)
2. Ethanol 95%(v/v)
3. Conc. Sodium hydroxide.

3.1.2.5 Determination of Ash

3.1.2.5.1 Apparatus

1. Silica dish
2. Bunsen flame in fume cupboard
3. Electrical balance
4. Muffle furnace at 500°c

3.1.3 Sensory Evaluation

3.1.3.1 Apparatus

1. Tempeh made from okara.
2. Tempeh made from soybeans
3. Cream crackers
4. Spoons
5. Water glasses
6. Ballet papers
7. Pens
8. Dishes

3.1.4 Handling of the Product for Microbiological Analysis

3.1.4.1 Coliform Test

3.1.4.1.1 Apparatus

1. Test tubes
2. Durham tubes
3. Pipettes
4. Autoclave
5. Incubator
6. Cotton plugs
7. Aluminum foils
8. Petri dishes
9. Parafilm

3.1.4.1.2Reagents

1. Brilliant Green bile broth
2. Distilled water

3. Prepared okara Tempe

3.1.4.2 Total plate count

3.1.4.2.1 Apparatus

1. Electronic balance
2. Mixer (Vortex mixer)
3. Electrical oven at 175^{0}C
4. Autoclave at 121^{0}C for 20 minutes
5. Incubator at 30^{0}C
6. Colony counting equipment (Dark field Quebec colony counter)
7. Petri dishes
8. Pipettes
9. Test tubes
10. Flasks

3.1.4.2.2 Reagents

1. Nutrient agar
2. Distilled water
3. Prepared Okara Tempe
4. Diluent (Peptone, NaCl, distilled water)

3.1.4.3 Yeast and Mold count

3.1.4.3.1 Apparatus

1. Electrical balance
2. Mixer (Vortex mixer)
3. Electrical oven at 170^{0}C
4. Autoclave at 121^{0}C for 20 minutes
5. Incubate at 25^{0}C
6. Colony counting equipment (Dark field Quebec colony counter)

7. Petri dishes
8. Flask
9. Test tubes
10. Graduated pipettes

3.1.4.3.2 Reagents

1. Yeast extract dextrose
2. Distilled water
3. Diluent (peptone, distilled water, NaCl)

3.1.5 Determination of pH value, Temperature, Formal value and Acid value

3.1.5.1 Determination of pH value

3.1.5.1.1 Apparatus

1. pH meter (Hanna, HI 8519)
2. Electrical balance
3. Pestle and motor
4. Muslin cloth
5. Beakers
6. Measuring cylinder

3.1.5.1.2 Reagents

Buffer solution

Distilled water

Prepared okara Tempe

3.1.5.2 Determination of Temperature

3.1.5.2.1 Apparatus

1. Thermometer

3.1.5.2.2. Reagents

1. Prepared okara Tempe

3.1.5.3 Determination of Acid Value

3.1.5.3.1 Apparatus

1. Burette with a stand
2. Titration flasks
3. White tile
4. Hot water both
5. Thermometer

3.1.5.3.2 Reagents

1. 0.05M NaOH solution
2. Phenolphthalein indicator solution
3. Distilled water

3.1.5.4 Determination of Formal value

3.1.5.4.1 Apparatus

1. Graduated pipettes
2. Titration flasks
3. White tile
4. Burette with a stand

3.1.5.4.2 Reagents

0.1M NaOH solution

40% Formaldehyde solution

Phenolphthalein indicator solution

Distilled water

3.2 Methodology

3.2.1 Preparation of Okara Tempe

Selected dry, quality soybeans were cleaned to remove damaged beans, and any other extraneous matter. Then cleaned beans were dried in the sun and passed through a properly speed burr mill for the dehulling. The hulls were blown off and soy splits were soaked in water:bean ratio of about 1 : 1, for over night at room temperature. These soaked splits were rinsed with clean water, followed by grinding while adding small quantities of hot water: beans ratio of about 9:1. The resulting slurry is then filtered through cheese cloth to separate soy-milk, and the residue, known as soy pulp or okara, that is used as major ingredient to make "Tempe".

