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by Siti Harnina Bintari

Submission date: 28-May-2018 11:38AM (UTC+0700)

Submission ID: 969394531

File name: 221-226.pdf (179.47K)

Word count: 3914

Character count: 21919

PJN

ISSN 1680-5194

PAKISTAN JOURNAL OF
NUTRITION

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Research Article

Development and Properties of Tegurt, a Yogurt-like Tempe Product

¹Siti Harnina Bintari, ²Nyoman Suci Widyastiti, ³Natalia Desy Putriningtyas, ⁴Rebriarina Hapsari and ⁵Kartika Nugraheni

¹Department of Biology, Faculty of Mathematics and Natural Science, Semarang State University, Semarang, Central Java, Indonesia

²Department of Clinical Pathology, Faculty of Medicine, Diponegoro University, Semarang, Central Java, Indonesia

³Department of Nutrition, Faculty of Health Science, Respati University of Yogyakarta, Yogyakarta, Indonesia

⁴Department of Clinical Microbiology, Faculty of Medicine, Diponegoro University, Semarang, Central Java, Indonesia

⁵Department of Nutrition, Faculty of Nursing and Health Science, Muhammadiyah University of Semarang, Semarang, Central Java, Indonesia

Abstract

Background: Soybeans and their derivatives are a major component of Indonesia's domestic food landscape and have been consumed in several forms. Tempe was originally intended to be eaten as plant-based protein source. In recent years, there have been some new innovations to make tempe more consumable as it has a high nutritional value and antioxidant content. **Objective:** This study aims to develop a yogurt that is partially substituted with tempe flour and to analyze its acceptability, nutritional content and antioxidant capacity. **Materials and Methods:** Tempe yogurt was made with varying amounts of tempe flour (2.5, 5, 7.5 and 10%). Fresh tempe was made using a double-pasteurisation method and dried to form flour. Tempe yogurt was analyzed for acceptability and nutrient and antioxidant contents. **Results:** Yogurt with 10% added tempe flour had the highest fat, protein, carbohydrate and flavonoid contents, while yogurt with 7.5% added tempe flour was the most acceptable according to hedonic tests. **Conclusion:** Tempe yogurt shows potential as a functional food, especially for consumers with metabolic diseases.

Key words: Food functional, soybean, tegurt, tempe, yogurt

¹⁵**Received:** December 12, 2016

Accepted: February 17, 2017

Published: March 15, 2017

³**Citation:** Siti Harnina Bintari, Nyoman Suci Widyastiti, Natalia Desy Putriningtyas, Rebriarina Hapsari and Kartika Nugraheni, 2017. Development and properties of tegurt, a yogurt-like tempe product. *Pak. J. Nutr.*, 16: 221-226.

Corresponding Author: Siti Harnina Bintari, Department of Biology, Faculty of Mathematics and Natural Science, Semarang State University, Indonesia

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Competing interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Soybeans and their derivatives are a major component of Indonesia's domestic food landscape. Indonesia's high soy consumption makes it one of the largest soy markets in Asia. Indonesians consume soybean products in several forms: Up to 50% as Tempe, 40% as tofu and 10% in other forms (fermented soy paste, soy sauce, etc.)¹. Tempe is made of whole soybeans fermented with fungi, such as *Rhizopus oligosporus*, *Rhizopus oryzae*, *Rhizopus stolonifer*, *Rhizopus chlamydosporus* and *Rhizopus arrhizus*. The Indonesian National Standards Authority (SNI) defines tempe as a soybean product that is fermented with *Rhizopus* sp. and formed into compact solid blocks and is generally white with a slight greyish tint and a distinct tempe aroma².

Soybean tempe is an Indonesian fermented specialty and an icon of Javan cuisine. It is also rich in active nutritional ingredients. The soybean fermentation process causes changes in taste, aroma, texture, nutritional content and digestibility³. The principal advantages of soybean lie in its nutritional composition, easily digested protein content and high levels of essential amino acids. Tempe also has lower concentrations of anti-nutritional components, such as antitrypsin and phytic acid, than do raw soybeans. The main disadvantage of fresh tempe is its relatively short shelf life. One method commonly used to extend its shelf life is grinding it into flour¹.

