

*Research Article*

## **Production and characteristics of canned tempe extract**

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### **Abstract**

Tempe is a traditional Indonesian food product derived from fermented soybeans using *Rhizopus oligosporus*. Tempe has many advantages, such as good nutritional value and organic compounds contributing to human health. Tempe's key benefits are related to its excellent protein content, high levels of essential fatty acids, numerous vitamins and minerals, dietary fibres and isoflavones. Tempe extract (tempe milk) is an example of a diversified tempe-based food. The purpose of this study is to produce canned tempe-extract. This study determined the sterilization time, nutrition facts, isoflavone contents, antioxidant capacity and production feasibility of canned tempe-extract. The results showed that the most favourable formula was tempe extract with 7%(w/v) honey as sweetener. The time needed to sterilize the canned tempe-extract (can size 202x308) was 25 minutes after the activation of the retort to reach sterilization level equivalent to  $F_0$  value of 2.62 minutes. The sterilization process did not affect the sensory characteristics of canned tempe-extract significantly. The proximate analysis results showed that canned tempe-extract contained 90.27% water, 0.06% ash, 3.66% protein, 0.98% fat and 5.03% carbohydrate. The isoflavones analysis results showed that canned tempe-extract contained total isoflavones of 5.09 mg/100g. The canned tempe-extract had antioxidant capacity of 7.13 mgAEq/150ml, which means 150 ml canned tempe-extract had equivalent antioxidant capacity to 7.13 mg ascorbic acid. The economic study showed the production of canned tempe-extract was feasible.

**Keywords:** soybean, sterilization, *Glycine max*, *Rhizopus oligosporus*, tempe milk, Indonesia.

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### **Introduction**

Tempe is an Indonesian traditional food derived from soybean (*Glycine max*) through fermentation using mold, especially *Rhizopus oligosporus*. Generally, tempe has white appearance caused by growth of the mold's mycelia. These mycelia connect the soybeans and therefore the compact texture of tempe is formed. In 2007, the consumption of tempe in Indonesia reached 21.70g/person/day [1].

There has been a lot of scientific evidence showing the functional properties of tempe in human health. This condition gives possibilities for tempe to be developed as a functional food. Tempe is a good source of nutrition, such as protein, essential amino acids, essential fatty acids, vitamin B and dietary fibre in adequate amounts. The latest research shows that tempe contains phytoestrogens called isoflavones. In the human body, isoflavones may act as antioxidants, anti-cancer, anti-osteoporosis and hypocholesterolemic agents [2]. Isoflavones may also act as an anti-aging agent and help post-menopausal women through problems caused by estrogen imbalance [3]. Isoflavones in tempe have higher bioavailability than isoflavones in soybean [4].

Currently, tempe is marketed in the form of fresh tempe (raw tempe). This condition is caused by low shelf life of tempe, therefore the marketing of tempe is limited. Tempe is classified as a perishable food and has a shelf life of about 72 hours at room temperature (25°C) [5]. Diversification of food derived from tempe is needed to give added value for this product. Tempe extract is one example of a diversified tempe-based food [6]. Tempe extract is made through extraction using water and is more practical for consumption than raw tempe. In addition, thermal processes can be applied in tempe extract production as a preservation method. Therefore, tempe extract has potential to be produced in order to diversify tempe-based food.

The aims of this study are (1) produce tempe extract with organoleptic characteristics (colour, aroma, taste and overall) acceptable to panelists, (2) determine the time needed to sterilize tempe extract at temperature 121°C, (3) determine nutritional content, isoflavone content and antioxidant capacity of canned tempe-extract, and (4) determine the production feasibility of canned tempe-extract.