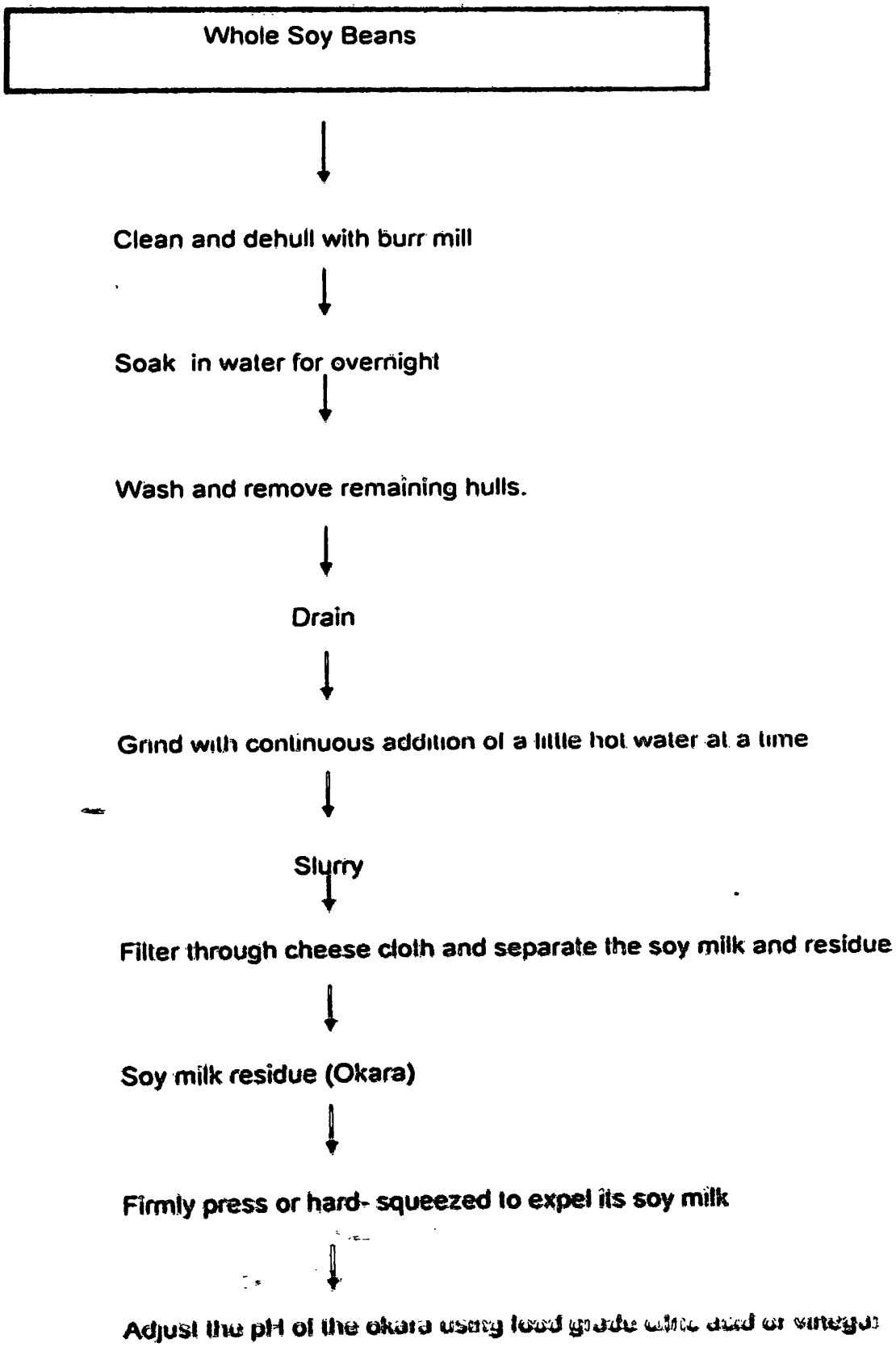
Coarse-textured okara which was firmly pressed or hard - squeezed to expel its soy-milk (reducing the moisture content by 80%) and impart a light crumbly texture.

Then okara was placed in a (Cloth-lined) preheated steamer, and steamed for 20 minutes to sterilize it, then it was re-pressed to expel excess moisture.

The pH of the okara substrate was adjusted to about 5.4 or 4.5 using food grade citric acid or vinegar, and allow to cool to body temperature before incubation.

The sterilized okara was inoculated with spores of *R. Oligosporus* , packed into pored polyethylene bags with perforations at 2.5mm intervals and sealed, spread 10mm thick, and incubated at room temperature for 36-48 hours in a Tempe racks.

The process flow for okara Tempe preparation is shown in figure 3.1



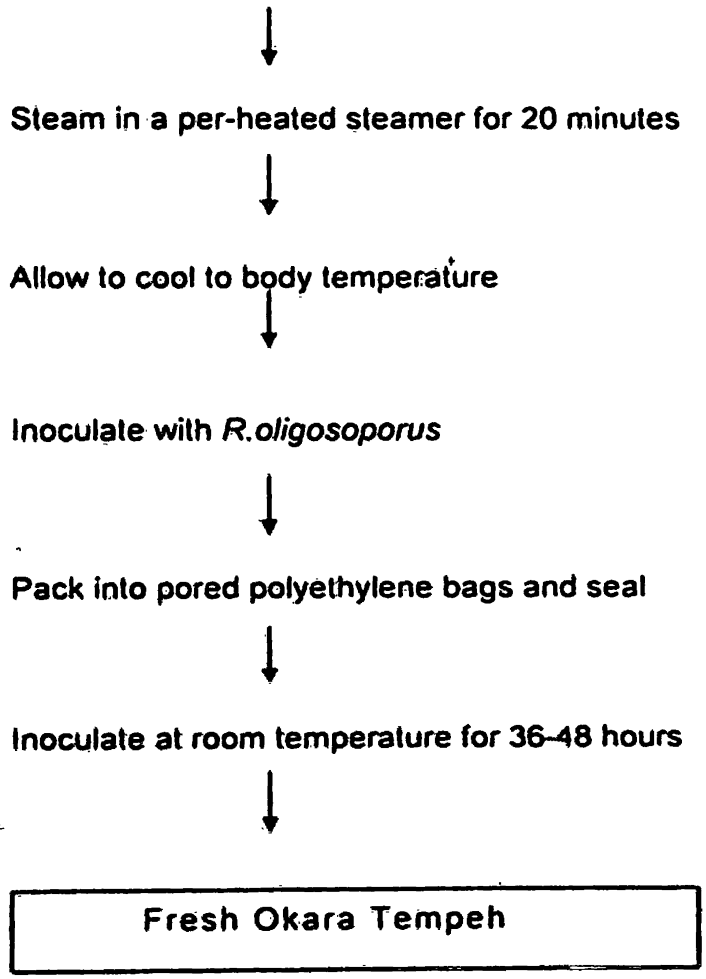


Figure 3.1 Process flow for Okara Tempe Preparation

Plate 3.1 Major ingredients and packets of fresh Okara Tempe.