Tempe is traditionally eaten with rice but in recent years there have been some new innovations such as tempe burgers, tempe salads and various third-generation products³. Tempe burgers and salads are generally regarded as second-generation derivatives. In these products, the shape, texture and taste of the tempe component are no longer apparent as the processing blends the tempe with other ingredients. Third-generation tempe derivatives are characterized by the effort to extract, isolate and purify the bioactive ingredients in Tempe¹. They take the form of useful bioactive compounds, such as antioxidants, that can be used to preserve foods, prevent cancer and reduce blood glucose levels. This study aims to explore tempe yogurt's technical characteristics and evaluate it for use as a probiotic product and functional food with health benefits.

MATERIALS AND METHODS

The production of tempe as the main ingredient of Tegurt is based on the tempe fermentation method described by Bintari⁴. This method involves pasteurization with two heating stages: One before and one after steeping.

The first heating stage runs at 100°C for 30 min, while the second runs at 60-70°C for 15-20 min. This pasteurization kills pathogenic bacteria, reduces the concentration of anti-nutrients and prevents protein denaturation⁴.

The two-stage cooking process generally begins with sorting the soybeans, followed by the first heating step to soften and remove the seed coat. The de-hulled beans are then washed and steeped before the second heat step (Pasteurization and leaking); fermentation is then started with a *Rhizopus* strain.

The pasteurization step makes use of a double pan, where a small plastic pan is inserted into a larger metal container that has been filled with water. This second heating step improves protein quality by reducing the concentration of trypsin inhibitors and increasing the amount of vitamin B12, maintaining the tempe's freshness for 80 h⁴.

The next stage is making the tempe yogurt, which we prepare with several different amounts of tempe flour (Table 1). Fresh tempe is dried, ground into flour and mixed with fresh milk. The mixture is strained through cheesecloth and then warmed to 43-45°C, while sugar is added to a concentration of 6.5%. The milk mixture is heated to 85-90°C and kept at that temperature for 30 min under constant stirring. After 30 min, the mixture is cooled to 45°C and inoculated with a yogurt starter culture. The culture is made of *Lactobacillus acidophilus* and *Lactobacillus casei* in a 50:50 milk mixture, incubated for 4-6 h and chilled for 12 h to stop the fermentation process. The resulting tempe yogurt is tested by semi-trained panelists prior to initial organoleptic testing.

The formulation for the tempe yogurt was defined in the Microbiology Laboratory of the Semarang National University and the Food Processing Laboratory at the Muhammadiyah University in Semarang. Proximate analysis and flavonoid content measurements were performed in the Food and Nutrition Laboratory at Gadjah Mada University. Organoleptic tests (of appearance, taste, aroma, texture and color) were performed in the Clinical Pathology Laboratory at the University of Diponegoro in Semarang using a 9 point hedonic scale with scores ranging from 0-9 (from "dislike extremely" to "like extremely"). The results of these tests were analyzed using SPSS and p-values of less than 0.05 were considered statistically significant.

Table 1: Possible compositions of tempe yogurt

| Groups | Ground tempe flour (%) | Cow's milk (%) | Sucrose (%) |
|--------|------------------------|----------------|-------------|
| T0 | 0.0 | 100.0 | 5 |
| T1 | 2.5 | 97.5 | 5 |
| T2 | 5.0 | 95.0 | 5 |
| T3 | 7.5 | 92.5 | 5 |
| T4 | 10.0 | 90.0 | 5 |

