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A Comparative Study of 'Tempe' Produced from Different Beans as A Protein Source in Malaysia and Japan

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Tempe is a traditional fermented food used widely in Southeast Asia especially in Malaysia and Indonesia. It has been consumed for a very long time since it is considered as a low-cost source of protein and can be consumed widely by people of every class. Tempe is generally made from dehulled, soaked solid soybeans which undergo fermentation by using Rhizopus subspecies. It has been classified as a nutrient-rich, easily assimilable, and tasty product in which it fulfills the needs of an increasing number of consumers who are looking for excellent meat substitutes. In spite of its many benefits, tempe has a short shelf life due to its microbial enzyme activity. To prolong shelf life, tempe were dried in an oven at a temperature of 80° C for 4 to 6 h. Therefore, the objective of this study was to produce three distinct varieties of tempe by using three types of beans which were; chickpeas, soybeans and Indian dahl. Indian dahl tempe resulted with the shortest time to boil and fully fermented. The nutrition analysis of the protein content of the beans were determined and soybeans were found to have the highest protein content (17 g). The sensory analysis for the three different types of tempe was also carried out, and soybean-fried tempe indicated the highest overall rating. This study provides an insight towards introducing tempe and its acceptance as a potential food choice in Japan since Japan is famously known for its 'koji', a starter culture for the production of shoyu and miso, fermented soybean foods and 'sake', a traditional alcoholic drink of Japan.

1. Introduction

1.1 Tempe

Tempe refers to a mass of precooked, fungus-fermented beans, grains, or other food processing waste that is bonded together by the mycelium of a living mould, primarily *Rhizopus sporangiospores* (Nout and Kiers, 2005). The most appealing features of tempe are its delicious flavour and texture, as well as some of its nutritional benefits (Nout and Rombouts, 1990). It should have a meaty, mushroom-like, and nutty flavour. Furthermore, tempe should have a dense structure that does not crumble when cut with a knife. The colour of the tempe should be white since the growth of the mycelium of Rhizopus sp. limits the amount of naturally occuring sporulation by the inoculant. The tempe should have a fresh smell without any ammonia odour (Ahnan-Winarno et al., 2021). Tempe consists of a minimum of 15 % (w/w) protein, a minimum of 7 % (w/w) lipid, a maximum of 2.5 % (w/w) crude fibre and a maximum of 65 % (w/w) moisture and (Food and Agriculture Organization- World Health Organization, 2017). It can substitute for meat or fish due to its high protein content (40–50 % of dry matter), which adds flavour to starchy staple meals like rice. Thus, tempe can be categorised as a dietary item that is pleasant, easily digested, and high in nutrition, and as such, it satisfies a growing demand from consumers seeking high-quality meat substitutes (Nout and Kiers, 2005).

1.2 Tempe substrates

The main step in the process of producing tempe is fermentation, which is followed by a number of other steps. Making tempe typically entails washing, soaking in water, dehulling, boiling, cooling, inoculating with the tempe

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starter, packaging, and incubating (Romulo and Surya, 2021). To produce tempe, yellow-seeded soybeans were the most popular and widely used raw material (Nout and Kiers, 2005). However, extensive study has demonstrated the utilisation of non-soybean materials as substrates for tempe manufacturing (Romulo and Surya, 2021). Other substrates that can be used to make tempe include maize (Cuevas-Rodríguez et al., 2004), rice (Cempaka et al., 2018), barley (Feng, 2006), and a variety of beans. Chickpea beans (Reyes-Moreno et al., 2000), yam bean (Azeke et al., 2007), cowpea (Annor et al., 2010), common bean (Reyes-Bastidas et al., 2010), jack bean (Puspitojati, 2019), faba bean (Polanowska et al., 2020), velvet bean (Ariani et al., 2016) can all be used to make tempe.

1.3 Nutritional content in tempe

Tempe is rich in nutrients that are beneficial to human health. The nutrition facts for the tempe are shown in Table 1. The quantity of protein in the tempe stands out as having the highest nutrient content in this table. Tempe is popular among health-conscious consumers due to its high protein content and nutritional richness. This is due to the market's current standards for food quality, which are based on the food's nutritious content.

