



# The Addition of Natural Colour Pigment Brazilin from Sappan Wood Extract (*Caesalpinia Sappan Linn*) in the Manufacture of Tempeh to Improve Tempeh Product Antioxidant Content

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**Abstract.** Sappan wood with the Latin name (*Caesalpinia Sappan Linn*) is a plant whose wood is used as a colorant in food. Brazillin, is a yellow crystal which is the colour pigment in the cup. Brazillin can inhibit surviving apoptosis inhibitor proteins, so that it is able to cancer. Tannins are components of very complex organic substances. The active compound of tannins functions. Saponins are complex glycosides with high molecular weights. Saponins function as substances so that they can help in the wound healing process. Alkaloids and Flavonoids, have a role as antioxidants, one of which is anthocyanins, secang wood dyes were taken by extraction with the maceration method because the dye in sappan wood is anthocyanin which is a type of flavonoid antioxidant that is not resistant to high temperatures to separate the extract from the solvent so that the concentration of the extract is more concentrated. The resulting secang extract contains many properties, one of which is antioxidants, namely as an antidote to free radicals which are very much needed in the current pandemic era. In this study, innovation was carried out by adding sappan wood extract as an additive to dye in soybean tempeh in order to increase its antioxidant content. The antioxidant content of ordinary soybean tempeh is around 85.97%, while the antioxidant content of soybean tempeh with secang extract is 88.27%. It can be said that the addition of Sappan wood extract in soybean tempeh succeeded in increasing its antioxidant content. Soy tempeh samples were further analysed for using the scoring method.

**Keywords:** Sappan Wood · Maceration · Antioxidant · Tempeh Antioxidant

## 1 Introduction

In the current pandemic era, the most important thing to maintain is physical health. In addition to carrying out health protocols, implementing a healthy lifestyle by eating nutritious foods will help maintain endurance. In the journal “How Nutrition Can Help to Fight Against the *Covid-19* Pandemic”, a balanced diet will ensure a strong immune

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**Table 1.** SNI 3144.2009 Tempeh Standard

No	Conditon Test Criterion	Unit	Regulation
1	Aroma	-	Normal, Special
	Colour	-	Normal
	Taste	-	Normal
2	Water Level (b/b)	%	Max. 65
3	Ash Level (b/b)	%	Max. 1,5
4	Fat Level (b/b)	%	Max.10
5	Protein Level (N x 6.25) (b/b)	%	Max. 16
6	Crude Fiber Level (b/b)	%	Max 2,5

Source: Directorate of Nutrition, Ministry of Health RI, 2009

system that can help resist viral attacks. Tempe is a typical Indonesian food that is very popular in the community. Soybean tempeh is processed from fermented soybeans with the fungus *Rhizopus sp.* Soybean tempeh on the market has a white, slightly bright yellow colour. The tempeh processing process starts with washing and cleaning soybeans, peeling, soaking, boiling, draining, cooling, and drying, inoculation, packaging, and fermentation with *Rhizopus sp.*, incubation be a tempeh product with a fermentation process at a temperature of 25 °C; for 80 h, a temperature of 25–37 °C; for 20–50 h, a temperature 32 °C; for 20–22 h, and a temperature of 35–38 °C; for 15–18 h. The process of fermentation produces a reaction.

The tempeh produced has a taste, texture, appearance, and aroma that is suitable for various types of cuisine, where tempeh contains complete nutrients with a distinctive meat-like flavour, is easily digested by the body, and can be used as a substitute for meat, tempeh. Not only safe but also has several functional compounds such as isoflavones, so that it can contribute to health in the body, in according with the tempeh standard SNI 3144-2009 shown in Table 1 Tempe Standard.

### 1.1 Definition of Sappan Wood

Sappan wood with the Latin name (*Caesalpinia Sappaan Linn*) is a leguminous tribe. Sappan wood plants come from Southeast Asia and are quite widely spread in several islands in Indonesia. Sappan wood is widely known as a plant that can grow wild in rocky mountainous areas that are not too cold or in open places on high land [19].

In This wild plant can grow up to 6 m and has brownish green round leaves and reddish woody stems. This red colour on the bark of the sappan tree gives red colour as an additional colouring agent for painting and gives colour to woven materials, cakes, drinks, or as ink [18] (Fig. 1).



