ANTIOXIDANT ACTIVITIES OF SOY YOGHURT PRODUCT IN COMBINATION WITH RED FRUIT (*Pandanus conoideus* Lam.)

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**ABSTRACT**

Red fruit pasta (*Pandanus conoideus* Lam.) contains β-carotene and α-tocopherol which are function as antioxidant compounds. This aims of this research to make food functional in which the predominance of soy milk yoghurt that combination with red fruit pasta which able to replace the consumption of animal milk production and have antioxidant activity. The methods were made of soy yoghurt using bacterial starter *Lactobacillus bulgaricus* and *Streptococcus thermophilus* for 3% and divided into 3 formulations of yoghurt. Formulations of yoghurt were examined by organoleptic then analyzed by SPSS version 21.0. The selected formulations of yoghurt were examined pH, water content, total solids, ash content, lactic acid levels and antioxidant activity. The results showed that formulation of yoghurt combined with red fruit pH: 3,91, water content: 38,97%, total solids: 61,02%, ash level: 0,71%, lactic acid: 1,01%, and IC50 21,32 ppm.

**Keywords:** Red Fruit; Yoghurt; Antioxidant

**ABSTRAK**

Pasta Buah Merah (*Pandanus conoideus* Lam.) mengandung β-karoten dan α-tokoferol yang merupakan senyawa antioksidan. Penelitian ini bertujuan membuat pangan fungsional dimana keunggulan formulasi yoghurt susu kedelai dengan kombinasi pasta buah merah dapat menggantikan konsumsi produk susu hewani dan memiliki aktivitas antioksidan. Penelitian ini menggunakan metode pembuatan soy yoghurt dengan menggunakan starter bakteri *Lactobacillus bulgaricus* dan *Streptococcus thermophilus* sebanyak 3% dan dibagi menjadi 3 formulasi yoghurt. Formulasi yoghurt diuji secara organoleptik lalu dianalisis dengan SPSS versi 21.0, yoghurt terpilih diuji pH, kadar air, total padatan, kadar abu, kadar asam laktat dan aktivitas antioksidan. Hasil penelitian formulasi yoghurt kombinasi buah merah menunjukkan pH 3,91, kadar air 38,97%, total padatan 61,02%, kadar abu 0,71%, Asam laktat 1,01%, dan IC50 21,32 ppm.

**Kata kunci:** Buah Merah; Yoghurt; Antioksidan
INTRODUCTION

Free radicals can cause damage to cell structure in the body so that it can cause the potential for various diseases to emerge. Cancer is a disease that arises due to abnormal growth of body tissue cells.

According to (Riskesdas, 2013) Cancer prevalence in Indonesia is 1.4 per 1000 population, the highest prevalence of cancer is in Yogyakarta Special Region (4.1%), Central Java (2.1%), Bali (2.1%) , Jakarta (1.9%) and Papua (1.1%).

The high incidence of cancer in Indonesia is caused by several risk factors, namely the prevalence of lack of physical activity (26.1%), the prevalence of smokers (36.3%), the prevalence of 10 years of age consuming less fruits and vegetables (93.5%) , as well as the prevalence of people aged> 10 years consuming alcoholic drinks (4.6%) (Riskesdas, 2013). The results of data on risk factors for people affected by cancer indicate that the importance of fruit and vegetable consumption as a source of antioxidants that can prevent oxidative stress (Setiati, 2003).

Red fruit extract (Pandanus conoideus Lam.) Contains β-carotene and α-carotene each 130 µg and 1,980 µg / 100 g of sample, with a high antioxidant content (α-tocopherol), which is 21.20 mg / 100 g sample (Suruno et al, 2006). Antioxidants are able to ward off and break the chains of free radical chains in the body and are destructive to Hadad et al, (2006).

Because the antioxidant content in red fruit is quite high and is considered capable of counteracting free radicals, it is necessary to innovate processed food products by utilizing red fruit paste ingredients so that the resulting food products are expected to be of good quality, both in terms of their efficacy and organoleptics.

Yoghurt is a fermented milk product using lactic acid bacteria, namely: Streptococcus thermophilus and Lactobacillus bulgaricus which can increase the nutritional content and nutrition of yoghurt for health as a probiotic for the body. Probiotics are live bacteria that are used as food supplements, which have beneficial effects for maintaining intestinal health and intestinal microflora of the human body (Widiyaningsih, 2011).