Table 1: Nutritional contents for 100 g of tempe (Source: USDA Nutrient Database for Standard Reference)

Nutrients	Unit	Nutrients	Unit	Nutrients	Unit
Water	54.9 g	Isoflavones	53 mg	Selenium, Se	8.8 µg
Energy	833 kJ	Calcium, Ca	93.0 mg	Vitamin C	0.0 mg
Protein	19.0 g	Iron, Fe	2.3 mg	Thiamine (B1)	0.131 mg
Fat	7.7 g	Magnesium, Mg	70.0 mg	Riboflavin (B2)	0.111 mg
Saturated fatty acids	1.11 g	Phosphorus, P	206 mg	Niacin (B3)	4.63 mg
Mono-unsat. fatty acids	1.7 g	Potassium, K	367 mg	Panthotenic acid (B5)	0.355 mg
Poly-unsat. fatty acids	4.3 g	Sodium, Na	6.0 mg	Vitamin B6	0.299 mg
Carbohydrates	17.0 g	Zinc, Zn	1.81 mg	Folic acid	52.0 µg
Fiber	4.8 g	Copper, Cu	0.67 mg	Vitamin B12	1.0 µg
Ash	1.4 g	Manganese, Mn	1.43 mg	Vitamin A	69 µg

The tempe's protein composition stands out as having the highest nutritional value. Protein is a macronutrient that is necessary for cellular structure, muscular development and recovery, and fluid control in our bodies. Proteins also produce enzymes, which are involved in a wide range of chemical reactions. Moreover, protein makes us feel fuller or satiated for a longer period of time, which helps us avoid overeating. Overall calorie consumption decreases as a consequence, which eventually helps in maintaining a healthy weight.

1.4 Sources of protein in Malaysia and Japan

In Malaysia, one of the sources of protein is from legumes. Legumes are regarded as an essential source of protein (Goldstein and Reifen, 2022). It has been demonstrated that legumes contain significant quantities of various nutrients, including soluble and insoluble fibre, potassium, magnesium, folate, selenium, and phosphorus, as well as a variety of bioactive co-nutrients. The Malaysian Dietary Guidelines (MDG) also strongly advise consuming legumes (Jaafar et al., 2023). These legumes are frequently used in the food industry, and some examples include soybeans, chickpeas, lentils, and kidney beans. The most popular soy-based goods on the market in Malaysia include tempe, firm tofu, soft tofu, egg tofu, and fu jook. As compared to other locally produced soy-based goods in Malaysia, tempe has reportedly shown to possess a significant quantity of isoflavone. The next food is chickpea, which have a moderate flavour and a pleasing texture in addition to being a good source of fibre and protein. This bean may be prepared as dried chickpeas, which simply need to be boiled after being soaked. It is often consumed as stir-fry food in Indian cuisine in Malaysia. Like other beans, lentils provide a wealth of nutrients, especially in terms of protein, folate, thiamine, and iron. They are delicious in soups and stews like dal, but they may also be fried, roasted, packed into bread, or crushed into flour. On the other hand, kidney beans are a fantastic low-fat source of protein and nutritional fibre that is suitable for use in desserts such as cendol. Shaved ice and coconut milk are the main ingredients of cendol, a popular snack food in Malaysia. On top of the shaved ice, the red kidney beans are usually presented with brown syrup and bright green jelly worms.

In Japan, soybeans are referred to as "the meat of the fields" because they are regarded as a rich source of high-quality protein. Soybeans are mostly utilised in Japan for feed, food oil, high protein components in soy products, and edible uses. According to a special report of Japan Soybean Market Intelligence, it stated that the use of soybeans in Japan in 2011 accounted 66 % for food oil, followed by food beans (30 %) and feed (4 %). Food beans are the raw materials used to make soy products including tofu, natto, miso, soymilk, and soy sauce (U.S. Soybean Export Council, n.d.). As a result, the tofu sector in Japan is the country's biggest consumer of food-grade soybeans. In Japan, firm (momen) and soft (kinu-goshi) tofu are the two varieties that are commonly sold there. Momen can be further processed into deep-fried tofu or freeze-dried tofu, whereas kinu-goshi has a higher water content and is consumed in its natural state. A higher protein content and larger seed often resulted in a higher yield of soymilk, an intermediate step in the manufacturing of tofu. Next, natto, a common breakfast meal in eastern Japan, is becoming more well-liked in western Japan due to perceptions of its affordability, convenience, and health benefits. In addition, Japan is home to more than a thousand different varieties of miso, which are often used as food condiments. Large to medium-sized soybeans with high protein content and water absorption, higher soluble carbohydrates, and lower oil and calcium concentrations are preferred by miso producers. Additionally, soybean meal or fermented soybeans are used to make soy sauce, a salty and delicious liquid condiment. For Japanese cuisine, soy sauce and soy sauce-based condiments are an essential seasoning (Sasatani, 2021).