## Materials and Methods

### Materials

The materials used in production of tempe were soybean var. Bromo with harvest-age 85 days, starter (*laru*) and water. The starter used was produced by Pusat Penelitian Kimia LIPI (Lembaga Ilmu Pengetahuan Indonesia) Bandung, with single culture *Rhizopus oligosporus* and spore amount of 106 colonies/g. The materials used in production of tempe extract were tempe derived from soybeans, water, granulated sugar bought from a traditional market in Jakarta, commercially packaged honey bought from a supermarket in Jakarta, brown sugar bought from a traditional market in Jakarta, synthetic vanillin powder bought from a supermarket in Jakarta, CMC (carboxymethylcellulose) bought from a chemical store in Bogor and aromatic pandanus leaves bought from a traditional market in Jakarta.

The materials used in analysis were K<sub>2</sub>SO<sub>4</sub>, HgO, Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub>, H<sub>2</sub>SO<sub>4</sub>, H<sub>3</sub>BO<sub>3</sub>, NaOH, HCl, potassiumhydrogenphthalate (KHP) crystal, indicator phenolphthalein, mixture indicator of methyl red and methylene blue in ethanol, hexane, isoflavone standard, HCl 1M, acetonitrile, DPPH, methanol proanalysis and solution of ascorbic acid standard.

The utensils used in production of tempe were stove, pan, bucket, scale, plastic bag, spoon and stirrer. The utensils used in production of tempe extract were stove, pan, scale, filtering thread, blender and thermometer. The utensils used in canning of tempe extract were retort, thermocouple, thermorecorder, exhaustor, double seamer and metal cans. The retort used was a vertical retort with dimension 325x733mm<sup>2</sup> with brand Sa Bertozzi Brugherio Milano series 14 886 B year 1982 made in Italy. The cans used were type 6Z sized 202x308 with tin as their basic material and oleoresin as their enamel. The main utensils used in analysis were electrical furnace, oven, distillation kit, Kjehldahl flask, HPLC-meter with column C-18 and Spectrophotometer UV-Vis. The HPLC-meter used was FlowCal 5000 HPLC Liquid Flow Meter made in USA with

dimension 138x76x45 mm<sup>3</sup>. Spectrophotometer used was double beam Biochrom Libra S60 made in Great Britain.

## Methods

This study was classified into four stages: (1) fermentation of tempe, (2) production of tempe extract, (3) canning of tempe extract and (4) production feasibility analysis of canned tempe-extract.

### *Fermentation of tempe* [7]

Soybeans were cleaned, boiled for 30 minutes, then soaked for 48 hours. The soybeans were then husked manually, boiled for 30 minutes, leached and cooled. Starter with amount of 5g/kg tempe was mixed with soybeans. The soybeans were then wrapped in holed plastic bags and fermented for 36 hours at room temperature (25°C) to produce raw tempe.

### *Production of tempe extract*

Tempe was cut into uniform pieces, boiled for 10 minutes then leached. Tempe pieces were then extracted with boiled water using a blender with ratio between water and tempe of 8:1(v/w). The puree was filtered and produced filtrate as tempe extract and cake as by product. Pandanus leaves (80 g/L tempe extract) and vanillin powder (20 g/L tempe extract) were added into the extract. The tempe extract was then boiled at 100°C for 2 minutes. Following boiling, the extract was cooled down until it reached a temperature of 50°C. Then, CMC (100 ppm) and sweetener (7%(w/v)) were added.

In this study, 3 formulae for producing tempe extract were used. The difference among them was the type of additive used as a sweetener. The items used as sweetener for each formula were granulated sugar, honey and brown sugar. In addition to these formulae, a soybean extract with granulated sugar as sweetener was made as a standard for comparison. The formulation of tempe extract with basis of 1 kg tempe is shown in Table 1.