Soy yoghurt products themselves are still lacking in demand by the public even though they have high nutritional value, besides that it is necessary to use soy milk for yoghurt to help diversify soybean processed as a source of quality protein (Jenie, 2003).

This study aims to make yoghurt from soy milk and combined with red fruit paste containing antioxidant compounds that can help prevent free radicals. It is hoped that the combination of red fruit paste and soy yoghurt can increase antioxidant activity and enhance the immune system in counteracting free radicals.

RESEARCH METHODS

Materials
Red fruit, soy milk, mineral water, skim milk, (granulated sugar, palm sugar, corn syrup) as a sweetener, a starter of yoghurt bacteria Streptococcus thermophilus & Lactobacillus bulgaricus in the form of dry yoghurt powder, DPPH, vitamin C.

Tools
Manual incubator, desiccator, thermometer, refrigerator, oven, UV/Vis spectrophotometer T-1601, nabertherm p300 Furnace.
Yogurt Production

Production of yoghurt was following the method (Wardhani, 2015) with formulation as in Table 1.

Table 1. Basic yoghurt formulations

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soymilk (ml)</td>
<td>F1 500</td>
</tr>
<tr>
<td>Skim Milk (% w / v)</td>
<td>5</td>
</tr>
<tr>
<td>Arabic Gum (% w / v)</td>
<td>0,6</td>
</tr>
<tr>
<td>Granulated Sugar Sweetener (% w / v)</td>
<td>5</td>
</tr>
<tr>
<td>Palm Sugar Sweeteners (% w / v)</td>
<td>-</td>
</tr>
<tr>
<td>Corn Syrup Sweetener (% w / v)</td>
<td>-</td>
</tr>
<tr>
<td>Yoghurt starter (% v / v)</td>
<td>3</td>
</tr>
</tbody>
</table>

The three soy milk yoghurt formulations were heated to a temperature of 80 °C for 20 minutes, carried out cooling until the yoghurt temperature dropped to 45°C, then carried out the addition of L. bulgaricus and S. thermophilus bacteria. The next step is to incubate soy milk yoghurt for 12 hours at 45 °C. The final stage of storing soy milk yoghurt in a refrigerator with a temperature of 10°C. For the yoghurt formulation selected, 5% (v / v) red fruit paste is added for antioxidant testing.

Evaluation of yoghurt characteristics

In this research, yogurt characteristic testing consists of several methods, namely organoleptic test on the three formulations of soy milk yogurt, conducted to determine the response to more specific formulation characteristics such as color, taste, aroma, and thickness and then the level of public acceptance or panelists of 30 people against yogurt formulations. The untrained panelist category was chosen randomly, followed by hedonic testing to determine the level of panelist preference for the yoga formulation. The assessment of preference (hedonic) tests on color, taste, aroma, viscosity starts from number 1 (dislikes), 2 (somewhat likes), 3 (neutral), 4 (very likes), 5 (very likes), then distribution the data using SPSS version 21 analysis Kolmogorov-Smirnov, seen the differences in the three formulations of yogurt with one way Anova where yogurt formulations that were further tested were only yogurt formulations selected.

The selected yogurt formulation was continued with measurements using a pH meter that had been calibrated with a buffer solution of pH 4 and pH 7 (Hadiwiyoto, 1994). Then the water content of the selected yogurt is tested using the formula of Askar, et al (2005):

$$\text{Water content (\%) = } \frac{\text{B - C}}{\text{B - A}} \times 100\%$$

Note:
A = Weight of the empty cup (g)
B = Weight of sample before roasting (g)
C = Weight of sample after roasting (g)

Solid total testing is carried out to determine the solid portion of a sample roughly. Total solids are calculated from the calculation of water content, using the formula from Askar, et al (2005):

$$\text{Total Solids (\%) = 100\% - Moisture Content}$$

Further testing is the ash content taken from the calculation of water content, where the cup is heated in an oven at 105 oC for 24 hours, then put into the furnace and heated at 550oC for 6 hours, after that it is cooled in a desiccator and weighed according to the formula from Askar, et al (2005):
The Ash content can be calculated using the formula:
\[
\text{Ash content (\%)} = \frac{D-A}{B-A} \times 100\%
\]

Information:
\( A \) = Weight of the empty cup (g)
\( B \) = Weight of ash sample before roasting (g)
\( D \) = Weight of ash sample after roasting (g)

The final test in evaluating the characteristics of yogurt is testing the level of lactic acid, where the large yield of lactic acid in soy yogurt is calculated using the formula from (Hadiwiyoto, 1994).

\[
\text{Lactic acid (\%)} = \frac{V \times N \times \text{BE Asam Laktat}}{(V \times 1000)} \times 100\%
\]

Information:
\( V \) = volume of NaOH (ml)
\( N \) = NaOH normality (0.1N)
\( \text{BE} \) = equivalent weight of lactic acid (0.09)

**Evaluation of antioxidant activity**

a. Sample preparation test, positive control, and negative control.