**Fig. 1.** Sappan wood

## 1.2 The Content and Benefit of Sappan Wood

This Sappan wood contains chemical compounds that have benefits, including:

1. Brazillian, is a yellow crystal which is the color pigment in the cup. Brazillian can inhibit surviving apoptosis inhibitor proteins so that it can treat cancer [19].
2. Tannins are components of very complex organic substances. The active compound of tannins functions as astringent, anti-diarrhea, anti-bacterial, and antioxidant [7].
3. Saponins are complex glycosides with high molecular weights. Saponins function as anti-inflammatory, anti-oxidant, anti-bacterial, and anti-fungal substances so that they can help in the wound healing process [9].
4. Alkaloids and Flavonoids, have a role as an antioxidant, one of which is anthocyanins. The antioxidant index of sappan wood water extract is higher than commercial antioxidants so it has the potential as a free radical scavenging agent. Antioxidant compounds from natural ingredients produce residues that are more easily degraded naturally than synthetic materials. Another benefit of sappan wood is that it can be used as an antibacterial, antiviral, anti-inflammatory, anticancer, and antitumor. Therefore, sappan wood water extract can be used as an herbal drink for health and for treating disease [19].

In the research that has been done, it is an innovation, namely by adding dyes from natural ingredients, in with is the natural colour of sappan wood (*Caesalpinia Sappan Linn*) which is rich in antioxidants and its availability is quite abundant in Indonesia. An innovative step in the use of natural dyes from plants that are easily available around the community so that they can be creative with various colours that will be very attractive and have economic value without reducing the nutritional content benefits for the human body, by accordance with the Regulations of the Food and Drug Supervisory Agency of the Republic of Indonesia (BPOM) Number 11 of 2019 concerning “Food Additives”. The extraction of sappan wood dye is carried out by extraction with the maceration method because the dye in sappan wood is anthocyanin which is one type of antioxidant of the flavonoid group which is not resistant to high temperatures [13].

The addition of dyes from sappan wood to soybean tempeh has an important effect on increasing antioxidant levels in soybean tempeh because sappan wood contains anthocyanins. The antioxidant index of sappan wood water extract is higher than commercial antioxidants so it has the potential as a free radical scavenging agent. In addition, antioxidant compounds from natural ingredients produce residues that are more easily degraded naturally than synthetic materials. Another benefit of sappan wood is that it can be used as an antibacterial, antiviral, anti-inflammatory, anticancer. Therefore, sappan wood water extract can be used as an herbal drink for health and treating disease [6]. In addition, the addition of this dye is also expected to increase the attractiveness of the tempeh itself.

## 2 Research Methodology

In this study, the extraction of food colouring was carried and the maceration method and concentrated a rotary evaporator. Then the resulting dye extract will be used as a variable in the innovation of making soybean tempeh.

This research was conducted to find the right formulation for adding sappan wood extract to produce good colour and, increase antioxidant levels without disturbing the performance of *Rhizopus sp.*

### 2.1 Research Design

In this study, experiments were carried out using soybeans as the main raw material for making tempeh and then varied with natural dye variables from sappan wood extract to determine the appropriate volume of addition of extract without disturbing the performance of *Rhizopus sp.* The steps taken are as follows:

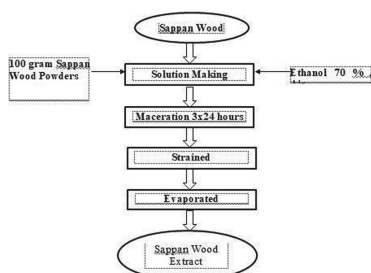
- a) Preparation of raw materials, where at this stage the preparation of raw materials such as soybeans, yeast, solvents, and sappan wood is carried out.
- b) Extraction of natural dyes from sappan wood using two different solvents by maceration method, then concentrated by means of a Rotary Evaporator. This dye will be used as a food additive for soybean tempeh colouring.
- c) Making tempeh with the addition natural dyes with variable volumes of sappan wood extract.
- d) Anthocyanin analysis, antioxidant analysis, moisture content analysis, ash content analysis, and hedonic analysis in the form of organoleptic analysis (taste, colour, aroma) (Table 2).

