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Black Soybean Tempeh Ice Cream (Glycine Max Var. Mallika) Rich in Antioxidants to Increase Endurance

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Low fat intake can contribute to preventing and reducing the risk of degenerative diseases and antioxidants can prevent oxidative stress and increase the body's endurance. Soybeans are a source of low-fat vegetable protein and contain isoflavones which have the potential to act as anti-inflammatory agents. Butterfly pea flowers are one of the plants that are used as a source of antioxidants. This research aims to develop ice cream that is low in fat and contains antioxidants by using black soybean tempeh milk and butterfly pea flower coloring. This research stage includes formula creation, sensory testing, analysis of physical characteristics, and analysis of antioxidant capacity. This research used three treatments with different dilution ratios of black soybean tempeh and water, namely P1 (15% dilution), P2 (20% dilution), P3 (35% dilution). Based on the results of the sensory test, P3 was the selected formula. The antioxidant capacity of black soybean tempeh milk ice cream with the addition of butterfly pea flower coloring is stated in IC50 at 6816.66 ppm or the equivalent of 13.56 mg Vitamin C/100 g. One serving of soy milk ice cream in this study can fulfill the low-fat claim and antioxidant.

1. Introduction

Tempeh is a processed soybean product that is popular in Indonesia, as a fermented soybean product using Rhizopus mold or better known as tempeh yeast. Making tempeh generally uses yellow soybean raw materials, but lately it is popular to develop processed tempeh products using raw materials for various types of beans, one of which is black soybeans.

In Indonesia the use of black soybeans (Glycine max var. Mallika) as a food raw material is still relatively more limited than yellow soybeans. This is because black soybeans contain anti-nutritional compounds, one of which is tannins whose levels are four times higher than yellow soybeans (Palupi &; Rahmatika, 2022)

Research by (Nurrahman et al., 2012) states that black soybeans mallika variety has the highest content of daidzein isoflavones, amino acids, glutamate, oleic acid, and linoleic acid compared to other soybeans. Tempeh is classified as functional food by having a variety of nutrients besides the fermentation process of tempeh with Rhizopus type mold can produce protease enzymes, amylase and lipase that can break down complex carbohydrates, proteins and fats into a variety of simpler compounds. The increase in folic acid and vitamin B12 resulting from the fermentation process of vegetable materials (Pinasti et al., 2020). Black soybean tempeh is widely developed into various kinds of processed food products. Black soybean tempeh has a protein content of 63.01%, fat 8.69%, and carbohydrates 25.65% while yellow soybeans have a lower protein content of 45.93% and higher fat and carbohydrate content of 16.49% and 32.09% (Setiawan et al., 2022).

The use of black soybeans into tempeh which is a typical Indonesian food has the potential to be developed. Previous research has shown that black soybean tempeh milk can be an alternative to processed products that are ready for consumption. Black soy tempeh milk is known to have health benefits in lowering malondialdehyde levels in liver fibrosis rats (Stephanie et al., 2019).

According to (Zainal Abidin et al., 2020) one form of processed tempeh that can extend shelf life, through efforts to diversify food, and improve the image and acceptability of tempeh, namely by processing it into milk or tempeh milk drinks and ice cream.

Ice cream is generally made from fresh cow's milk which is high in cholesterol. Ice cream that can be developed is to substitute cow's milk with vegetable protein sources, so that it can be an alternative food to replace ice cream products containing cow's milk. Soy milk acts as a source of protein similar to cow's milk protein, but low in fat (An et al., 2024).

Cow's milk as the basic ingredient of ice cream can be substituted with black soybean tempeh milk as a substitute for protein in cow's milk, because, black soybean tempeh milk is superior in fiber content compared to cow's milk. Therefore, processed food that can be developed is ice cream with the basic ingredients of black soybean tempeh milk combined with telang flowers. While telang flowers are used as natural coloring ice cream products, because the natural color of black soybean tempeh milk is not attractive. In addition, by adding eagle flowers, it can add to the nutritional value of ice cream products, namely antioxidants (Nurcholis et al., 2023); (Yanti et al., 2023). Therefore, black soybean tempeh milk ice cream products can help prevent and reduce the risk of degenerative diseases and prevent oxidative stress (Stephanie et al., 2019) and increase endurance (Surya et al., 2021).