**Table 1. Formulation of tempe extract (basis 1 kg tempe).**

Materials	Amount			
	Formula I	Formula II	Formula III	Soybean extract
Tempe	1 kg	1 kg	1 kg	Soybean (1 kg)
Water	8 litre	8 litre	8 litre	8 litre
Sweetener	Gran. sugar (560 g)	Honey (560 g)	Brown sugar (560 g)	Gran. sugar (560 g)
CMC	800 mg	800 mg	800 mg	-
Pandanus leaves	80 g	80 g	80 g	-
Vanillin powder	20 g	20 g	20 g	-

### *Canning of tempe extract*

Tempe extract with volume of 150 ml was filled into a can. Exhausting, seaming and sterilization, respectively, were then applied. Analysis of heat distribution inside the retort and heat penetration inside the product were needed to determine the sterilization time. This analysis was done by measuring the profile of heat increase in the retort and product using a thermocouple [8]. The sufficient sterilization time of canned tempe-extract was determined by plotting time of heating and lethal rate calculated from the temperature. The area below the curve shows the value of Fo, the time needed to sterilize canned tempe-extract at temperature 121°C. During this stage, sensory analysis and chemical analysis were also done. The sensory analysis included hedonic rating test of formulae in Table 1 (70 untrained panelists, category scale 1-7, sig. 5%) and triangle test

between unsterilized tempe extract and sterilized tempe extract (30 untrained panelists, sig. 5%)<sup>[9]</sup>. The chemical analysis included pH measurement, proximate analysis, isoflavone analysis and antioxidant capacity analysis. Measurement of pH was done by using a pH-meter [10]. The proximate analysis included analysis of water content (gravimetric), analysis of ash content (gravimetric), analysis of protein content (Kjehldahl method), analysis of fat content (Soxhlet method) and analysis of carbohydrate content (by difference) [11]. Isoflavone analysis was done by using HPLC-meter with genistein and daidzein as standards [12]. Antioxidant capacity analysis was done by using DPPH assay [13].

#### ***Production feasibility analysis of canned tempe-extract*** [8]

Production feasibility analysis of canned tempe-extract included the quantification of payback period, net present value, profitability index and break-even point. These criteria were quantified based on assumption of middle-level factory with total area of 75 m<sup>2</sup> and worker amount of 10 people.

## **Results and Discussion**

#### ***Determination of chosen tempe extract formula***

Based on hedonic rating test, it can be concluded that the chosen tempe extract formula is formula II (tempe extract with honey 7%(w/v) as sweetener). This formula had the highest rating in overall parameters. Additionally, this formula is the only formula which had an overall rating higher than soybean extract as standard of comparison. The chosen tempe extract formula (formula II) has overall rating of 5.7 out of 7. The responses of panelists in hedonic rating test are shown in Table 2.

**Table 2. Responses of panelists in hedonic rating test of tempe extract formulae and soybean extract.**

Sample	Rating			
	Colour	Aroma	Taste	Overall
Tempe extract (granulated sugar)	5.5 <sup>b</sup>	5.1 <sup>a,b</sup>	5.3 <sup>b</sup>	4.9 <sup>b</sup>
Tempe extract (honey)	5.8 <sup>c</sup>	5.7 <sup>c</sup>	5.8 <sup>c</sup>	5.7 <sup>d</sup>
Tempe extract (brown sugar)	4.4 <sup>a</sup>	5.3 <sup>b</sup>	4.7 <sup>a</sup>	4.4 <sup>a</sup>
Soybean extract	5.7 <sup>c</sup>	5.0 <sup>a</sup>	5.4 <sup>b</sup>	5.2 <sup>c</sup>

#### ***Determination of heat sufficiency in canned tempe-extract sterilization***

The target of sterilization used in this study was the microbe *Clostridium botulinum*. This decision was taken due to the canned tempe-extract having a pH value of 7.48. Therefore, canned tempe-extract was classified as low acid food. The thermal process which must be applied to low acid food is sterilization [8]. In sterilization, the targeted microbe is *C. botulinum* [14]. The Do value of *C. botulinum* is 0.2 minutes [14] Do value means the time needed to reduce the amount of *C. botulinum* by one logarithmic cycle at 121°C [14]. In this sterilization process, it was targeted that the amount of *C. botulinum* in the product would be reduced by 12 logarithmic cycles. Therefore, the Fo value targeted in this sterilization was 0.2x12=2.4 minutes.