1.5 Purpose of study

The purpose of this study was to evaluate the nutritional value of three different types of tempe made from three different bean varieties: chickpeas, soybeans, and Indian dahl. The three beans were chosen as they are widely available in Malaysia and were known to be the potential substrate for tempe. Therefore, these three different varieties of beans were used in this study to investigate the potential and feasibility of producing tempe from other legume substrates. The variations using these types of beans in the production of tempe may be identified, including texture, duration of the fermentation process, flavour, and nutrient content. In addition, to ascertain which variety of tempe is more favoured by individuals, sensory analyses of the three varieties of tempe were also conducted.

2. Methodology

Soybeans, chickpeas, and Indian dahl were obtained from a local supermarket in Shah Alam, Selangor. *R. oligosphorus* was used as a starter for tempe.

2.1 Preparation of tempe

The production of tempe was an important aspect of this research. The tempe was produced using the standard procedure. Basically, 1 cup of dry beans were prepared and soaked in water for 6–8 h or overnight. This soaking process served many purposes: it increased the moisture content of the beans, made them edible, extracted bitterness, naturally produced antibacterial compounds (saponins), and enabled microbial activity during the fermentation process (Nout and Kiers, 2005). Next, the inclusion of bean hulls in finished tempe is regarded as a contaminant by CODEX; hence, the beans were dehulled as it is a crucial step after that (Food and Agriculture Organization–World Health Organization, 2017). Dehulled beans were also necessary for optimal fermentation by tempe starters (Romulo and Surya, 2021). After that, the beans were cleaned and boiled until fully cooked, which usually took 30 minutes to an hour. Boiling was a crucial step in the manufacturing of tempe since it eliminated antinutrient components, caused denaturation of proteins, removed pathogens and spoilage organisms that could represent a threat to food safety and interfere with the fermentation process, as well as removed the raw flavor. After the beans had been cooked, the hot water was immediately drained, and the hot beans were spread out on trays to be steamed off and rapidly chilled to 20–25 °C in 30 min to 1 h. The removal of free water was necessary to prevent microbial spoilage during the process' later stage, which was fermentation, as it required an ideal level of 62 % humidity and 0.99 to 1.00 water activity (aw). The cooled beans were then inoculated with *R. oligosporus*. 2 g of this tempe starter were required for 1 kg of beans. This inoculation caused the mycelium biomass to develop densely and become harvestable before it sporulates (Ahnan-Winarno et al., 2021). Finally, the inoculated beans were packed into plastic bags with perforations, where they fermented for 48 h at room temperature (32 °C). As a result of completing all of the processes, three types of tempe were produced.

2.2 Drying

To determine the nutritional content, a dried sample of tempe was made. The tempe produced in Section 2.1 was thinly sliced and dried for 15 hours in an air oven at 60 °C. After the tempe slices were completely dry, they were crushed until they became powder.

2.3 Determination of nutritional content in tempe

The nutrients, which were protein, lipids, fiber, ash, and moisture for three types of tempe, were compared using the AOAC International Official Methods of Analysis 2019. Protein content was determined using the Kjeldahl method to convert the amount of nitrogen in food into protein. Lipid content was determined using Soxhlet extraction, which used an organic solvent to elute and measure lipids in food. The fiber content was determined using the Prosky method, which involved precipitating, filtering, drying, and weighing the undigested eluted components generated during the enzymatic treatment. The determination of ash and moisture content were based on the weighed samples before and after they were heated in the muffle furnace and air oven. Then, based on the findings from the experiment, the percent content per 100 g of tempe was computed to determine the nutritional content of tempe. All calculations were done in triplicate, and the average values were taken as the final data.

2.4 Sensory analysis

In the sensory analysis, 15 Malaysian undergraduate students, age ranging from 20 to 23 years old participated in this study. The respondents tasted all three different types of tempe fried in oil and were asked to answer questions with a rating range from 1 to 9. The ratings were: 1: dislike extremely, 2: dislike very much, 3: dislike moderately, 4: dislike slightly, 5: neither like nor dislike, 6: like slightly, 7: like moderately, 8: like very much, and 9: like extremely. As for the questionnaire, they were tested based on appearance, smell, taste, flavor, texture, and an overall rating of all three types of tempe produced.

3. Result and discussions

3.1 Making tempe

Figure 1 shows the result of the process of fermenting tempe, where (a) is chickpea, (b) is soybean, and (c) is Indian dahl. After 24 h, the inside of the package becomes damp and slightly warm. After sometime, mycelium started to surround the spaces between the beans. As a result, Indian dahl was the fastest formed into tempe after 24 hours compared to other beans. However, all types of tempe were completely fermented after 48 hours, indicating that all types were successfully produced within the standard fermentation time. Mycelial development of *R. oligosporus* and the tightness of the raw materials are two potential causes of varied fermentation times (Erkan et al., 2020). By comparing the production process, Indian dahl required the shortest boiling time and the shortest time to fully ferment.