2. Research Method

2.1. Place and Time of Research

This research was carried out from September – December 2023 at the Agricultural Product Technology Laboratory of Lampung State Polytechnic, Bandar Lampung, Lampung.

2.2. Tools and Materials

The tools used in research, namely tools used in making ice cream consist of ice cream makers, mixers, filter cloths, scales, stoves, pots, basins, knives, and cool boxes. Equipment for testing the physical characteristics of ice cream includes a 100 ml cup glass, scale, refractometer and stopwatch. The sensory test requires stationery in the form of pens, organoleptic forms, spoons, and ice cream cups. Equipment for the analysis of antioxidant capacity includes analytical balances, porcelain dishes, aluminum cups, weighing paper, UV-Vis spectrophotometers, glass funnels.

The ingredients for making black soybean tempeh consist of black soybeans obtained from black soybean farmers in East Lampung district, Lampung province, water and yeast. The ingredients used in making black soybean tempeh milk are black soybean tempeh and water. The ingredients used to make coconut milk cream are grated coconut and water. The ingredients used to make the striped flower extract are telang flower and water. The ingredients used to make ice cream consist of major ingredients (black soy tempeh milk, coconut milk cream, and sugar) and minor ingredients (soy lecithin, gum arabic, vanilla extract, and telang flower extract). The material used in sensory tests and physical analysis is ice cream samples. In addition, the chemicals used are H_2SO_4, H_3BO_3 4%, NaOH 40%, HCl 0.1 N, NH_4SO_4, Na_2CO_3, $Na_2HPO_4.2H_2O$, $NaH_2PO_4.2H_2O$, NaN_3, selenium mix, hexane, aquades,

indicators of methyl red and methyl blue.

2.3. Research Stages

The study was conducted by making black soybean tempeh (Glycine max var. Mallika) based on processing methods from previous studies (Nurhidajah, 2010) and (Rahma &; Sutrisno, 2017) which were modified. Fermentation is carried out for 42 hours inside PP plastic packaging at room temperature. The process of making soy tempeh milk refers to (Filiyanti, 2013) and (Jauhari et al., 2014).

Steamed black soybean tempeh is mashed with a blender with the addition of water with a ratio of tempeh and water (15%, 20%, 35%) so that tempeh porridge is obtained. The tempeh pulp is then filtered with a filter cloth to separate the tempeh pulp from tempeh milk. The tempeh milk is then packed in sealed containers and stored in a chiller (4°C).

Planning ice cream formulas by modifying standard ice cream making recipes based on (Ahmed et al., 2023) and (Widiantoko &; Yunianta, 2014). Ice cream formulas that generally use cow's milk are fully substituted with black soybean tempeh milk as a substitute for protein and fat substitutes in cow's milk to form the texture and texture of telang flowers. Black soybean tempeh milk ice cream formulation (Glycine max var. Mallika) with the addition of telang flower extract can be seen in Table 1.

Table 1. Black Soybean Tempeh Milk Ice Cream Formulation

Bahan	Sum	Unit
Black soy tempeh milk	100	%
CORNSTARCH	6	%
Coconut coconut milk	40	%
Lesitin Soya	1	%
Arabic gum	1	%
Gula pasir	25	%
Vanilla	0,5	%
Coloring (cross flower extract)	8	%

2.4. Trial Plan

The experimental design used in this study was a Complete Randomized Design (RAL) with a factor of differences in the concentration of black soybean tempeh and water. The ratio of the concentration of black soybean tempeh with water consists of three levels, namely P1 (15% dilution), P2 (20% dilution), P3 (20% dilution). The response modifiers in this study were sensory properties, physical properties and antioxidant capacity of black soybean tempeh

milk ice cream.