### 2.2 Observation

This study will use variations in the composition of Secang Wood Extract to obtain good quality tempeh data without disturbing the performance of *Rhizopus Sp* and according to the tempeh standard of SNI 3144:2015. Some of the analyses carried out are as follows:

**Table 2.** Table of Variations in Composition of Addition of Secang Wood Extract Standard

Type of Solvent	Amount of Extract (ml)	Weight of Soybean (gram)	Yeast Weight (gram)	Inoculation Time (hour)
Ethanol	10	250	0.25	40
	30	250	0.25	40
	50	250	0.25	40
	70	250	0.25	40
Water	0	250	0.25	40
	10	250	0.25	40
	50	250	0.25	40
	70	250	0.25	40

**Fig. 2.** Stages of colour extract making

1. Yield Analysis [1].
2. Anthocyanin Analysis with a Ph Differential Method
3. Water Content Analysis [10].
4. Antioxidant Activity Analysis
5. Ash Content Analysis [1].

### 3 Material and Method

#### 3.1 Research and Procedure

##### Colour Extract Making

Making colour extract from Sappan Wood by maceration process. According to the research procedure conducted by [13] with the following stages as shown in Fig. 2.

##### Stages of Making Tempeh

The soybean tempeh fermentation process is carried out according to the tempeh standard of [8]. Figure 3 shows the stages of making soybean tempeh.

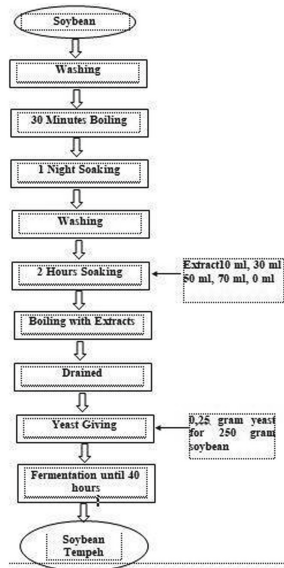


Fig. 3. Stages of making soybean tempeh

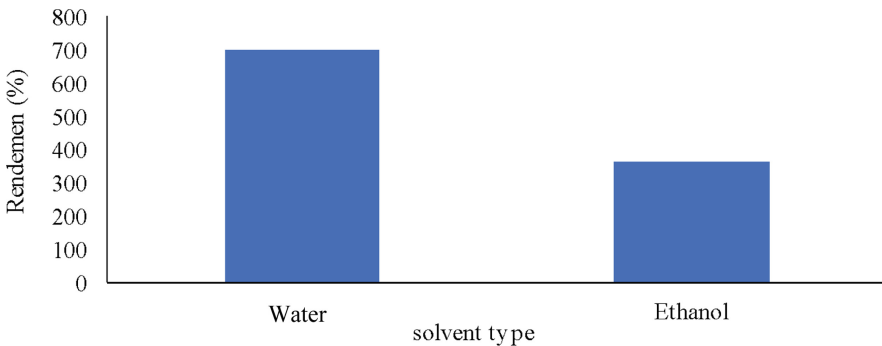


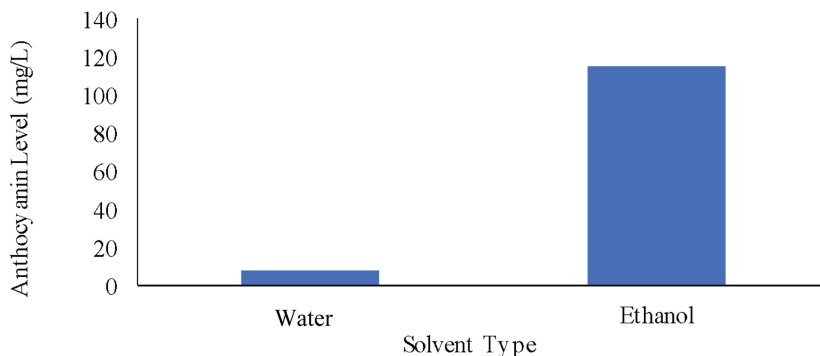
Fig. 4. Analysis of Solvent Types on Yield of Sappan wood Extract

## 4 Results and Discussions

### 4.1 Analysis of Yield

Sappan wood with extract solution with water as a solvent produced a higher yield of 700.77 or 7.007%, while with ethanol as a solvent it produced a lower yield of 361.195 g or 3.61% (Fig. 4).

