***Original Research Article***

# Evaluation of physicochemical and nutritional contents in Soybean fermented food tempeh by *Rhizopus* oligosporus


**ABSTRACT:**

*Rhizopus oligosporus* is considered as most preferred species in tempeh fermentation among the tempeh culture on top of it is most widely used and one of the known molds in vegetal substrates fermentation. A fermented product "Tempeh" was prepared using mixed culture fermentation of soaked and cooked soybeans or cereal grains by means of diverse group of microflora like bacteria, yeasts and molds. *Rhizopus oligosporus,* NRRL-2710 was used for the preparation of tempeh. The culture was maintained on potato dextrose agar media prepared by adding 3.8 grams PDA in 100 ml distilled water followed by heating. The culture was incubated at 30-37 ° C in an oven for 24 hours and then stored in refrigerator at 4 °C. Soya beans soaked overnight were boiled for 10-15 minutes. The hot beans were spread in a thin layer and cooled to room temperature then they were inoculated with a spore suspension of *R. oligosporus.* The inoculated soya beans were packed firmly in perforated plastic bags, and incubated at 30°C for 24 h, yielding fresh tempeh. The physicochemical characteristics and nutritional content in soybean fermented food tempeh were then assessed. Filamentous fungi are used in the preparation of fermented foods to improve the taste and the nutritional value of the product. The aim of the study was to verify the viability *of Rhizopus oligosporus* in soybean fermented food tempeh. Tempeh is a striking product from sensual, health and cost-effective points of view. The present work determined the sensory analysis and nutritional viability evaluated the effects of pH, temperature, relative humidity, aeration, moisture and ash contents, inoculums size, shelf life and its mineral values including protein, carbohydrate and lipid content. The mold growth was rapid at 30 $^{o}$C. When *R. oligosporus* was inoculated at approximately $10^{4}$ spores/g moist substrate, a tempeh cake with dense mycelial growth was obtained.



**Keywords:** Molds, Tempeh, fermented food product, Nutritional contents, physicochemical analysis

## Introduction:

The food industry has incorporated the consumption of residues that are resulting from food production in the last decades. Cheese industry being one of the major sectors of food industry generates bulk of milk serum as the remains of cheese production. A number of studies have been carried out to optimize an environmental friendly use of milk whey that helps to reduce the pollution caused by its disposal. It emerges as a source of environmental degradation due to the high content of organic materials and high biological demand of oxygen which is necessary for lactose deterioration. Ahmad *et al*., (1995) reported that high nutritional value of a product contains considerable amount of proteins and lactose. The beverages production by the use of milk serum has been studied by several researchers. Bakhit *et al.*, (1994) explored cost effective methods of production to acquire a product with enriched characteristics and chemical composition. The production of different fermented soy food products including Tempeh usually involves the activity of molds, aspergillus, rhizopus, mucor, actinomucour and numerous species of yeasts and lactic acid bacteria. Many nutrients in the food including proteins, fats, vitamins, minerals, and phytonutrients are converted due to the action of microorganisms during the fermentation process. In fermented soy foods, proteins are often more digestible minerals and become more soluble including many phytonutrients such as isoflavones like genistein and daidzein. When fermentation changes the digestibility of protein in soy foods they develop a unique health supportive property of their own. For example, an important storage protein Conglycinin and its fellow, glycinin, account for as much as 80% of the total proteins in soybeans. It is often broken down into smaller peptides that serve as antioxidants that boost immune function, and prevent excessive inflammatory response during the process of fermentation. These whole food-based forms of soy stand in clear peculiarity of highly processed varieties of soy like soy protein essence. At the same time, researcher's provision for the health benefits of soy foods is even robust for fermented versus non-fermented soy foods. Tempeh is one of the most appropriate options in such case.

Tempeh is fermented soy food that came from the island of Java in Indonesia at least hundreds of years ago and is fermented with the mold *Rhizopus oligosporus*. For the fermentation of tempeh, a period of several days or longer is utilized and usually carried out at temperatures of 85-90°F/29-32°C. Tempeh is usually obtained in a cake-like form bounded with dense white

mycelium. Pakistan is a developing country where there are roughly 35 million people living below the poverty line and facing massive food crisis with around 20% food inflation rate. Tempeh being a cost effective protein source can be used as a substitute of meat. Tempeh encompass a variety of important nutrients that are tied to an impressive array of health benefits including decreased risk of heart disease and strokes, osteoporosis, cancer and digestive disorders. It also helps in losing excess weight in addition to easing some of the symptoms of menopause. All these health benefits are due to the low fat content in tempeh.