RESULTS AND DISCUSSION

Raw soybeans have a mildly bitter taste but the steeping, washing and enzymatic modifications during the fermentation process reduce the beany flavor. Tempe is a fermented soybean product cultured with *Rhizopus* sp. This microorganism can grow at low concentrations (0-2%) at an optimum temperature of 37°C⁵. The fermentation process decreases the concentration of anti-nutrients, such as phytic acid; deactivates the lipoxygenase enzyme and increases nutrient bioavailability. Phytic acid is reduced to one-third of its original concentration in raw soybeans under the tempe fermentation process with the bacteria *Rhizopus oligosporus*⁶. Tempe is a traditional food produced through the fermentation of soybeans with *Rhizopus* moulds of the species *Rhizopus oligosporus*, *R. oryzae* and *R. stolonifer* (known as tempe moulds). Soybeans fermented by the mould *Rhizopus oligosporus* experience a change in protein structure, while *Rhizopus oryzae* can produce amylase and *Rhizopus stolonifer* can produce pectinase⁷. Soybeans fermented with *Rhizopus oligosporus* are bound into a solid cake by the mould's mycelium⁷.

The main benefits of soybean fermentation are improvements in organoleptic qualities and nutritional value and a reduced need for preservatives. The steeping, boiling, cooking and fermentation of soybeans in tempe production greatly decrease phytic acid and antitrypsin concentrations. Reducing phytic acid is very important for increasing the bioavailability of minerals. Fermentation can reduce phytic acid concentration to a low enough level that calcium, zinc and iron become significantly more available⁸.

One common issue with fresh tempe is its short shelf life; grinding tempe into flour is one approach for extending its shelf life¹. Fresh tempe has a very high water content (55-65%) that supports a living and growing microbial community; thus, reducing this water content is a major issue. One kilogram of fresh tempe can be expected to yield approximately 700 g of tempe flour⁹.

Only 100 g of tempe can provide 37% of the recommended daily intake of protein. This study's analysis of tempe flour suggests an even higher protein content of 41.08% (Table 2). The variety of amino acids in tempe is also quite complete. Its most abundant amino acids are glutamic acid, aspartic acid and leucine. The fermentation process also decreases the level of fat saturation; thus, tempe contains a good amount of polyunsaturated fatty acids (PUFAs)¹.

Tempe contains 220 mg of omega-3 fatty acids and 3590 mg of omega-6 fatty acids per 100 g. Tempe is also a plant source of vitamin B12 (cyanocobalamin), which is more commonly found in animal food products. Other vitamins found in tempe include B2, B6, B1, niacin, folic acid and fat-soluble vitamins (vitamins A, D, E, K). Vitamin B12 levels can increase 9 to 33-fold during fermentation; riboflavin increases 8 to 47-fold; pyridoxine increases 4 to 14-fold; niacin increases 2 to 5-fold; folic acid increases 4 to 5-fold; and pantothenic acid increases two-fold. The vitamins produced during tempe fermentation are not by-products of the mould but rather of contaminating bacteria, such as *Klebsiella pneumoniae* and *Citrobacter freundii*¹⁰.

The fermentation agent in tempe is a fungus that breaks down the soy proteins into amino acids, making them easier to digest³. This alters the overall protein content in tempe and especially impacts the absorption process. Tempe has a higher nutritional value and can be consumed as an affordable protein source⁸. This increased digestibility leads to a qualitatively better nutritional value than that of unprocessed soybeans. The activity of proteolytic enzymes increases the water solubility of the proteins¹⁰.

Table 2 shows the nutritional composition of tempe flour. Raw soybeans contain approximately 38-44% protein¹¹. By comparison, the protein content of the tempe flour used in this research is 41.08%. Fermentation during tempe preparation increases the amount of individual free amino acids between 1 and 85 times above the levels in raw soybeans¹². Other nutritional components show no significant changes.

Table 3 shows the results of a nutritional analysis on tempe yogurt. The tempe yogurt formulations with the highest fat, protein, carbohydrate and flavonoid contents are, respectively; T4, T1, T4 and T4. According to these results, the T4 formulation is most recommended for consumption, although organoleptic tests with semi-trained panellists show that most of the panellists preferred "tegurt" with a composition of 7.5% tempe flour and 92.5% cow's milk (T3).