2.5. Data Processing and Analysis

Data processing using Microsoft Excel 2016 was then analyzed using Statistical Product and Service Solution (SPSS) 16.0 for Windows. Organoleptic test data were analyzed with One Way ANOVA test and Duncan's Multiple Range follow-up test if ANOVA test results showed a noticeable treatment effect ($p<0.05$). Analysis of antioxidant capacity data using the Independent Sample T-test to see the effect of differences in black soybean tempeh milk ice cream diluted with black soy tempeh milk ice cream without dilution. The Independent Sample T-Test is conducted to determine the effect of a variable of two unpaired groups on other variables shown by real differences or not. The results of the analysis are said to differ significantly if the p-value is smaller than ($p<0.05$).

3. Result and Discussion

3.1. Hedonically Sensory Characteristics of Black Soybean Tempeh Milk Ice Cream

The hedonic test of ice cream assessment based on liking uses a hedonic scale consisting of nine scales, namely 1 (very, very dislike) to 9 (very, very like) against seven parameters, namely the appearance of color, aroma, texture, taste, mouthfeel, and overall acceptance in the hedonic test (Nurjanah et al., 2020). The product tested was black soybean tempeh milk ice cream with the addition of telang flowers consisting of three treatments of black soybean tempeh dilution and water, namely P1 (15% dilution), P2 (20% dilution), and P3 (35% dilution).

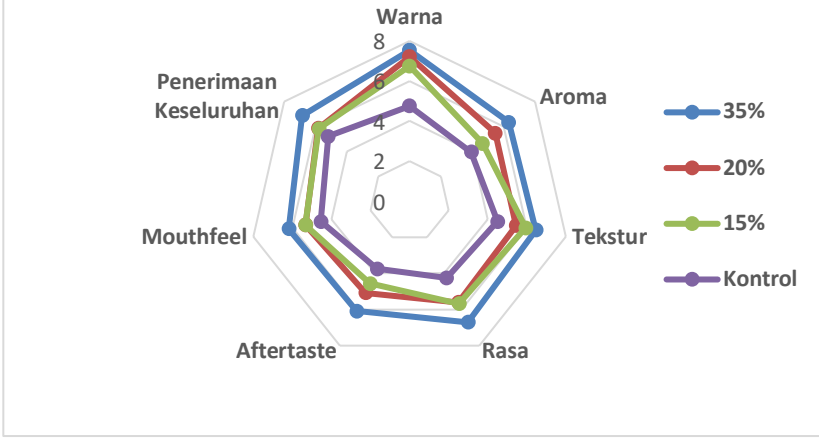

Figure 1 Hedonic sensory testing results of black soy tempeh milk ice cream product

Hedonic tests were conducted on all three ice cream treatments using a line scale starting from

lowest to highest intensity. The hedonic test aims to determine the level of preference of panelists for the three treatments without comparing between formulas. The test was conducted by semi-trained panelists or those who had gained knowledge related to organoleptic tests by 33 semi-trained panelists who were students of the Food Technology Study Program, Lampung State Polytechnic. Panelists gave feedback on their likes or dislikes of the product using a hedonic scale ranging from very, very dislike to very, very like. Tests were carried out on several hedonic test attributes, namely appearance or color, aroma, texture, taste, aftertaste, mouthfeel, and overall acceptance (Sari Putri &; Mardesci, 2018).

Based on sensory testing on the parameters of color, aroma, texture, taste, aftertase, mouthfeel, and overall acceptance of black soybean tempeh milk ice cream with the addition of telang flowers showed that on average panelists gave color values 6.75 - 7.53 (somewhat like), aroma 4.64-6.33 (somewhat dislike), texture 5.45-6.46 (neutral-somewhat like), taste 5.61-6.71 (neutral-somewhat like), aftertaste, 4.56-6.08 (somewhat dislike), mouthfeel 5.33-6.19 (neutral-somewhat like), and overall acceptance of 5.80-6.85 (neutral-somewhat like), with the highest acceptance value in P3 ice cream being the treatment with the most dilution of water addition compared to black soybean tempeh. Based on the results of statistical tests, it is known that there is no noticeable difference in the intensity of color, aroma, texture, taste, aftertase, mouthfeel, and overall acceptance between black soybean tempeh milk ice cream treatments ($p>0.05$). However, the treatment with the highest average yield is P3 which has a higher proportion of added dilution water than P1 and P2.