3,2.2 Proximate Analysis

3.2.2.1 Determination of moisture

The metal dish, dried in an oven and cooled in a desiccator was weighed by using an electrical balance. Five grams of the sample was placed on weighed dish (W1) and weighed using the balance (W2). This was put an oven maintained at 105°C for 3 hours, cooled in a desiccator to room temperature and weighed. Drying, cooling, Weighing were repeated until a constant weight (W3) obtained.

Calculation

$$\text{Percentage of moisture (dry basis)} = \frac{(W2-W3) \times 100}{W_3-W_1}$$

3.2.2.2 Determination of Fat

Sample of okara Tempe was weighed (W) and placed in 50ml beaker. Then 2ml of 95% ethanol and 10ml of HCl (Prepared by adding 25ml of Conc. HCl and 1ml of water) were added and mixed thoroughly. The mixture in beaker was placed in a hot water both at 70° to 80°C and frequently stirred for 30 minutes.The beaker was washed with three portions of 25ml of ether and added onto the pre-weighed dry flask (w1). The flask was stopped with a cork and shaken vigorously for few minutes and also with 25ml of petroleum ether. The flask was stood until a clear layer of pet ether appeared. The upper layer was taken into a clean, dry previously weighed flask by using a pump. The content was dried in a water bath at 90°C until a constant weight (W2) was obtained.

Calculation

$$\text{Percentage of total fat} = \frac{(W2-W1)}{W}$$

3.2.2.3 Determination of Protein

Weighted de-fatted sample (W) was carefully transferred into a cleaned, dried kjeldhal digestion flask. One kjeldhal tablet, which contained Selenium and 10ml of Conc. sulfuric acid to added to it. The flask was connected to the fume trap and attached it to the pump. The flask was heated for 4 hours for digestion process and then allowed to cool for 1 hour.

The sample was dissolved with 15ml of ammonia free distilled water and transferred it into a semi-micro kjeldhal distillation apparatus which has been previously conditioned by passing steam through it for several minutes. Then distillation was done after adding 8ml of $NaOH/Na_2S_2O_3$ solutions to the flask. 4% of boric acid solution and 3 drops of indicator were taken into a titration flask and kept it at the end of the digestion apparatus. The resulted solution was titrated with concentration (C) of 0.1m HCl solution. The end point was in pink color. The used HCl volume (V) was recorded.

Calculation

$$\text{Percentage of Nitrogen (N)} = \frac{V \times C \times 100}{W}$$

Percentage of protein = Nitrogen x 6.25

3.2.2.4 Determination of crude fiber

W1 amount of defatted, dried sample was transferred into boiled 200 ml of 0.128 mol l^{-1} Sulfuric acid continued boiling for one minute. The sample was poured into the funnel and washed with boiling water until the washings were no longer acidic to Litmas papers. The sample was added into the flask that containing boiled 200 ml of 0.128 mol l^{-1} Sodium hydroxide and boiling was continued for one minute. The boiled sample was washed with distilled water until alkalinity was removed. The residue was transferred into boiling water and transferred into the crucible. Then the residue was washed with 15 ml ethyl alcohol. Oven drying, cooling in a desiccator and weighing were repeated until a

constant weight (w3) was obtained. The crucible was transferred in the furnace and the content of crucible was incinerated. Then the sample was cooled in a desiccator and final weight (w2) was obtained by using an electrical balance.

Calculation

$$\text{Percentage of crude fiber} = \frac{(W3 - W2) \times 100}{W1}$$

3.2.2.5 Determination of Ash content

5.00 g of the sample (W1) was placed in a weighed (W2) clean and dry silica dish. It was ignited slowly over a Bunsen flame until no more fumes evolved. The dish was transferred to the muffle furnace set at 500°C. The content was in cinerated for 3 hours until sample was free from back carbon particles. The process of igniting was done in the muffle furnace, cooling in a desiccator and weighing was repeated until a constant weight (W3) was obtained.

Calculation

$$\text{Ash content (\%)} = \frac{(W3-W2) \times 100}{W1}$$

3.2.3 Determination of pH value, Temperature, Formal value and Acid value

Inoculated okara substrate with *R. oligosporus* was allowed to ferment, and changes in temperature were recorded at the time of incubation and at 4 hours intervals for 48 hours.

3.2.3.1 Determination of pH value

Inoculated okara with *R. oligosporus* was allowed to ferment. A 5g of sample was withdrawn at a time from the time of incubation and at 4 hours intervals for 48 hands, for determination of pH value variations.

The sample was put in 45ml of distilled water and mixed in a blender for 1 minute. The mixture was filtered through muslin cloth to remove coarse materials. The pH of the filtrate was determined using an Acid-base analyzer type pH meter, equipped with glass electrodes.