The aims and objectives of the present study were to:

- To optimize process parameters such as acidity, temperature, relative humidity, inoculums size, depth of beans, time course, and aflatoxin
- To evaluate nutritional content of tempeh
- To determine moisture content and ash content
- To isolate *Rhizopus oligosporus* from local soil samples
- To assess tempeh fermentation using soybean as substrate
- To study the shelf life of tempeh
- To optimize organoleptic evaluation of tempeh

**Microflora of Tempeh**

The culture of tempeh includes *Rhizopus oligosporus, R. oryzae, R. arrhizus, R. stolonifera, Mucor spp., lactic acid bacteria, Citrobacter freundii* or *Klebsiella pneumonia and* probiotic *Lactobacillus reuteri. Rhizopus oligosporus* is the dominant tempeh fungus (Sharma and Sarbhoy, 1984), although some other moulds, such as *R. oryzae* and *Mucor* spp., may also contribute to the flavor, texture or nutritive value (Wiesel *et al.*, 1997). Lactic acid bacteria may contribute to the microbial safety (Nout *et al.*, 1987; Ashenafi, 1991). Vitamin $B_{12}$ production by using bacteria such as *Citrobacter freundii* or *Klebsiella pneumoniae* has received special attention (Liem *et al.*, 1977; Okada *et al.*, 1985; Suparmo, 2008; Keuth and Bisping, 1993; Wiesel *et al.*, 1997). However, these two species are potentially pathogenic (Struve and Krogfelt, 2004). Recently, the probiotic *Lactobacillus reuteri* was reported to produce vitamin $B_{12}$. Yeasts are frequently detected in tempeh, but their role is still unknown (Samson *et al.*, 1987).

***Rhizopus oligosporus***

For the first time in 1895, Dutch scientist Prinsen Geerligs, identified the tempeh most active mould. *R. oligosporus* is considered as most preferred species in tempeh fermentation among the tempeh culture (Ahmad and Sarbhoy, 1984), due to its properties such as rapid growth at high temperature (30-42C°), an inability to ferment sucrose, high photolytic and lipolytic activities and production of strong antioxidants (Steinkraus *et al.*, 1983). During tempeh fermentation, the soybean is degraded by *R. oligosporus* enzymes, such as carbohydratases (e.g. polygalacturonase, endocellulase, xylanase, arabinanase and small quantities of α-D-galactosidase, β-B-galactosidase, β-D-xylosidase, α-L-arabinofuranosidase and α-D-glucosidase), lipases, proteases and phytases (Nout and Rombouts, 1990). In contrast, Rehms and Barz (1995) reported that *R. oligosporus* did not produce α-galactosidase and consequently cannot degrade flatulence-causing compounds such as stachyose and raffinose. *R. oligosporus* can inhibit the growth and aflatoxin $B_1$ accumulation of *Aspergillus flavus* and *A. parasiticus* (Nout, 1989). *R. oligosporus* has been reported to produce 4 to 5 anti-bacterial compounds during soybean tempeh fermentation (Anon, 1980; Wang *et al.*, 1969; Nowak & Steinkraus, 1988). The fungus also produces phenolic compounds that inhibit the growth of pathogenic bacteria such as *Helicobacter pylori* (Correia *et al.*, 2004). An antibacterial protein has been purified from *R. oligosporus*, with activities against *Bacillus* spp. (especially against *Bacillus subtilis*), *Staphylococcus aureus* and *Streptococcus cremoris* (Kobayasi *et al.*, 1992).

## Materials and Methodology

### Organism:

*Rhizopus oligosporus,* NRRL-2710 was used for the preparation of tempeh. The culture was maintained on potato dextrose agar media. Potato dextrose agar media was prepared by adding 3.8 grams PDA in 100 ml distilled water followed by heating. Potato dextrose agar media was purchased from ACROS Chemical Corporation. Then culture was incubated at 30-37 ° C in an oven (MEMMERT 854, West Germany) for 24 hours. After incubation, culture was stored in refrigerator at 4 °C.

### Preparation of tempeh:

Soya beans were soaked overnight at room temperature (25°C), and then boiled for 10-15 minutes. Then the hot beans were spread in a thin layer and cooled to room temperature; subsequently, they were inoculated with a spore suspension of *R. oligosporus.* The inoculated soya beans were packed firmly in perforated plastic bags, and incubated at 30°C for 24 h, yielding fresh tempeh.