According to the panelists, the three treatments almost have something in common, namely there is still a distinctive langu smell of soybeans and still a little icy, but the treatment with the highest acceptance value is P3 because it has a lower proportion of black soybean tempeh than P1 and P2. This is related to the low fat content in soy milk, while fat has an important role in inhibiting the formation of ice crystals during freezing to produce a soft texture. According to the panelists, the ice cream flavor is better without well accentuating the soy flavor.

Control test results based on color parameters obtained a score of 4.75 which was preferred by panelists, aroma sample score 3.94, texture 4.5, taste 4.25, aftertaste 3.75, mouthfeel 4.55 and overall acceptance obtained a score of 5.2. Overall, control samples or undiluted black soy tempeh milk ice cream obtained results that panelists were less favorable to than diluted black soy tempeh ice cream samples. This is because black soybean tempeh milk ice cream has an aroma similar to herbal medicine so that it can change the overall perception of panelists in

the assessment of black soy tempeh milk ice cream samples.

Determination of the selected formula based on the product preferred by the panelists according to the results of sensory tests, namely hedonic tests. The selected treatment based on the results of the hedonic test showed marked differences in the attributes of color, aroma, texture, taste, aftertaste, mouthfeel and overall acceptance. The average value of each attribute for the three treatments shows that P3 has a higher value than P1 and P2, so P3 has better acceptance than P1 and P2. Consideration of the results of the hedonic test, the selected treatment was P3, namely black soybean tempeh milk ice cream with a dilution of 35%.

The characteristics of the main ingredient of ice cream, namely black soy tempeh milk, are taken into consideration, so that sensory attributes in the form of aroma, aftertaste, and sandy texture are important aspects in determining the chosen formula. Panelists preferred ice cream with a low intensity of langu aroma typical of soybeans. Aroma has an important role in increasing the attractiveness of a product in addition to color attributes, because volatile compounds can be received easily and quickly by the human sense of smell (Zainal Abidin et al., 2020). In addition, soybeans have a strong aftertaste so that they can reduce the preference for processed soybeans (Subali et al., 2023). Therefore, formulas with the best aroma and aftertaste values have a higher chance of being accepted by the wider community.

3.2. Physical Characteristics of Black Soybean Tempeh Milk Ice Cream

Analysis of the physical characteristics of black soybean tempeh milk ice cream was carried out by comparing the products produced with the quality requirements of ice cream based on SNI 01-3713-1995. The physical characteristics analyzed against black soybean tempeh milk ice cream consisted of overrun, melting time, and total dissolved solids. The results of the analysis of physical characteristics are presented in Table 2.

Table 2. Physical characteristics of black soybean tempeh milk ice cream

Physical Characteristics	Treatment		SNI Es Krim (3713:1995)
	P0 (without dilution)	P3 (dilution 35%)	
Overrun (%)	26,76 ± 0,22a	23,74 ± 0,36b	35 - 50
Melt time (minutes)	9.45 ± 0.46a	10,78 ± 0,39a	15 - 25
Total Dissolved Solids (^{obrix})	21,50 ± 0,71a	24,75 ± 0,35b	≥ 30

Information:

a, b : Different letters in the same line show a noticeable difference ($p<0.05$)

P : Dilution treatment of black soybean tempeh

3.2.1. Overrun

The overrun value of ice cream is known as the development of volume from ice cream dough to ice cream. This is related to the ice cream processing process which involves beating or stirring to produce a homogeneous dough (Halim, 2022). The homogenization process serves to capture air and turn it into small bubbles. The air capture process cannot be separated from the use of a stabilizer which acts as a free water binder, making it easier for air to be trapped (Fikri &; Firmansyah, 2022). One stabilizer that can be used is cornstarch to increase the viscosity of ice cream and prevent the formation of ice crystals so that they do not melt easily (Ahmed et al., 2023).