3.2.3.3 Determination of Temperature

The temperature variations of inoculated mass were recorded using a thermometer. The thermometer was carefully inserted into the inside of the sealed Okara Tempe packets and observations were taken at 4 hours intervals for 48 hours.

3.2.3.3 Determination of Acid value

Inoculated okara with *R. oligosporus* was allowed to ferment. Samples were withdrawn at the time of incubation and at 4 hours intervals for 48 hours. Then samples were blanched in boiling water to inactivated enzyme activity. Samples were dehydrated at temperature below 50°C using a hot air circulation laboratory drier.

18g (dry basis) of the sample was put in 200ml of distilled water and mixed in a blender for 1 minute. This mixture in a conical flask was placed in a hot water bath for 1 hour with periodic agitation. This was filtered. 100ml of clean filtrate was titrated with 0.05 M NaOH solution after adding three drops of phenolphthalein, as the indicator. The titrated amount was noted at the end point where color changed from colorless to pink color.

Calculation

$$\text{Acid value} = \frac{\text{Titrated 0.05M NaOH amount} \times 5.61}{\text{Weight of the sample used}}$$ (Pearson.D, 1976)

3.2.3.4 Determination of Formal value

Samples were prepared according to the procedure given in determination of acid value in 3.2.3.3.

18g (dry basis) of the prepared sample was put in 200ml of distilled water and mixed in a blender for 1 minute. This mixture in a conical flask was placed in a hot water bath for 1 hour with periodic agitation. This was filtered. 100ml of clean filtrate was taken into a stopped conical flask by using a 10ml pipette. Few drops of phenolphthalein were added and then it was titrated with 0.1M NaOH to rose pink. 5ml of 40% formaldehyde was also neutralized to the same tint with 0.1M NaOH solutions were mixed in the stopped conical flask and allowed to stand for 5-10 minutes. When pink color has disappeared, titrated back with 0.1M NaOH and phenolphthalein, to the same tint. The amount of 0.1M NaOH solution required for the final titration was only recorded.

Calculation

Formal Value = Amount of 0.1M NaOH solution (in ml), required for the final titration Only.

3.2.4 Sensory Evaluation of the Okara Tempe

Sensory evaluation of okara Tempe was performed by 20 panelists of the Food Research Unit. They were asked to mention the degree of likeness for okara Tempe and soybean Tempe. The two samples were cooked according to same manner. The two samples used for comparison were coded with three digit random numbers.

581- Okara Tempe

716- Soybean Tempe

Panelists were assigned two samples for evaluation. They were provided with a ballet paper, which is given at Appendix 1.

3.2.5 Handling of the product for Microbiological Analysis

3.2.5.1 Coliform Test

- Preparation of single - strength Brilliant green bile broth.

- The Durham tubes were introduced in all tubes and were sterilized after filling the tubes with culture medium without air bubbles. All sets of tubes were sterilized in an autoclave at 121°C, is plsg for 20 minutes. The test tubes were covered with cotton plugs and aluminum foil before sterilized in an autoclave.

- 1ml of the prepared okara Tempe sample was inoculated at 37°C were examined the gas formation in the Durhum tubes at 24 hours and 48 hours.

3.2.5.2 Enumeration of Yeast and molds

- Diluent was prepared by dissolving the components in the water, adjusted the pH. 9ml of Diluent was dispensed into flask and sterilized in an autoclave at 121°C, 15 psig for 20 minutes.

- Preparation of culture medium and that was dispensed into culture flasks and sterilized at 121°C, 15 psig for 20 minutes.

- Preparation of the test samples.

- 2 sterile petri dishes were taken. Using a sterile pipette, 1ml of the test sample was transferred to each dish.

- Two other petri dishes were taken using a fresh sterile pipette, 1ml of the first dilution (10^{-2}) of the initial suspension was transferred to each dish.

- Repeated the procedure with other dilution using a fresh sterile pipette for each dilution.

- About 15ml of yeast-extract-dextrose-chloromphenical agar medium was poured into each petri dish.

- The inoculum with the medium were carefully mixed and allowed to solidify placing the petri dishes on a clean horizontal surface.

- The dishes were incubated at 37°C, counted the yeast and mold colonies on each plate after 3, 4 and 5 days of incubation.

- The obtained data were calculated according to the Sri Lanka standards No 393.

Calculation

$$N = \frac{\Sigma}{(n1 + 0.1\ n2)\ d}$$

Where;

Σ = is the sum of colonies counted on all plates

n1 = is the number of plates retained in the first dilution

n2 = is the number of plates retained in the second dilution

d = is the dilution factor corresponding to the first dilution.

3.2.5.3 Enumeration of Total Plate Count Technique

The total plate count technique for the product was carried out according to the Sri Lanka standards for the product concerned and SLS 393.

Calculation

Calculation was also done according the method given in SLS 393.

CHAPTER 4

RESULTS AND DISSCUISION

4.1 Preparation of Okara Tempe

Soy milk residue or Okara that is the by-product of the tofu industry was major ingredient of the developed Tempe by extending the usefulness and nutritional quality of Okara.

Soaking step involves to reduce the anti-nutritional factors and acid fermentation is also taken place. During bacterial acid fermentation the pH of the bean falls to a range of 4.5 to 5.3.It does prevent the development of undesirable bacteria that might spoil the Tempe.