According to the research of [5], this may occur because the residual water solvent is quite high due to incomplete evaporation, while ethanol solvent is a volatile compound with a lower boiling point so it will be easier to evaporate.



**Fig. 5.** Analysis of Solvent Types on Anthocyanin Levels of SappanWood Extract

## 4.2 Analysis of Anthocyanins

Anthocyanins are bioactive components of the flavonoid group that can give red, purple, blue colours to flowers, tubers, fruits and vegetables [8]. Anthocyanin levels were tested using the Differential pH method, namely by measuring the absorbance of the extract at a wavelength of 510 nm and a wavelength of 700 nm at pH 1 and pH 4.5.

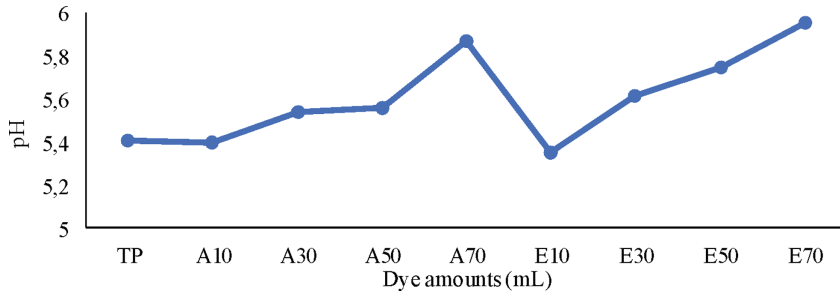
In this study, the anthocyanin content of the secang wood extract with water as a solvent was 8.33323 mg/L while the ethanol solvent contained more anthocyanins, namely 115.0386 mg/L. This happened because the effectiveness of the extraction was influenced by the type of solvent used. Ethanol solvent has almost the same polarity as anthocyanins so that it is easily absorbed by cell membranes, where the function of the solvent in the extraction is to break the membrane contained on the surface of the sappan wood particles so that a lot of sappan wood tissue is broken, the more anthocyanin levels will be.

## 4.3 Analysis of Degree of Acidity (pH)

The degree of acidity (pH) is an important factor that affects the colour of anthocyanins, under acidic conditions (low pH) anthocyanins are red, while in alkaline conditions (high pH) anthocyanins are blue [5].

Measurement of the degree of acidity (pH) using a pH meter. Sappan wood extract with 70% Ethanol solvent contains a more acidic pH of 6.5 with a reddish-orange extract colour while the sappan wood extract with water solvent contains a neutral pH of 7 with a dark red colour.

Sappan wood extract is then added to the soybean soaking process to give it colour. The addition of this sappan wood extract done by varying the amount of 0 ml, in each extract solution with different solvents. Then the soybeans and the extract are boiled together until cooked. Boiling water that still contains sappan wood extract is measured for its acidity using a pH meter. The following is a graph of the ratio of pH to the quantity of sappan wood extract (Fig. 6).



**Fig. 6.** Analysis of the amount of dye on the pH of boiled water



**Fig. 7.** Before and After Fermentation

Sample Code:

A70 = Soybean tempeh extract a cup of water solvent 70 ml

E70 = Tempeh soybean extract cup of ethanol solvent 70 ml

TP = Soy Tempeh without colouring

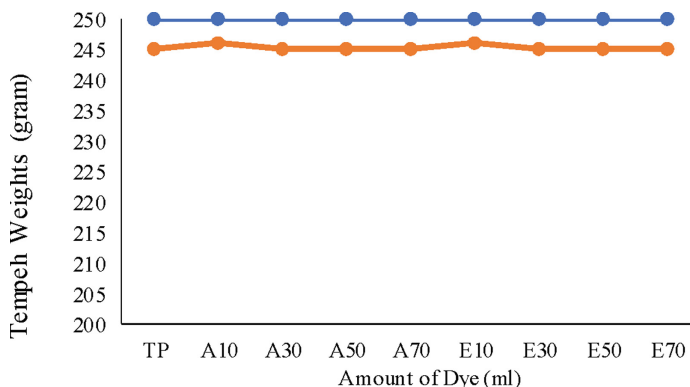
In this study, the pH of the sappan wood extract which had been varied decreased to a pH of 5.35–5.95. This happens because of the influence of the soybean soaking process which is a pre-fermentation process in which lactic acid bacteria grow spontaneously. This soybean pre-fermentation process results in a decrease in the pH of soybean cooking water [8].