**Acidity:**

The initial pH before inoculation was ranging between 4, 4.5, 5, 5.5 and 6. Acetic acid was added to adjust the pH.

**Temperature:**

For the determination of suitable temperature for fermentation process, the inoculated cotyledons were incubated at 25 °C, 28 °C and 30 °C.

**Effect of Aeration:**

To determine the aeration rate during fermentation process, the depth of soybean was varied from 1.0 cm to 4.5 cm in sterilized plastic bags.

**Effects of Inoculums Size:**

Inoculums Size were varied from 10 µl to 500 µl per 15 grams soybeans in each sterilized plastic bags to know the best concentrations of inoculums for fermentation of soybean.

**Relative Humidity:**

Relative humidity of inoculated cotyledons were maintained by using refrigerator incubator (FOC 225I, Italy) at 20, 40 and 60.

**Time Course of Fermentation:**

To determine the fermentation time course, tempeh was incubated for 18, 20, 22 and 24 hours.

**Shelf Life:**

Tempeh was stored for 24 hours and was evaluated for flavor, taste, appearance, and texture.

**Moisture and Ash Content:**

The known weight (10.9g) of fermented sample was placed in petri dish and placed in oven at 100 $^{o}$C for 24 hour. Final weight of sample was determined and moisture content of tempeh was expressed in percentage (%). Ash content was determined by the method outlined by AOAC (1984). Pre-weight sample ash was heated at 500 $^{o}$C in a muffle furnace until residue turned whitish grey. The ash content per unit weight was calculated and expressed as percentage (%).

**Protein and Fat Content:**

Protein analysis was carried out by kjeldahl method. Tempeh was fermented with sulfuric acid in the presence of mercury oxide or copper sulphate which reduced organic nitrogen in the presence of catalysts which reduced organic nitrogen into ammonium sulphate, followed by distillation in the presence of sodium hydroxide, liberating ammonia gas. Then distillate was collected into boric acid solution, and the borate anions formed were titrated with standardized hydrochloric acid solution. The mill equivalents of acid required for titration were used to calculate the nitrogen content in the sample (Chang, 1998). Fat analysis of tempeh was carried out by Soxhlet extraction method of prepared tempeh. Tempeh was placed inside thimble and loaded into the main chamber of the Soxhlet extractor. Then Soxhlet extractor placed onto a flask containing the ethanol. The solvent was heated to reflux and traveled to distillation arm in form of vapors, and flood into the chamber housing the thimble of solid. After the chamber filled with warm solvent and some of the desired compound dissolved in solvent in every cycle. After many cycles the desired compound concentrated in the distillation flask. After extraction the solvent is removed, typically by means of a Rotary evaporator, yielding the fat content.

**Aflatoxin analysis:**

Aflatoxin was determined by the method described by Pons et al., (1966). Tempeh sample was prepared in laboratory and was tested in Pakistan council for scientific and industrial research (PCSIR) for aflatoxin analysis.

**Results**

**Physicochemical analysis**

**Effects of pH on tempeh fermentation:**

The low acidic pH of substrate during the production of tempeh is very important in controlling the growth of pathogen or food spoilage organisms. Acidity was varied from 4.0-6.0. The growth of mold at pH 4.0 was thick white mycelium. At the end of fermentation, the beans were bound together by mycelium forming a firm cake like products. The taste of tempeh was acceptable.

**Effects of depth of beans on tempeh fermentation:**

The supply of oxygen proved to be very essential for the mold growth. Effects of different depth of soybean for fermentation were evaluated in polythene bags. Thickness was varied from 1.0 cm to 4.5 cm. The mold growth was rapid while the thickness of the cake was 2.0 cm because oxygen supply was sufficient for tempeh fermentation.

**Effects of temperature on tempeh fermentation:**

Incubation temperature had great influence on the growth rate of mold culture. The inoculated soybean was incubated at 25 $^{o}$C, 28 $^{o}$C and 30 $^{o}$C. The mold growth was rapid at 30 $^{o}$C. The fermented product was not of good quality than that at high temperature soybean tends to dry out because the mold growth was suppressed.

**Effects of inoculums size on tempeh fermentation:**

Inoculum size is an important factor in tempeh fermentation. Excess inoculum promoted rapid and uniform tempeh fermentation and too little inoculum allowed bacteria to grow which suppress *Rhizopus oligosporus* growth. In the present study, inoculum size was varied from 10 µl to 500 µl. Optimum inoculum size was 90 µl for tempeh fermentation in polythene bags.