Pre-cooking under steam pressure makes Okara a very sterile substrate and develops a slightly better flavor and texture. The quality of the final product improves nutritionally, functionally and organoleptically by destroying enzymes and harmful microorganisms.

Moisture content and pH value of the Okara substrate and hygienic practices play important roles in making okara Tempe, rather than soybeans Tempe. Moisture content of the Okara substrate after re-pressing should be about 43%. Therefore, it is important to reduce the moisture level as well as possible by dehydration in the drier at 60^{0}C for about 1 hour. But the problem is that it increases cost of the product. The pH of the product should be about 4.5 to 5.00. Citric acid or vinegar can be used as Acidulants. The acid will prevent deterioration due to bacteria.

Basically, three factors are responsible for the manufacturing of Tempe from okara. Firstly, Okara should be made suitable for the growth of Tempe mold. Secondly, a proper starter should be added to the processed Okara and lastly, proper environmental conditions should be provided for the growth of mold on the substrate. The shelf-Life of Okara Tempe is limited to one to two day period day.

4.2 Proximate Analysis

These nutritional data should be absolute due to personnel errors and instrumental errors, which may have occurred.

4.2.1 Determination of Moisture

Weight of the dish (W 1)	= 106.28 g
Weight of the sample with the dish (w2)	= 111.28 g
Constant weight of sample with the dish (w3)	= 107.56 g

The mean value for moisture content is 74.2 %.

4.2.2 Determination of Fat

Weight of sample (W)	= 5.00 g
Weight of dry flask (w1)	= 136.78 g
Constant weight of fat with flask (w2)	= 136.92 g

The total fat percent is 2.8.

4.2.3 Determination of Protein

Weight of the de-fatted sample (W)	= 10.863 g
Volume of 0.1 M HCl titrated (V)	= 0.85 ml
Concentration of HCl (C)	= 0.1 mol/ l
Percentage of Nitrogen	= 0.782

The protein percentage is 4.890

4.2.4 Determination of Crude fiber

Weight of the de-fatted sample (W_1)	= 5.0211 g
Weight of fiber + organic matters + crucible (w3)	=18.244 g
Final constant weight of fiber with crucible (w2)	= 18.033 g

The crude fiber percentage is 4.202

4.2.5 Determination of Total Ash

Weight of the sample (w1)	= 5.001 g
Weight of silica dish (w2)	= 16.756 g
Constant weight of dish with (w3) Ash	= 16.82 g

The total ash percentage is 0.919

4.3 Determination of changes in pH value, Temperature, Acid value and Formal Value during Fermentation of Okra Tempeh

4.3.1 Changes in Temperature, during fermentation of okra Tempeh

Temperature variations of inoculated okara mass were recorded for 48 hours at 4 hours of intervals.

- Temperature changes during Okara Tempe fermentation were shown in Table 4.1

Table 4. 1 Chahges in temperature during fermentation of Okara Tempe

Time (hrs)	Temperature (^{0}C)
4	26.0
8	24.0
12	26.5
16	27.5
20	32.0
24	36.0
28	34.0
32	30.5
36	29.5
40	29.5
44	31.0
48	31.5

- Changes in temperature during fermentation of okara Tempe are shown in fig. 4.1

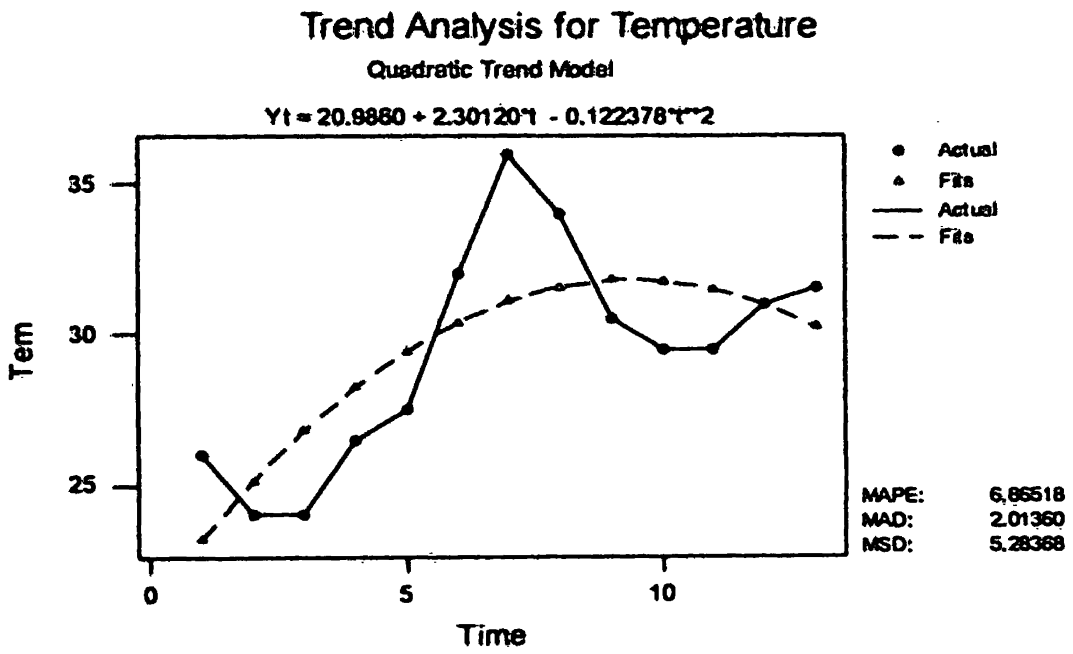


Figure 4.1 Changes in temperature during fermentation of Okara Tempe.