Based on the result of analysis of heat distribution, the retort had venting time of 15 minutes and come-up time of 22 minutes after the activation of the retort. Results of analysis of heat penetration showed that the time needed to sterilize canned tempe-extract was 25 minutes after the activation of the retort. This thermal application was equivalent to Fo of 2.62 minutes.

***Nutritional content of canned tempe-extract***

Based on the results of proximate analysis, it was revealed that canned tempe-extract contained 90.27% water, 0.06% ash, 3.66% protein, 0.98% fat and 5.03% carbohydrate. Therefore, one package of canned tempe-extract (net volume 150 ml) contained 5.49 g protein, 1.47 g fat and 7.54 g carbohydrate with total energy of 65 Kal. Generally, a person is suggested to consume 2000 Kal/day, 50 g protein/day, 55 g fat/day and 325 g carbohydrate/day [15]. Based on this suggestion, it can be known that consumption of one package of canned tempe-extract may contribute to 3% of daily energy needs, 11% daily protein needs, 3% daily fat needs and 2% of daily carbohydrate needs. Canned tempe-extract still contained nutrition in adequate amounts and can therefore be used as a good source of nutrition.

***Changes in sensory characteristics of canned tempe-extract caused by sterilization***

Based on the result of the triangle test, the amount of panelists which can distinguish sterilized canned-tempe extract from unsterilized canned-tempe extract were 9 panelists. This amount is lower than the minimum amount of panelists required from the binomial probability table ( $p=0.33$ ;  $n=30$ ;  $\alpha=0.05$ ), which is 15 panelists. Therefore, it can be concluded that the sterilization process did not affect the overall sensory characteristics of canned tempe-extract significantly in degree of confidence of 5%.

Application of thermal processes in protein-based food may lead to changes in sensory quality, such as browning and sedimentation of protein. Browning can be caused by Maillard reaction between amine groups of proteins with carbonyl groups of reducing sugars catalyzed by heat. This reaction may lead to forming of brown pigment called melanoidine which may cause browning in food. The sedimentation of protein may be caused by the denaturation of protein by heat. Denaturation of protein may decrease its solubility in water. Therefore, the protein will settle down and form sediment [16]. There was no detected change in sensory characteristics of canned tempe-extract by the panelists because the protein and carbohydrate content of canned tempe-extract was relatively low. Therefore, the reaction between them may not contribute to the sensory characteristics of canned tempe-extract significantly.

***Isoflavone content of canned tempe-extract***

Based on the results of isoflavone analysis, it was revealed that canned tempe-extract contained total isoflavones of 5.09 mg/100 g (wet basis). This amount is divided into glycoside isoflavones of 1.94 mg/100 g (daidzin 0.77 mg/100 g and genistin 1.17 mg/100 g) and aglycone isoflavones of 3.15 mg/100 g (daidzein 1.56 mg/100 g and genistein 1.59 mg/100 g). Therefore, one package of canned tempe-extract (net volume 150 ml) contains about 7.64 mg isoflavones. Some nutrition experts suggest the minimum daily human consumption of isoflavones should be 30 mg [4]. Based on this suggestion, it can be concluded that one package of canned tempe-extract may contribute to about 25% of the suggested daily isoflavone consumption.

***Antioxidant capacity of canned tempe-extract***

Based on the results of antioxidant capacity analysis using DPPH assay, it was revealed that canned tempe-extract had antioxidant capacity of 7.13 mg AEq/150 ml, which means 150 ml canned tempe-extract had equivalent antioxidant capacity to 7.13 mg ascorbic acid. Comparison between antioxidant capacity of canned tempe-extract with other food cited from literature can be seen in Table 3.