**Moisture and Ash content:**

Tempeh fermentation is considered as exothermic reaction because of the release of moisture during and after fermentation. The moisture content of tempeh was determined by oven drying tempeh sample at 100 $^{o}$C for 24 hours. The moisture content of tempeh sample was 62.38%. Ash content was determined by pre-weight tempeh sample ash by heating at 500 $^{o}$C in a muffle furnace until residue was whitish grey. The ash content per unit weight was 4.12 %.

**Nutritional contents**

**Protein and Fat content:**

*Rhizopus oligosporus* produce a variety of enzyme like proteases which causes significant increase in protein content of tempeh. Kjeldahl method was used for determination of protein content in tempeh sample. Result showed that protein content in tempeh sample was 37.38 %. During tempeh fermentation, *Rhizopus oligosporus* produce lipases enzymes which break down lipids in tempeh and *Rhizopus oligosporus* consumes these small fatty acid molecules for their energy requirement. Fat content was determined by soxhlet extraction method and calculated value of fat content in tempeh samples was 17.31%.

**Aflatoxin content:**

Tempeh sample was prepared in laboratory and was tested in Pakistan council for scientific and industrial research (PCSIR) for aflatoxin analysis. Results showed that tempeh was free of aflatoxin because mould *Rhizopus oligosporus* does not produce aflatoxin itself as well as inhibits the growth of those species which produce aflatoxin (Kontessis, 1990).

**Table No 1: DETERMINATION OF SOME OF PARAMETERS AFTER TEMPEH FERMENTATION**

| Assays | Values |
|---|---|
| Humidity | 20-60 R. h. |
| pH | 4.0-6.0 |
| Temperature | 25-30 $^{o}$C |
| Moisture content | 62.38% |
| Ash content | 4.12% |
| Proteins | 37.38 % |
| Lipids | 17.31% |
| Aflatoxin | Absent |

# Discussion

The present study describes the propagation of *Rhizopus oligosporus* on dehulled soybeans as substrates. Soybean Tempeh was prepared by *Rhizopus oligosporus* NRRL-2710.

**Acidity:**

In normal condition, pH of tempeh varies from 4-6. The initial pH increased from 4.5 to 6.0 after 26 h at 28 °C or 18 h at 38 °C. Tempeh fermented for 48 h at 28 °C or 20 h at 38 °C resulted in the pH leveling off around 7.5 to 8.0. *R. oligosporus* can also grow at pH 3 as at pH 4 or 5. However, there was a significant difference between pH 5 and pH 6. Thus, this mold could be used in a protected fermentation. This would be desirable in circumstances where sterilization is not possible due to lack of equipment or cost (Omosaiye et al., 1978). The most favorable pH range for the growth of most fungi is from pH 4 to 7 (Liem, 1997). The preferable pH of beans is of a range of 4.0 to 5.0. At this pH range, the growth of contaminating bacteria would be inhibited, but not that of the tempeh mold. The tempeh mold will be inhibited when the pH drops below 3.5.

**Temperature:**

Temperature is another important factor in tempeh preparation. According to the Frankland et al., (1982), the speed of fermentation is determined by the incubation temperature. Incubation temperatures above 40 °C and below 25 °C will not produce good tempeh whereas temperature of 37 - 38 °C will produce tempeh within 22 hr; a temperature of 28 - 30 °C will take up to 48 h to produce tempeh. When fermentation temperature is as low as 25 °C, an acceptable tempeh could be produced. However, the fermentation required as long as 5 days to complete. In contrast, fermentation at 37 °C required only 1 day. Thus, it can be concluded that a temperature slightly above room temperature is the best for *tempeh* fermentation (Liu, 1997).

**Relative humidity:**

Relative humidity is defined as the ratio of the partial pressure of water vapors in an air parcel of air to the saturated vapor pressure of water vapor at a prescribed temperature. Wiesel (1997) reported a pilot plant process requiring 18 h incubation at 35 - 38 °C and 75-78% relative humidity (R.h.). Optimum Relative humidity was reported as 60 %, 65%, 75% and 90% (Steinkraus, 1985). At relative humidity >75% undesirable fungal sporulation were observed**.** Messina (1994) elevated the relative humidity by placing a tray of water in the bottom of an incubator set at 31 °C. A similar procedure using black common beans was conducted at 37 °C with a relative humidity of 70%. Relative humidity was maintained at 75% by wetting a Whatman No. 1 filter paper disc with a saturated solution of sodium chloride (Rockland, 1960).