(a) (b) (c) (a) (b) (c) (a) (b) (c)

0 h 24 h 48 h

Figure 1: Fermentation process for a) chickpea, b) soybean, and c) Indian dhal for 2 days

3.2 Nutritional analysis

The results of the nutritional content calculated for three different tempe were indicated in Table 2. Triplicates samples of the tempe from each species of the beans were utilized in the study to get a precise result. The nutritional contents of soybean tempe determined only slightly varied when compared to the original nutritional content listed in Table 1, with the exception of the energy nutrient.

Table 2: Nutritional contents for 100 g of three different tempe

Nutrients	Soybean	Chickpea	Indian Dahl
Water (g)	59.4	59.8	68.6
Energy(kcal)	214.3	153.3	145.0
Protein (g)	17.0	9.3	9.1
Fat (g)	9.1	6.3	1.0
Carbohydrates (g)	12.9	30.2	29.8
Fiber (g)	6.2	7.0	9.8
Ash (g)	1.5	3.5	1.5
Sugar (g)	12.9	11.2	19.9

The results in Table 2 showed that the highest protein level in tempe was found in soybeans, followed by chickpeas and Indian dahl. This was in accordance with previous research as chickpea tempe had a higher protein content than lentil tempe (Erkan et al., 2020). Compared to soybean and Indian dahl tempe, chickpea tempe showed a higher ash level. Nevertheless, according to other studies, the ash level of chickpea tempe was lower than that of soybean tempe, and there was only a statistically significant difference between the two (Erkan et al., 2020). Also, Indian dahl tempe was particularly rich in sugar, which was consistent with prior research that found lentil tempe had the highest sugar level when compared to soybean tempe (Erkan et al., 2020).

3.3 Sensory analysis

Sensory analysis of three different types of tempe was conducted after they were successfully produced. This sensory analysis was performed to examine the properties of the three different tempe, including their appearance, smell, taste, flavor, and texture, using the respondents' senses of sight, smell, taste, and touch. Since the sensory analysis involved many ratings from 1 to 9, it will only be considered to take the results based on the total respondents' ratings of 7, 8, and 9 in order to determine the most preferable tempe for each characteristic. From the result, fried soybean tempe had the highest rating for appearance (12 respondents) and texture (8 respondents), followed by fried chickpea tempe and fried Indian dahl tempe. This might be because the mycelium, which keeps the grains together, was less formed in chickpea and Indian dahl tempe, where there was minimal microbial development (Erkan et al., 2020). Aside from that, the taste of beans in fried chickpea tempe was the most pleasant (7 respondents), with a slight variation from fried soybean tempe. This result might be due to the size of the beans, as Indian dahl was the largest, followed by soybeans and chickpeas. Thus, the respondents might prefer the medium-sized beans in the production of tempe for their better characteristics of texture, appearance, and taste of beans. Following that, fried Indian dahl tempe received the highest ranking in terms of smell (10 respondents). This could be because Indian dahl beans have a good sense of smell. Furthermore, the fried soybean tempe had the most like compared to the others for its taste (9 respondents) and flavour intensity (10 respondents). Finally, the highest overall rating was obtained by fried soybean tempe (10 respondents), followed by fried chickpea tempe (8 respondents), and fried Indian dahl tempe (7 respondents). As a result, tempe produced from soybeans received the highest ranking for the majority of characteristics, as well as the most preferable tempe.

4. Conclusions

In the production of tempe, it was observed that Indian dahl is practically ready as tempe after 24 h, while soybeans and chickpeas took 48 h to become entirely fermented. The experimental value of nutritional content for each sample has shown significant variation when compared with standard nutritional content in tempe. Based on the overall result of the sensory analysis, it was observed that fried soybean tempe obtained the highest ranking, followed by fried chickpea tempe and fried Indian dahl tempe. Soybean tempe showed the best performance based on its highest protein content and results from sensory analysis. However, for other types of tempe like chickpea and Indian dhal, they were still acceptable to certain individuals. As a result, this demonstrated that tempe is possible to be produced using other legume substrates. Since each tempe has individual characteristics in terms of nutritional content and sensory content, it is of interest to develop products that take advantage of these characteristics and conduct further detailed research. As such, these tempe can be used as a supplementary food ingredient in the Japanese and Malaysian cuisines, which use soybeans as their base raw material.

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