#### 4.4 Tempeh Analysis Before and After Fermentation

The soybean tempeh fermentation process begins after adding yeast to every 250 g of soybeans that have been given a variation in the amount of dye. During the fermentation, there is a chemical reaction in soybeans. These changes occur because the soybean substrate (protein, fat, carbohydrates, and other compounds) is decomposed into smaller molecules by enzymes produced by the fungus *Rhizopus sp.*

This fungus *Rhizopus sp.* will form white hyphae and cover the entire surface of soybean seeds, then coalesce to form a white mycelium [6]. Figure 7 shows the picture of soybean samples that have been coloured and fermented for 2 days.





**Fig. 8.** Analysis of the Amount of Dyes on Changes in Fermentation Weight

From Fig. 5, it can be seen that soybean tempeh after fermentation has a white colour due to the growth of mycelium from *Rhizopus sp* so that the soybeans that have been treated with Sappan wood dye are covered. Of the 9-growth fungus *Rhizopus sp*. that mushrooms will thrive at pH 3.5–6, so the fermentation process will occur perfectly.

This fermentation process results in changes before and after fermentation as shown in Fig. 8.

Sample Code:

A70 = Soybean tempeh extract a cup of water solvent 70 ml

E70 = Soy tempeh extract a cup of ethanol solvent 70 ml

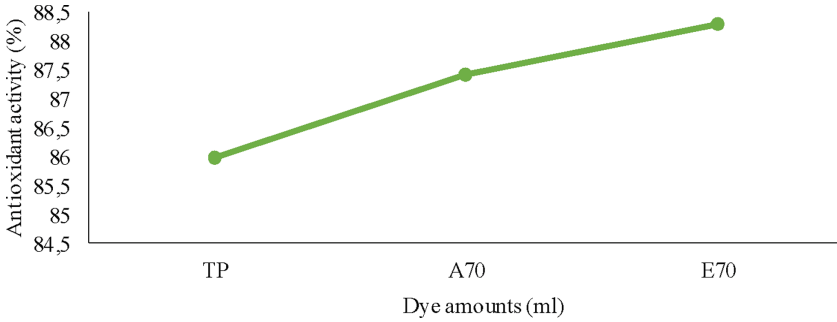
TP = Soy tempeh without dye

In this study, the antioxidant activity of soybean tempeh without dye was 85.97%. Meanwhile, soybean tempeh with water and ethanol as a solvent contained 87.4% and 88.27% antioxidants, respectively. The highest antioxidant increase occurred in soybean tempeh which was dyed with sappan with ethanol as a solvent. This happens because the antioxidant compounds in sappanwood are phenolic flavonoids [12]. Where the hydrogen from the hydroxyl group of the phenolic compound can bind to the hydroxyl group of the ethanol compound, increasing the solubility of phenolic compounds in ethanol [12].

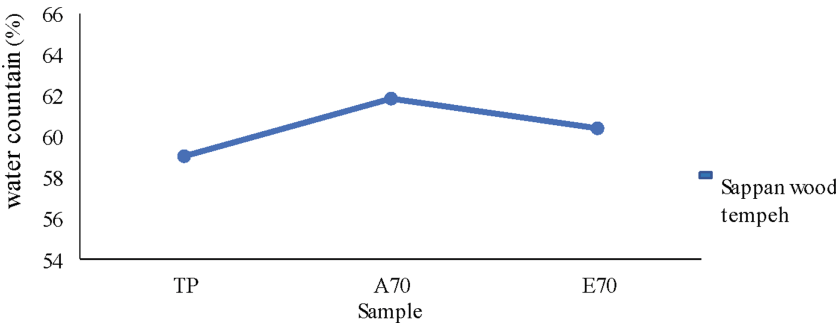
#### 4.5 Analysis of Antioxidant

Antioxidants are molecules that can inhibit the oxidation of other molecules or can counteract free radicals [3] Antioxidant levels were measured by means of a UV-Vis spectrophotometer using the DPPH method with absorbance monitoring at a wavelength of 517 nm. Results the antioxidant activity test of soybean tempeh with sappan wood dye can be seen in Fig. 9.