**Inoculums size:**

The inoculation levels of *R. oligosporus* strongly influenced tempeh fermentation**.** The excess inoculums promoted rapid and uniform fermentation. Wang *et aI*. (1975) concluded that if too little inoculums were used; bacteria would be allowed to grow. With inoculation at approximately $10^2$ spores/g moist, the fungus grew more slowly and a tempeh cake with dense mycelial growth was not obtained until after 28 to 32 h (Nout and Kiers, 2005). When *R. oligosporus* was inoculated at approximately $10^4$ spores/g moist substrate, a tempeh cake with dense mycelial growth was obtained after 20 h (Nout and Kiers, 2005). For optimal fermentation, Wang et al. (1975) recommended that 1 x $10^6$ spores per 100 g of cooked soybeans were used. On the other hand, fermentation failures and excessive heat production were reported to be caused by insufficient packing density with pockets of air and heavy inoculation. However, the growth was uneven probably due to oxygen limitation in the center (Nout and Kiers, 2005).

**Aeration:**

Aeration is one of the imperative factors for production of tempeh which affects the quality of tempeh. Most of the researchers prepared tempeh within range of 2-5 cm of bean's thickness. Frankland et al., (1982**)** performed experiment at laboratory scale in which he placed soybeans in a plastic bag and flatten the contents out to a cake about 2.0 cm thick and reported that the area of the cake is not important but the thickness should always be about 2.0 cm to have food quality of tempeh. Same as Keuth *et al.,* (1993) inoculated beans were spread on stainless steel pans (25.4 X 35.6 X 6.4 cm) to a depth of approximately 2.54 cm and covered with metal covers and incubated at 37 °C. A freeze-dried starter culture of *R. oligosporus* NRRL 2710 was added at 1% (w/w) of the wet substrate and mixed thoroughly for 3 min. The inoculated substrates were packed into sterile plastic petri plates (diameter 87 mm, depth 12.5 mm (1.25 cm), each plate containing approximately 42 g. The inoculated substrate is transferred to a confined space and a slight pressure is applied from outside. Traditionally, this is achieved by wrapping small quantities in plant leaves or by covering 4-6 cm thick beds with banana leaves or polythene sheets, which may be weighted down with clay bricks (Nout and Rombouts, 1990). American vegetarians' consume tempeh burgers of about 1.5 cm thickness beans and starter are mixed homogeneously into 3–5 cm thick beds (Brandi 1992).

**Moisture and Shelf life:**

Tempeh fermentation is an example of solid substrate fermentation that involves the growth of microorganisms on solid organic materials in the absence or near absence of free water. In general, high relative humidity and good absorbency of the substrate are absolutely needed for proper Tempeh. The production of polysaccharidases as well as their specific activities during tempeh fermentation was found to depend on water activity of the soybean substrate (Liu, 1997). The nutritional implications of the tempeh fermentation reported that fresh tempeh contains 60% moisture. Fresh tempeh cakes must be consumed within 1 or 2 days or the mold proteolytic enzymes will cause ammonia to form, which results in an undesirable taste. Storage stability of tempeh can be extended by drying, frying, dehydration, freezing, and other preservation methods. Wang and Hesseltine (1979) reported that shelf life could be prolonged by freezing, drying, or canning. Steinkraus *et al*., (l965) cut the fermented tempeh into 2.5 cm squares and placed the squares into a hot-air dryer in order to lower the moisture level to 2 to 4%. It is reported that that tempeh remained stable without refrigeration for 24 to 48 h after harvesting. Therefore, freshly made tempeh can be stored for several days at room temperature without adversely affecting the nutritional or organoleptic properties. Steinkraus *et al*., (1965) reported that dehydrated tempeh could be stored in plastic storage bags for several months at room temperature without noticeable changes in color or flavor whereas changes in reducing substances, soluble solids, and soluble nitrogen content of tempeh occurred. Horbar *et al*., (2002) studied the storage stability of tempeh using canning and found that a shelf life of 10 weeks could be attained without significant alterations in the acceptability of tempeh resulting. Having an effect or making an impression on sense organs; usually used in connection with subjective testing of foods and drug products known as organoleptic evaluation. Organoleptic evaluation includes testing of tempeh by aroma, taste, appearance, texture and mycelia growth. Organoleptically, tempeh scored best at the end of the first phase of fermentation (30 h at 32 °C), kept its good quality during the second phase (one additional day at 32 °C), and deteriorated rapidly during the third phase (Suparmo *et al*., 2008). Signs of deterioration appeared as loss of pleasant taste, smell and texture.