Based on the results of the analysis, it is known that the overrun value of the control treatment ice cream (P0) is significantly different ($p<0.05$) with the overrun value of selected treatment ice cream (P3). The overrun value of P0 (without dilution) is higher with a value of 26.76% compared to P3 (dilution 35%) with a value of 23.74%, so that the dilution treatment of black soybean tempeh milk in the formulation gives a significant difference to the overrun value of ice cream. However, the results of both treatments have overrun values that are not in accordance with standards according to (BSN SNI 01-3713-1995, 1995), namely good quality ice cream for home industries overrun values ranging from 35-50% which are referred to as soft ice cream.

3.2.2. Melting Time

Melting time is one of the parameters of measuring ice cream quality related to the overall time it takes for ice cream to melt completely at a certain volume (Ahmed et al., 2023). Based on the results of the analysis, it was found that the melting time of the control treatment ice cream (F0) was not significantly different ($p>0.05$) from the melting time of selected treatment ice cream (P3). The time it takes P0 (without dilution) to melt completely is 9.45 minutes, while P3 (dilution 35%) which takes approximately 10.78 minutes to melt completely, so there is a trend of increasing melting time along with dilution. Previous research results showed that the higher the dilution, the longer the melting time of ice cream. This is because the total dissolved solids can increase the viscosity of the dough so that the water frozen in the dough is reduced, which causes ice cream not to melt easily (Satar &; Nistia, 2023). Stabilizers can increase the viscosity of ice cream, thereby slowing down the time for ice cream to melt (Ahmed et al., 2023). The melting time is also affected by the overrun associated with the fat content of ice cream. Increasing the overrun value will increase the length of melting time, because the high volume of air will slow down heat transfer, so that ice

cream does not melt easily (Satar &; Nistia, 2023). However, ice cream in this study had low fat content and ice cream overrun values that did not qualify. According to (BSN SNI 01-3713-1995, 1995), the melting time or resistance of ice cream to melting is good for 15-25 minutes. Therefore, both P0 and P3 treatment formulas have not met the ice cream quality requirements in the aspect of melting time.

3.2.3. Total Dissolved Solids

Total dissolved solids indicate the total components of substances dissolved in water, namely glucose, fructose, sucrose, and water-soluble proteins (pectin) (Filiyanti, 2013). The main components indicated in total dissolved solids are sugars, while other components such as organic acids and proteins. Therefore, the higher the sugar content in a food, the total dissolved solids will increase. The pasteurization process carried out on ice cream dough can also affect the amount of dissolved sugar, because the heating process can increase the components extracted from raw materials (Fikri &; Firmansyah, 2022). Based on the results of the analysis, it was found that the total dissolved solids of the control treatment ice cream (P0) was significantly different ($p<0.05$) from the total dissolved solids of selected treatment ice cream PF3). P0 (without dilution) has less total dissolved solids at 21.50obrix than P3 (35% dilution) which has a total dissolved solids of 24.75obrix, so the dilution treatment makes a significant difference to the total dissolved solids of ice cream. The results of this study are in line with research (Halim, 2022), in which the total dissolved solids in cow's milk kefir increased along with the addition of red dragon fruit juice. This is related to the sugar content contained in red dragon fruit, namely glucose, fructose, and oligosaccharides which are quite high compared to white dragon fruit. According to (BSN SNI 01-3713-1995, 1995), the quality requirement of good ice cream is to have a total dissolved solids of ≥30 obrix. Therefore, both P0 and P3 treatments have not met the ice cream quality requirements in terms of total dissolved solids.