Do Amaral Santos *et al.*¹³ developed a new functional fermented beverage from peanut and soy using mixed cultures, including *L. acidophilus* and showed that it can be consumed by all ages. Shori¹⁴ showed that soybean yogurt made from cow's and camel's milk has antioxidant activity. This study also revealed that tempe yogurt shows promise as an antioxidant-rich beverage.

Table 2: Analysis of components in tempe flour

| Sample | Water content (%) | Ash content (%) | Fat content (%) | Protein content (%) | Flavonoid (ppm) |
|-------------|-------------------|-----------------|-----------------|---------------------|-----------------|
| Tempe flour | 5.41 | 2.66 | 22.46 | 41.08 | 70.85 |

Table 3: Analysis of components in tempe yogurt

| Groups | Nutrition | | | | | | |
|--------|-----------|---------|---------|-------------|--------|---------------|---------------------------------|
| | Water (%) | Ash (%) | Fat (%) | Protein (%) | CH (%) | Flavonoid (%) | Calories (cal g ⁻¹) |
| T0 | 83.68 | 0.78 | 2.24 | 2.04 | 11.26 | 0.006870 | 861.06 |
| T1 | 83.18 | 0.66 | 2.65 | 3.01 | 10.51 | 0.008444 | 821.98 |
| T2 | 83.66 | 0.66 | 3.02 | 2.58 | 10.09 | 0.009173 | 825.14 |
| T3 | 83.40 | 0.65 | 2.91 | 2.61 | 10.43 | 0.009606 | 889.69 |
| T4 | 82.94 | 0.68 | 3.12 | 2.70 | 10.55 | 0.011146 | 907.66 |

Table 4: Microbiological tests on tempe yogurt

| Groups | Microbiological analysis | | | |
|--------|--------------------------|---------------------------------------|------------------------------------|-----------------------------------|
| | pH | Bacterial TPC (CFU mL ⁻¹) | Fungal TPC (CFU mL ⁻¹) | Yeast TPC (CFU mL ⁻¹) |
| T0 | 4.60 | 1.48 × 10 ⁸ | <10 ¹ | 3.5 × 10 ⁶ |
| T1 | 4.49 | 2.42 × 10 ⁸ | <10 ¹ | 9.0 × 10 ⁶ |
| T2 | 4.41 | 2.60 × 10 ⁸ | 0 | 4.7 × 10 ⁷ |
| T3 | 4.40 | 2.60 × 10 ⁸ | 0 | 3.9 × 10 ⁷ |
| T4 | 4.43 | 3.59 × 10 ⁸ | 0 | 3.4 × 10 ⁷ |

Yogurt is viewed as a highly nutritious dairy food product due to its low carbohydrate content and high levels of protein, vitamin B-complex, phosphorus, magnesium and potassium. Yogurt's lactic acid bacterial cultures can also improve digestive conditions, including gut microflora growth, bowel transit time and immune response in the alimentary tract¹⁵.

Table 4 shows an increase in lactic acid bacteria in all groups of tempe yogurt. It also shows that the *Lactobacillus* are clearly stimulated by the addition of tempe flour to the yogurt. The growth of mould-a detrimental microorganism in yogurt-is inhibited by the addition of tempe flour via this induction of lactic acid bacteria. The yeast and fungi in these tempe yogurt products were by-products of the tempe fermentation process; yeast was detected in the tempe yogurt because it grows well in the yogurt's acidic environment.

Lactobacillus is known to modify gut flora and affect metabolic enzymes, such as bile hydrolase, azoreductase and β -glucuronidase. Previous study also shows that fermented milk containing *Lactobacillus* can reduce cholesterol levels in mice serum and liver^{15,16}.