**Nutritional content**

**Soybean source of protein:**

Protein is one of most essential nutrients among other nutritive elements. Tens and thousands of children in developing countries die every day due to disease caused by protein deficiencies**.** Soybean is considered by many agencies, including the US food and Drug administration, to be a source of complete protein. A complete protein is one that contains significant amounts of all the essential amino acids that must be provided to the human body because of the body's inability to synthesize them. For this reason, soy is a good source of protein, amongst many others, for many vegetarian and vegans or for people who cannot afford meat. All around the world, soybean are known due to their rich protein content but increasingly, soyfoods are being recognized as having potential roles in the prevention and treatment of chronic diseases, most notably cancer and heart disease. There are also potential roles for soyfoods with respect to osteoporosis and kidney disease. Soybean is thought of Asian origin (Turecki *et al*., 1998). Soybean was taken to the united states in 1804, but there was little commercial production until the $20^{th}$ century .since then, soybean has been processed into comparatively simple food products; processing includes water extraction (soy milk)with coagulation calcium salt(tofu), roasting (kinako) and fermentation (miso, natto, tempeh, and soy sauce).Of all legumes, soybean crop proteins have reached the highest degree of refinement and extent of development; and are added to a wide variety of processed foods (Varzakas *et al*., 1999). Soybean is rich with following nutrients: protein 39% (crude protein 44%), lipids 17-20%, carbohydrate 18%, digestible fiber 40% and minerals 5%. Soybeans have played very important role in Asian culture, both as a food and as a medicine. In comparison to most other legumes, soybeans are much higher in protein (~35% of energy), which may be particularly important for developing countries. However; it is not only the amount of protein in soybeans that is notable, but also the amino acid pattern of soy protein. Soy protein is very efficiently produced; approximately 25, 10 and 5 times more protein is produced by soybeans per acre as compared with beef, milk and wheat production, respectively. Because of the proteins semi digestive state, it makes a good protein source for people with gastro-intestinal upsets (i.e. POW's, AIDS, third world countries) (Varzakas, 1999). From a nutritional perspective, soy protein may hold many advantages over animal proteins above and beyond the fact that soybeans are low in saturated fat and, of course, cholesterol-free. Of utmost importance is the hypocholesterolemic effect of soy protein (Bakhit *et al*., 1994) with the help of this property of soybean, soy protein represents a safe, viable and practical non pharmacologic approach to lowering cholesterol. The hypocholesterolemic effects of soy protein may be of

particular benefit to patients with chronic renal in sufficiency, because elevated levels of cholesterol can exacerbate disease progression**.** The oxidation of low-density lipoprotein (LDL) cholesterol may play a critical role in this regard; consequently, the suppression of LDL-cholesterol oxidation by soyprotein may be still another benefit of soy protein not only to kidney disease patients, but also to the general public. For this reason, the kidney disease patients would benefit as much by substituting soy protein for animal protein as by restricting overall protein intake. Soy protein may also help to promote bone health. Factors affecting urinary calcium excretion play critical roles in determining calcium balance and bone mineral density. The hypercalciuric effect of protein has been proposed as one factor contributing to the high rates of osteoporosis in Western countries (Abelow *et al.*, 1992), where protein intake greatly exceeds requirements. However, in comparison with animal proteins, soy protein causes much less calcium to be excreted in the urine. Parenthetically, the isoflavones in soybeans may also directly inhibit bone absorption (Brandi, 1992). Research on the potential health benefits of soyfoods is particularly intriguing with respect to cancer prevention and treatment. Epidemiologic data suggest the consumption of as little as one serving of soyfoods (i.e., one cup soymilk, 5 cup tofu) per day lowers risk for a wide range of cancers (Messina *et al.,* 1994).

**Conclusion:**

From the present study, it can be concluded that with the growing demand for soy foods, tempeh is now becoming more and more available throughout the country. Plain soy tempeh was prepared from soy and Rhizopus mold with and without the addition of soy-grain combinations. The tempeh mold inhibited when the pH drops below 3.5. Kjeldahl method was used for determination of protein content in tempeh sample. Result showed that protein content in tempeh sample was 37.38 %. Fat content was determined by soxhlet extraction method and calculated value of fat content in tempeh samples was 17.31%. The inoculation levels of *R. oligosporus* strongly influenced tempeh fermentation**.** The excess inoculums promoted rapid and uniform fermentationTempeh that says "pre-cooked" and "ready to eat" foods contains a good source of protein, phosphorus, vitamin $B_{12}$, and magnesium which are also more delicious, healthier, digestible and absorbable form due to the process of fermentation.