As shown in the figure 4.1, during incubation, the period from zero hour to 10th hour, there has been a stationary phase. Subsequently, there has been an increasing temperature from 27^{0} C to 35^{0} C from the 10 th hour to 24 th hour. Beyond this, there has been a sharp decrease in the temperature from 36^{0} C to 29.5 ^{0}C in about 12 hours.

The first phase is characterized by the germination of the spores of the *R. oligosporus* and the initiation of the growth of mycelia. The second phase was characterized by the rapid multification of the fungus. At the peak, the temperature reached as high as 35^{0}c – 36^{0}c. The increased rate of respiration, most probably could have contributed to the rapid increased in temperature. By this time, the okara substrate is already knitted into a compact mass by mold mycelia and the Tempe is ready to be harvested.

Variation of temperature was analyzed using Time series analysis. The fitted trend line was not good for the temperature variation. When Tempe mold grows on okara substrate, the temperature fluctuations taken place.Therefore, good fitted trend line could not obtain for the temperature variation.

4.3.2 Changes in pH during fermentation of Okara Tempe.

- Changes in pH values are shown in Table 4.2

Table 4.2 Changes in pH values during fermentation of Okara Tempe

Time (hrs)	pH value
4	5.5
8	6.94
12	7.04
16	7.04
20	7.10
24	7.19
28	7.26
32	7.25
36	7.45
40	7.56
44	7.70
48	7.84

- Changes in pH value, during fermentation of okra substrate are shown in fig. 4.2

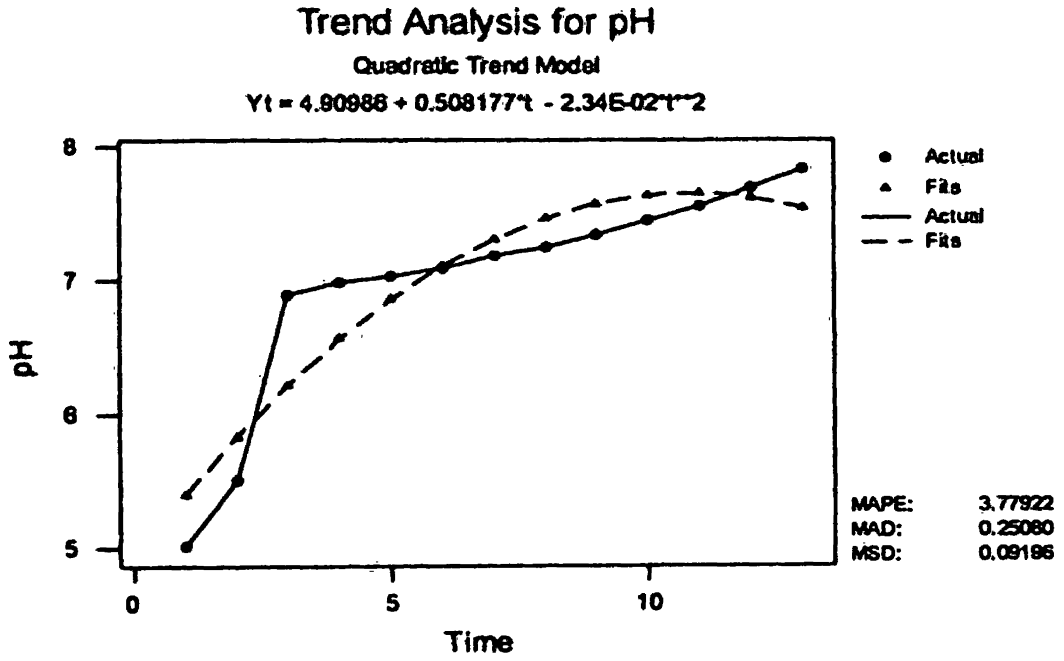


Figure 4.2 Changes in pH values during fermentation of Okara Tempe.

During the entire fermentation, the pH rises from 5.5 at the initial stage to 7.9 at the final stage. The data of pH values showed an increasing of pH during the second stage of Tempeh fermentation (Fig. 4.2).

It seems that deamination processes by mold and bacteria are quite active and able to increase the pH of Tempe. Mainly this process caused the spoilage of Tempe. The optimum pH for good quality Tempe is in the range of 6.3-6.5.

4.3.3 Changes in Formal Value during fermentation of Okara Tempe

- Changes in formal values are shown in Table 4.3

Table 4.3 Changes in Formal value in during fermentation of Okara Tempe

Time (hrs)	Formal value
8	0.0500
12	0.0550
16	0.0600
20	0.0625
24	0.0675
4	0.0425
28	0.0700
32	0.0750
36	0.0775
40	0.0826
44	0.0900
48	0.125

Formal value data show an increasing percentage (Fig. 4.3). It proves that may be the proteolytic activities of bacteria are high and the deaminations processed have increased.