Phytoestrogens are found in various fruits, beans and vegetables. There are three broad classes of phytoestrogens, namely, isoflavones, lignans and coumestans. Isoflavones are flavonoid compounds commonly found in beans, especially soybeans. Phytoestrogens are structurally similar to oestradiol, with a non-steroidal structure and a phenolic ring that enables binding to oestrogen receptors (ERs) as well as the ability to act as either an agonist or an antagonist oestrogen¹⁷.

Flavonoids are bioactive phenols commonly found in fruits, vegetables and parts of plants and have a major role as antioxidants¹⁸. Fermentation increases antioxidant activity and thus the functional value of the foodstuff in question. A study

by McCue and Shetty¹⁹ shows that fermentation causes a transformation of glycosides accompanied by an increase in glycosidase and glucuronidase activity along with the release of potential antioxidants through flavonoid transformation. Table 4 shows that the T4 group has the highest flavonoid content among the tempe yogurt formulations tested.

These results show that fermentation has increased the bioavailability of isoflavones during the digestion process. The high bioavailability comes from the activity of microorganisms, which degrade macromolecular compounds into simpler forms that are easier to digest and absorb. During fermentation, isoflavones change from glycosides into aglycones, while proteins are broken up into peptides and amino acids. This change increases the anti-diabetic activity of the fermented soy product²⁰. Isoflavones as a flavonoid component, can influence cellular insulin secretion capacity¹⁸. Insulin secretion in DM type 1 patients has been shown to improve under glucose control²¹.

There are two main forms of isoflavones in soybeans: β -D-glycosides known as genistein and daidzein. These glycosides are biologically inactive. Isoflavone glycosides are hydrolysed by β -glycosidase bacteria on the intestinal wall into bioactive aglycone forms (genistein and daidzein). The aglycone forms can be absorbed by the intestines, which improve their biological activity. Daidzein can be rapidly metabolized into equol and O-demethyangolensin, while genistein is metabolised into p-ethyl phenol. Genistein, daidzein, equol and O-demethyangolensin are the primary isoflavones found in human and animal blood and urine²¹. Healthy adult humans can absorb isoflavones quickly and efficiently²². The effective half-lives of daidzein and genistein-9.3 and 7.1 h, respectively indicate that isoflavones and their metabolites are rapidly excreted²³.

Polyphenolic flavonoids display strong antioxidant activity²⁴. Genistein and daidzein can increase plasma insulin levels in test animals with type 1 diabetes. Isoflavones are active flavonoids that can increase antioxidant activity by increasing the level of cellular antioxidant enzymes, such as superoxide dismutase (SOD), catalase and glutathione peroxidase²⁵.

The flavonoid and lactic acid bacteria content in this tempe yogurt gives it potential as a functional food product. Flavonoids, especially tempe isoflavones, have antioxidant activity that can help protect against free radicals and prevent clinical complications from metabolic diseases, such as diabetes mellitus, dyslipidemia and metabolic syndrome²⁶⁻²⁸. A high-isoflavone diet is also possibly beneficial for accelerating the apoptosis of cancer cells²⁹. The lactic acid bacteria in tempe yogurt can provide relief from diarrhoea and other digestive tract diseases³⁰.

This study shows that tempe can be used to create a nutritious and healthy beverage. Tempe yogurt is suitable for consumption not only by healthy people but also by those who have metabolic diseases, such as dyslipidemia and diabetes as it contains isoflavones, fibre, protein and lactic acid bacteria that could help decrease blood sugar, cholesterol and triglyceride levels. This study has many limitations that could be improved by further research, especially regarding the safety and recommended dosages for certain health conditions.

CONCLUSION

Tempe yogurt, containing many beneficial and nutritious components, has a good chance of getting a positive reception from the public. It shows potential as a functional food that could be used as part of a person's daily intake or as a dietary intervention for free radical-related diseases.

ACKNOWLEDGMENT

This study received grant funding from the University of Diponegoro budget in 2016 with award number 1051-23/UN7.5.1/PG/2016 in the name of Dr. Nyoman Suci Widyastiti, Sp.PK, M.Kes.

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