**References:**

1. Abelow, B. J., Holford, T. R., & Insogna, K. L. (1992). Cross-cultural association between dietary animal protein and hip fracture: a hypothesis.*Calcified tissue international*, *50*(1), 14-18.
2. Ahmad, N., & Sarbhoy, A. K. (1995). New powdery mildews from India.*Mycological Research*, *99*(3), 374-376.
3. Añón, M. C., & Calvelo, A. (1980). Freezing rate effects on the drip loss of frozen beef. *Meat science*, *4*(1), 1-14.
4. Ashenafi, M. (1991). Growth of Listeria monocytogenes in fermenting tempeh made of various beans and its inhibition by Lactobacillus plantarum. *Food Microbiology*, *8*(4), 303-310.
5. Bakhit, R. M., Klein, B. P., Essex-Sorlie, D., Ham, J. O., Erdman Jr, J. W., & Potter, S. M. (1994). Intake of 25 g of soybean protein with or without soybean fiber alters plasma lipids in men with elevated cholesterol concentrations. *The Journal of nutrition*, *124*(2), 213-222.
6. Brandi, M. L. (1992). Flavonoids: biochemical effects and therapeutic applications. *Bone and Mineral*, *19*, S3-S14.
7. Brockerhoff, H., Hoyle, R. J., Hwang, P. C., & Litchfield, C. (1968). Positional distribution of fatty acids in depot triglycerides of aquatic animals. *Lipids*, *3*(1), 24-29.
8. Chang, I. S., & Lee, C. H. (1998). Membrane filtration characteristics in membrane-coupled activated sludge system—the effect of physiological states of activated sludge on membrane fouling. *Desalination*, *120*(3), 221-233.
9. Correia, M., Silva, M. R., Matos, I., Magalhães, R., Lopes, J. C., Ferro, J. M., & Silva, M. C. (2004). Prospective community-based study of stroke in Northern Portugal Incidence and case fatality in rural and urban populations. *Stroke*, *35*(9), 2048-2053.
10. Frankland, J. C., Hedger, J. N., & Swift, M. J. (1982). *Decomposer basidiomycetes: their biology and ecology*. Cambridge University Press.
11. Horbar, J. D., Badger, G. J., Carpenter, J. H., Fanaroff, A. A., Kilpatrick, S., LaCorte, M., ... & Soll, R. F. (2002). Trends in mortality and morbidity for very low birth weight infants, 1991–1999. *Pediatrics*, *110*(1), 143-151.

12. Keuth, S., & Bisping, B. (1993). Formation of vitamins by pure cultures of tempe moulds and bacteria during the tempe solid substrate fermentation.*Journal of Applied Bacteriology*, *75*(5), 427-434.
13. Kobayashi, M., Nagasawa, T., & Yamada, H. (1992). Enzymatic synthesis of acrylamide: a success story not yet over. *Trends in biotechnology*, *10*, 402-408.
14. Kontessis, P., Jones, S., Dodds, R., Trevisan, R., Nosadini, R., Fioretto, P., & Viberti, G. (1990). Renal, metabolic and hormonal responses to ingestion of animal and vegetable proteins. *Kidney international*, *38*(1), 136-144.
15. Kowal, J. M., & Kassam, A. H. (1978). *Agricultural ecology of savanna. A study of West Africa*. Oxford University Press.
16. Liem, I. T., Steinkraus, K. H., & Cronk, T. C. (1977). Production of vitamin B-12 in tempeh, a fermented soybean food. *Applied and environmental Microbiology*, *34*(6), 773-776.
17. Liu, K. (1997). Chemistry and nutritional value of soybean components. In*Soybeans* (pp. 25-113). Springer US.
18. Messina, M. J., Persky, V., Setchell, K. D., & Barnes, S. (1994). Soy intake and cancer risk: a review of the in vitro and in vivo data. *Nutrition and cancer*, *21*(2), 113-131.
19. Nout, M. J. R., & Kiers, J. L. (2005). Tempe fermentation, innovation and functionality: update into the third millenium. *Journal of Applied Microbiology*, *98*(4), 789-805.
20. Nout, M. J. R., & Rombouts, F. M. (1990). Recent developments in tempe research. *Journal of Applied Bacteriology*, *69*(5), 609-633.
21. Nout, M. J. R., & Rombouts, F. M. (1990). Recent developments in tempe research. *Journal of Applied Bacteriology*, *69*(5), 609-633.
22. Nout, M. J. R., De Dreu, M. A., Zuurbier, A. M., & Bonants-van Laarhoven, T. M. G. (1987). Ecology of controlled soyabean acidification for tempe manufacture. *Food Microbiology*, *4*(2), 165-172.
23. Nowak, J., & Steinkraus, K. H. (1988). Effect of tempeh fermentation of peas on their potential flatulance productivity as measured by gas production and growth of Clostridium perfringens. *Nutrition reports international*, *38*(6), 1163-1171.
24. Okada, Y. (1985). Surface deformation due to shear and tensile faults in a half-space. *Bulletin of the seismological society of America*, *75*(4), 1135-1154.