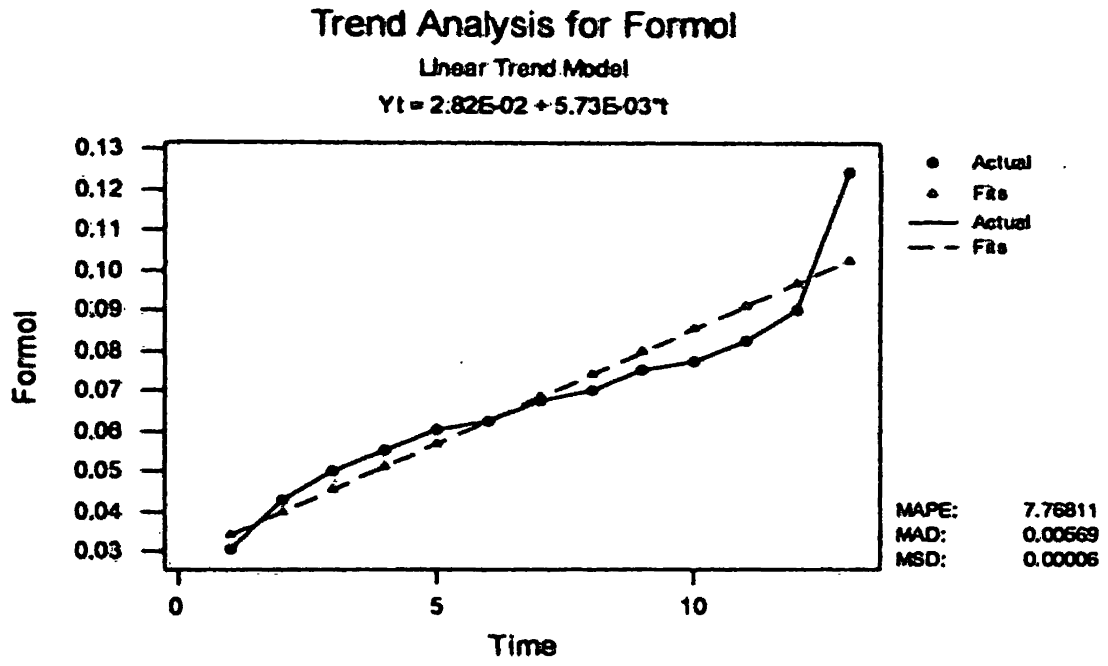


Figure 4.3 Changes in Formal values during fermentation of Okara Tempeh

4.3.4 Changes in Acid value during Fermentation of Okara Tempe

Table 4.4 Changes in Acid value during fermentation of Okara Tempe

Time	Acid Value
0	2.20
4	3.6
8	5.0
12	6.21
16	7.45
20	8.80
24	10.00
28	11.10
32	12.40
36	13.45

40	14.40
44	15.10
48	15.80

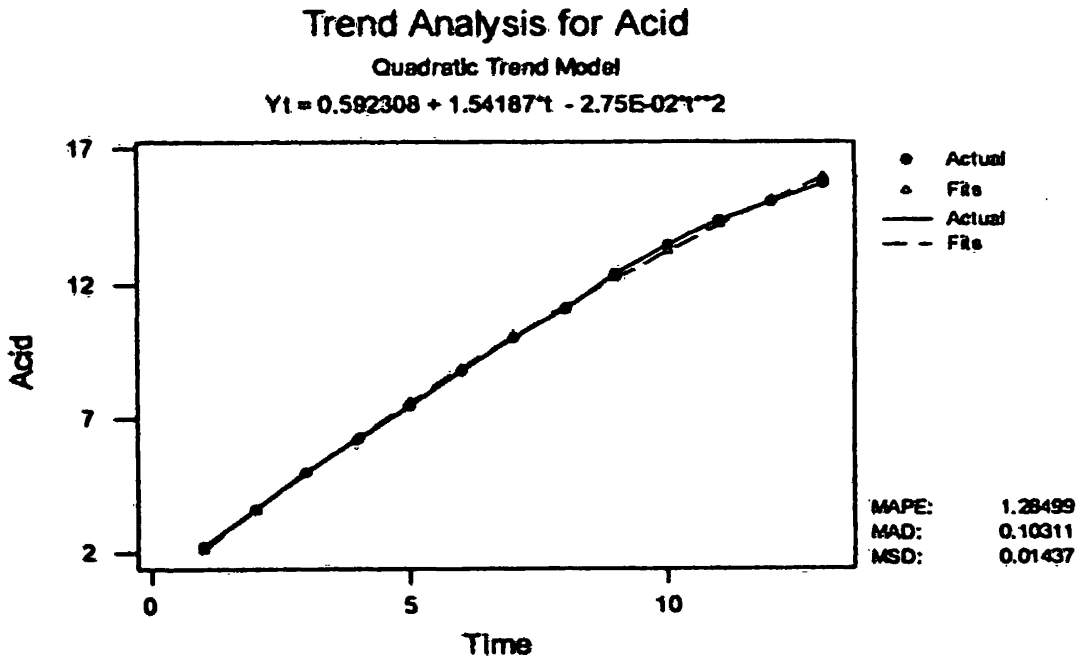


Figure 4.4 Changes in Acid value during fermentation of Okara Tempe

Acid value data denoted an increasing percentage (figure 4.4). It seems that lypolytic activities of bacteria after the rapid growth of mold are high.