3.3. Antioxidant Capacity

Analysis of antioxidant capacity was carried out in the control treatment and selected treatment, namely P3 with the DPPH method (1,1-diphenyl-2-picrylhydrazil) because this method is accurate, simple, and easy to apply in a short time and is able to measure antioxidant capacity in solid and liquid samples, and has good enough sensitivity to allow evaluation of antioxidant capacity in samples with small concentrations (Nurcholis et al., 2023). Testing with the DPPH method was carried out as many as two repetitions in each formula. Antioxidant capacity is represented by an IC50 (Inhibition Concentration) value which indicates the ability of an extract to inhibit free radicals by 50% at a certain concentration

(Surya et al., 2021). Striped flowers have a distinctive color, namely blue, which comes from the content of betacyanin pigments which have a role as antioxidants, as well as betalains, carotenoids, and flavonoids which are also the main value of antioxidants in eagle flowers (Nurcholis et al., 2023). Meanwhile, tempeh's antioxidant capacity is 67.45 ppm, which is relatively strong because the IC50 value is in the range of 50-100 ppm (Chitisankul et al., 2022). Table 3 presents IC50 and AEAC values.

Table 3 Results of antioxidant capacity of black soybean tempeh ice cream

Sample	P0 (without dilution)	P3 (dilution 35%)
IC50 (mg/ml)	10523,95 ± 679,10a	6816,66 ± 992,05b
Ek (MGR. K/100K)	8.73 ± 0.63a	13,56 ± 2,28b

Information:

a-b Average values followed by different letters in the same row show a noticeable difference ($p<0.05$)

The results of the analysis showed that the antioxidant capacity of the selected formula (P3) was stronger than the control treatment (P0). The IC50 value of P0 is 10523.95±679.10 mg/ml while P3 is 6816.66±992.05 mg/ml. The results of statistical tests show that there is a significant difference between the antioxidant capacity of P0 and P3, so that dilution in soy milk ice cream gives a significant difference to the antioxidant capacity of ice cream ($p < 0.05$). In accordance with previous studies, antioxidant capacity increases along with the increase in the addition of additional ingredients containing flavonoid compounds (Chitisankul et al., 2022), (Surya et al., 2021), (Widodo & Lo, 2023). However, the results of the antioxidant capacity test in this study are known to be very weak. The lower the IC50 value indicates the greater the ability of the extract to inhibit free radicals. Antioxidant capacity is categorized as very strong if it has an IC50 value of <50 ppm, strong if IC50 is 50-100 ppm, medium (moderate) if IC50 is worth 101-150 ppm, and weak if IC50 is valued at 151-200 ppm (Molyneux, 2004). Previous research showed a low antioxidant capacity of 132298 ± 29802 ppm in the addition of dragon fruit juice as much as 20% in soy milk ice cream (Halim, 2022).

The antioxidant capacity in a food can be equivalent to vitamin C or commonly expressed as Ascorbic Acid Equivalent Antioxidant Capacity (AEAC). Based on the results of equalizing antioxidant capacity with vitamin C, it is known that there is a significant difference in the AEAC value of the two treatments, with a higher P3 value than P0. This is in accordance with the IC50 value that has been obtained in this study. P3 treatment contains antioxidant capacity equivalent to 13.56 mg vitamin C / 100 g, while antioxidant capacity P0 equivalent to 8.73 mg vitamin C / 100 g. Higher P3 capacity compared to P0 is supported by the presence of bioactive

compounds that have the potential as natural antioxidants in telang flowers such as vitamin C, vitamin B, vitamin E, flavonoids, carotenoids, anthocyanins, and polyphenols (Nurcholis et al., 2023). Based on previous research, it is known that there is vitamin C of 20.00 ± 1.33 mg / 100g of fruit which functions as an antioxidant (Xu &; Li, 2024).

4. Conclusion

Variations in the percentage of water addition for dilution of black soybean tempeh had a significant effect on the attributes of color, aroma, and non-sandy texture, taste, aftertaste, mouthfeel and overall acceptance on the hedonic test. The dilution treatment of black soybean tempeh as much as 35% was chosen as the selected formula based on the results of sensory tests. The overrun value decreased and the melting time and total solids of the ice cream increased with the addition of water for dilution, but there was no noticeable difference between the selected formula and the control formula.

Black soybean tempeh milk ice cream with the addition of telang flowers has the potential to be a healthy snack. Further studies are needed to overcome the aroma and taste that is strong enough, and further studies are also needed for physical characteristics so as to produce better soy milk ice cream.

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