In this study, the antioxidant activity of soybean tempeh without dye was 85.97%. Meanwhile, soybean tempeh with water and ethanol as a solvent contained 87.4% and 88.27% antioxidants, respectively. The highest antioxidant increase occurred in soybean tempeh which was dyed with sappan with ethanol as a solvent. This happens because the



**Fig. 9.** Effect of Secang Wood Extract with Water and Ethanol Solvent on Antioxidant Activity of Tempe



**Fig. 10.** Effect of Sappan wood Extract with Water and Ethanol Solvent on Tempe Water Content

antioxidant compounds in sappanwood are phenolic flavonoids [9], where the hydrogen from the hydroxyl group of the phenolic compound can bind to the hydroxyl group of the ethanol compound, increasing the solubility of phenolic compounds in ethanol [3].

#### 4.6 Analysis of Water Content

Moisture content a test method used to measure the water content in a material. Moisture content can also determine food quality and resistance to damage [2]. The analysis of the moisture content of soybean tempeh uses the thermogravimetric method, by drying the material in an oven at 105 °C for 3 h and then calculating the decrease in sample weight during drying [3]. Analysis of the water content of soybean tempeh was accordance with the quality standard of tempeh which refers to [12] published by the National Standardization Agency (BSN). Figure 10 shows the graph of the analysis of the amount of dye on the water content:

Samples A70, E70, and TP were in accordance with the quality standard of SNI 3144:2015 of soybean tempeh with a maximum moisture content of 65%.

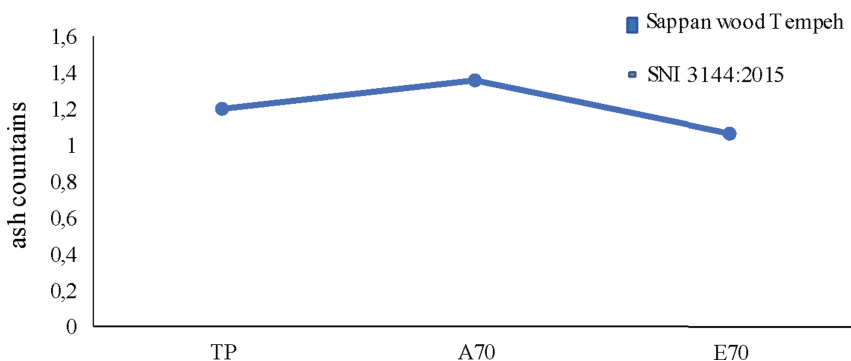


Fig. 11. Effect of Sappan wood Extract

#### 4.7 Analysis of Ash Content

Ash content is a test method for a material to measure the content of mineral or inorganic salts in it. The higher the ash content, the more inorganic material content in it. Analysis using the ashing method in a furnace at a temperature of 600 °C for approximately 4 h [1].

Analysis of ash content was carried out to determine whether the ash content of soybean tempeh is in accordance with the quality standard of soybean tempeh which refers to [10] issued by the National Standardization Agency (BSN). Figure 11 shows the graph of the analysis of the amount of dye on the ash content of soybean tempeh with Water and Ethanol Solvent on Tempe Ash Content.

In this study, the ash content in the A70 sample was 1.35%, the E70 sample was 1.06% and TP sample was 1.19%. Sample A70 has the highest ash content while sample E70 has the lowest ash content because the A70 soybean tempeh sample contained sappan wood dye with water as a solvent, where water was an inorganic compound containing minerals [4]. The ash content of samples A70, E70, and TP was by the quality standard of [12] from soybean tempeh with a maximum ash content of 1.5%.

## 5 Conclusion

Extraction of sappan wood dye with ethanol solvent is more optimal in dissolving anthocyanin active compounds in sappan wood compared to using water solvent because ethanol solvent has almost the same polarity as anthocyanin so it is easily absorbed by cell membranes, where the solvent function in extraction is to break down the membrane contained on the surface of the sappan wood particles so that a lot of sappan wood tissue is broken, the more anthocyanin levels will be.

Soy tempeh with sappan wood dye contains more antioxidants than ordinary soy tempeh. This indicates that the addition of sappan wood dye in the process of making soybean tempe increases the antioxidant content in soybean tempeh.

Soybean tempeh with sappan wood dye has complied with the quality requirements of tempeh which refers to [12] in terms of water content and ash content which does not exceed the maximum limit that has been set.

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