4.3.5 Overall Discussion for the Variation of pH, Temperature, Formal value and Acid Value.

Variation of pH, Temperature, Formal value and Acid value were analyzed using Time Series Analysis.

According to the above figures, quadratic trend was observed in the pH and acid values. Linear positive trend was observed in the formal values.

Fitted trend lines are accurate, since MAPE, MAD, MSD values are small and the accuracy of the models are good.

Where,

MAPE - Mean Absolute Percentage Error
MAD - Mean Absolute Deviation
MSD - Mean Square Deviation.

When graph was plotted for the temperature variations, fitted trend line was not in good state. Because, Tempe mold grows on okara substrate, temperature fluctuations were taken place as a result of their metabolic activities and release of heat. Therefore, a good fitted trend line could not obtain for the temperature variations.

4.4 Sensory Evaluation

Table 4.5 Results of paired-comparison test

	Rank for samples	
judges	Okara Tempe	Soybean Tempe
1	8	8
2	7	8
3	6	7
4	8	8
5	8	9
6	8	8
7	7	8

8	8	8
9	8	8
10	7	8
11	8	8
12	7	8
13	7	8
14	8	8
15	7	8
16	8	8
17	7	8
18	7	8
19	8	8
20	8	8

4.4.1 Out put result of sensory evaluation

Mann-Whitney Confidence Interval and Test

```
first       N = 20      Median =       8.0000
second      N = 20      Median =       8.0000
Point estimate for ETA1-ETA2 is        0.0000
95.0 Percent CI for ETA1-ETA2 is (-1.0002,0.0001)
W = 324.0
Test of ETA1 = ETA2  vs  ETA1 not = ETA2 is significant at 0.0207
The test is significant at 0.0030 (adjusted for ties)
```

According to the above results of sensory evaluation, there is no significant difference between samples at 5% level of significance. Therefore, there is no significant difference of consumer acceptability for taste testing of okara Tempe at 5% level of significant.

4.5 Handling of the product for Microbiological Analysis

4.5.1 Coliform Test

Presumptive test, the first step of Coliform test was negative, because there are no gas formation inside Durham tubes. Therefore, further testing is unnecessary. During the steam cooking of Okra, the sterilization process was taken place at about 121^{0}C temperature for 20 minutes.

Most probably, deep- frying is the common cooking method of fresh okra Tempe,
In here, the product was subjected to about 175^{0}C temperature. Both of sterilization by steaming of the product and deep frying of the product helps to assure that there was no chances to post fecal contamination the product.

4.5.2 Enumeration of yeast and Molds

Yeast and mold count at 27^0 C gave the following results:

10^{-2} dilution : 97 and 105 colonies
10^{-3} dilution : 58 and 75 colonies

$$N = \frac{\Sigma}{(n_1+0.1n_2)\, d}$$

$$= \frac{97+105+58+75}{(2+0.1*2)*10^{-2}}$$

$$= \frac{335}{0.22}$$

$$= 15227.272$$

Rounding the results as specified above gave 15.000.

The estimated number of yeast and molds /ml was therefore $1.5*10^{-4}$ in the product.

4.5.3 Enumeration of Total plate count technique.

Total plate count at 27^0C gave the following results.

10^{-2} dilution : 170 and 220 colonies.
10^{-3} dilution ; 65 and 78 colonies

Sum of colonies counted on all plates (Σc)	= 533
No. of plates retained in the first dilution (n1)	= 2
No. of plates retained in the 2 nd dilution (n2)	= 2
Dilution factor (d)	= 10^{-2}

Rounding the results as specified above gave 24,000.

The estimated number of micro organisms/ ml was therefore $2.4^{*}10^{-4}$ in the product.

CHAPTER 5

CONCLUTIONS AND RECAMONDATIONS

5.1 Conclusions

The study on utilizing soymilk residue (okara) to manufacture Tempe is a solution for the tofu manufacturing industries that remove okara as a by-product. According to the results of sensory evaluation, this product has similar consumer acceptability as of soybean Tempe. The okara Tempe contains 74.2%of moisture, 2.8% of fat, 4.9% of protein 4.2% of crude fiber and 0.9% of total ash. The product serves as a meat-substitute in the vegetarian diet and it is ideal for use in Sri Lanka like developing countries as an inexpensive source of protein, but shelf-life of the product is limited to one to two days.

5.2 Recommendations for further studies.

1. Aflatoxin analysis for the product should be carried out.
2. Costing for the production should be done.
3. A market research should be carried out for consumer acceptability of the product.
4. Shelf life studies should be carried out further more to increase the shelf life of the product.
5. Further studies should be proceeding to obtain optimum moisture content of the substrate.

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APPENDIX 1

Questionnaire for Paired-comparison Test

Name : .....

Date : .....

Product : .....

Taste these two samples and check how much you like or dislike each samples.

9	Like extremely
8	Like very much
7	Like moderately
6	Like slightly
5	Neither like nor dislike
4	Dislike slightly
3	Dislike moderately
2	Dislike very much
1	Dislike extremely

Sample No. 581

.....

Sample No. 716

.....

Comments :

Thank you

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