**Table 3. Comparison of antioxidant capacity of tempe-extract with other food products.**

Product	Antioxidant capacity	
Canned tempe-extract	4.75	mgAEq/100ml
Commercial UHT soymilk [17]	4.09	mgAEq/100g
Soy ice cream [18]	7.2	mgAEq/100g
Soybean [19]	30.69-75.88	mgAEq/100g
Green tea [20]	218	mgAEq/100ml
Black tea [20]	146.5	mgAEq/100ml

Based on the table, there is no significant difference of antioxidant capacity of canned tempe-extract among soybean-based food (commercial UHT soymilk and soy ice cream). Canned tempe-extract had lower antioxidant capacity than soybean because of the dilution in canned tempe-extract production. Nevertheless, canned tempe-extract had much lower antioxidant capacity compared with green tea and black tea. Based on *in vitro* assay, antioxidant components of green tea and black tea had high antioxidant capacity. However, based on *in vivo* assay, tea had very low bioavailability due to the limitation of intestinal mucosa's permeability in absorbing tea catechine [21]. Isoflavone, the main component contributing to antioxidant capacity in canned tempe-extract had high bioavailability. Its bioavailability was higher than catechine in tea and also had the polyphenol molecule having the highest antioxidant capacity [22]. Therefore, it can be concluded that canned tempe-extract was soybean-based food with good antioxidant capacity, but cannot be used as the main antioxidant source for human consumption.

#### ***Production feasibility of canned tempe-extract***

In this economic quantification, it was established that the price of one package of canned tempe-extract was Rp3,500.00. Based on the result of this study, it was determined that each day, 2,800 cans tempe-extract can be produced. The results of quantification of canned tempe-extract's production feasibility and the standards used can be seen in Table 4.

**Table 4. Quantification of production feasibility of canned tempe-extract.**

Parameter	Value	Feasibility requirements
Payback period (PP)	2 years	PP<5 years [23]
Net present value (NPV)	Rp342,169,455.00/5 years	NPV has positive value [8]
Profitability index (PI)	2.16	PI>1 [8]
Break-even point (BEP)	16,470 products/month	-

From the table, it was revealed that commercial production of canned tempe-extract was feasible. The value of PP, NPV and PI fulfilled the standards of production feasibility cited from the literature. The value of BEP was 16,470 products per month. Compared with daily production which may reach 2,800 products, this number had high probability to be achieved. The BEP can be passed in six days of production if the all of the products produced can be sold. Therefore, it can be concluded that the production of canned tempe-extract was economically feasible.

#### **Conclusions**

Formula of tempe extract which panelists preferred was tempe extract with 7%(w/v) honey as sweetener. This formula then was canned using can size 202x308 with net volume 150 ml. The sterilization of canned tempe-extract was 25 minutes after the activation of the retort to reach sterilization level equivalent to Fo value of 2.62 minutes. Sterilization process did not affect the sensory characteristics of canned tempe-extract significantly. Canned tempe-extract contained 90.27% water, 0.06% ash, 3.66% protein, 0.98% fat and 5.03% carbohydrate. Total isoflavones content of canned tempe-extract was 5.09 mg/100 g, which contributed to about 25% of the suggested daily isoflavone consumption. Canned tempe-extract had antioxidant capacity of 7.13

mgAEq/150 ml. The production feasibility analysis showed that production of canned tempe-extract was feasible to be done.

It is suggested that, to further develop the formula of canned tempe-extract, studies such as the application of flavouring agents into the formula to increase its hedonic rating should be undertaken. The other formula development can be considered is acidification of canned tempe-extract formula until pH<4.6 is reached. Therefore, pasteurization can be applied to this product as a method of preservation. Pasteurization is cheaper, easier and more feasible to be applied in small-scale industry. Additionally, analysis and development of tempe cake (by product) as a functional food still can be considered because tempe cake still contains nutritional and non-nutritional compounds, such as protein, fat, dietary fibre and isoflavones.

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