25. Omosaiye, O., Cheryan, M., & Matthews, M. E. (1978). Removal of oligosaccharides from soybean water extracts by ultrafiltration. *Journal of Food Science*, *43*(2), 354-360.
26. Pons, W. A., Robertson, J. A., & Goldblatt, L. A. (1966). Objective fluorometric measurement of alfatoxins on TLC plates. *Journal of the American Oil Chemists' Society*, *43*(12), 665-669.
27. Rehms, H., & Barz, W. (1995). Degradation of stachyose, raffinose, melibiose and sucrose by different tempe-producing Rhizopus fungi.*Applied microbiology and biotechnology*, *44*(1-2), 47-52.
28. Rockland, L. B. (1960). Saturated salt solutions for static control of relative humidity between 5° and 40° C. *Analytical Chemistry*, *32*(10), 1375-1376.
29. Samson, S. D., & Alexander, E. C. (1987). Calibration of the interlaboratory 40 Ar□ 39 Ar dating standard, MMhb-1. *Chemical Geology: Isotope Geoscience Section*, *66*(1), 27-34.
30. Sharma, K., McCue, P., & Dunn, S. R. (2003). Diabetic kidney disease in the db/dbmouse. *American Journal of Physiology-Renal Physiology*,*284*(6), F1138-F1144.
31. Steinkraus, K. H. (1983). Lactic acid fermentation in the production of foods from vegetables, cereals and legumes. *Antonie van Leeuwenhoek*,*49*(3), 337-348.
32. Steinkraus, K. H. (1983). Lactic acid fermentation in the production of foods from vegetables, cereals and legumes. *Antonie van Leeuwenhoek*,*49*(3), 337-348.
33. Struve, C., & Krogfelt, K. A. (2004). Pathogenic potential of environmental Klebsiella pneumoniae isolates. *Environmental microbiology*, *6*(6), 584-590.
34. Suparmo, P. M. (2008). Uji ketergunaan situs web jaringan perpustakaan asosiasi perguruan tinggi Katolik di Indonesia (APTIK) bagi mahasiswa yang sedang menulis skripsi pada tahun akademik 2006/2007 di Universitas Sanata Dharma Yogyakarta. *Info Persadha*, *6*(1).
35. Turecki, G., Grof, P., Cavazzoni, P., Duffy, A., Grof, E., Ahrens, B., ... & Vojtechovsky, M. (1998). Evidence for a role of phospholipase C-y1 in the pathogenesis of bipolar disorder. *Molecular psychiatry*, *3*(6).
36. Udoidem, I. U. (2016). *Characteristics of Traditional Snacks Produced From Citrullus vulgaris S, Glycine max L, Arachis hypogea L and Sclerotium tuberygii* (Doctoral dissertation).

37. Varzakas, T., Escudero, I., & Economou, I. G. (1999). Estimation of endoglucanase and lysozyme effective diffusion coefficients in polysulphone membranes. *Journal of biotechnology*, *72*(1), 77-83.
38. Vatten, L. J., & Skjærven, R. (2004). Is pre-eclampsia more than one disease?. *BJOG: an International Journal of Obstetrics & Gynaecology*,*111*(4), 298-302.
39. WANG, H. L., Swain, E. W., Hesseltine, C. W., & Heath, H. D. (1979). Hydration of whole soybeans affects solids losses and cooking quality.*Journal of Food Science*, *44*(5), 1510-1513.
40. Wang, J. C. (1969). Variation of the average rotation angle of the DNA helix and the superhelical turns of covalently closed cyclic λ DNA. *Journal of molecular biology*, *43*(1), 25-39.
41. Wiesel, P., Mazzolai, L., Nussberger, J., & Pedrazzini, T. (1997). Two-kidney, one clip and one-kidney, one clip hypertension in mice.*Hypertension*, *29*(4), 1025-1030.