The best yogurt formulation, negative control (yogurt base) and positive control (yogurt added with vitamin C) were weighed as much as 5 mg, each test sample was put into a 50 ml volumetric flask, added with methanol solvent p.a to the mark flask markers.

b. Preparation of sample test solutions.

The test solution for each sample was made at a concentration of 100 ppm by piping as much as 1, 2, 3, 4, 5, 6, 7 ml of the test solution to a 10 ml volumetric flask and then adding the methanol solvent p.a to the flask mark limit.

c. Preparation of a 100 µM DPPH stock solution

DPPH powder were weighed as much as 2 mg, then put into a 50 ml volumetric flask and dissolved with methanol solvent p.a.

d. Testing the antioxidant activity of yogurt samples

Each 5 ml yogurt sample solution is put into a vial tube, then a 5 ml DPPH solution is added as much as 5 ml, then dissolved until homogeneous and wait for 30 minutes to settle in a dark room. Next uptake of antioxidant activity was measured using a UV / Vis spectrophotometer at maximum wavelength.

e. Determination of maximum wavelength

According to Molyneux in his research "The Use The Stable Free Radical Diphenypicyryhlydrazil (DPPH) for Estimating Antioxidant Activity" in 2004, the maximum wavelength of DPPH is 515-520 nm, so in this study the wavelength of 517 nm was used to see the effectiveness of antioxidant yogurt samples. at DPPH wavelengths.

f. Calculation of percentage of inhibition

Determination of antioxidant activity is done by determining the value of percent inhibition of yogurt formulation results, where the greater the value of percent inhibition obtained, the greater the value of antioxidant activity. Percent of inhibition is calculated by the following formula:

\[
\text{Inhibition (\%)} = \frac{(\text{Abs K}-\text{Abs P})}{\text{Abs K}} \times 100\%
\]

Information:
\( \text{Abs K} \) = absorbance of overall DPPH
Abs P = absorbance of DPPH after reacting with the sample

g. Calculation of IC<sub>50</sub> sample values (Inhibitory Concentration)

IC<sub>50</sub> value calculation is done by taking the results of the percentage of inhibition that has been obtained from each yogurt formulation then entered on the x and y axis in the linear regression equation. In determining the IC<sub>50</sub> value the regression equation \( y = (b) x + a \) is used, where \( y \) is the percent of inhibition that is 50 and \( x \) is the IC<sub>50</sub> value.

**Data analysis**

The test results found were statistically analyzed with one way ANOVA using SPSS for windows version 21.0, organoleptic test data presented in the narrative, while antioxidant testing was analyzed in linear regression using Microsoft Excel.

**RESULTS AND DISCUSSION**

**Testing the characteristics of yogurt**

The hedonic test results showed that 30 panelists preferred the visual form or appearance of the first formulation of yogurt formulation (F1) with sugar sweetener, then continued with the initial statistical test of the Kolmogorov-Smirnov normality test, this normality test aimed at testing whether the data obtained from each group has a normal distribution. The results of all test groups including color, taste, aroma, and viscosity were treated normally distributed (p,00.05), so the analysis was continued to the one way Anova test.

Anova one way testing aims to test three or more samples that are not interconnected, where group data from color, taste, aroma, and viscosity when tested show the results of all three treatments are identical or not identical. The results of the significance test on the color, taste, and viscosity of yogurt are 0.000 showing that if sig (≤0.05) it can be concluded that the colors of the three yogurt formulations are not identical or have different assessments, while the significance test on the aroma of yogurt shows the result of the significance of 0.499 where sig (≥0.05) so that it can be concluded that the aroma of the three formulations of yogurt is identical or has the same rating that is flavorful in soy.

From the hedonic analysis test data strengthen the results of organoleptic testing with the selection of the first formulation (F1), namely yogurt with sugar sweetener which has the most dominant score. Then the selected yogurt basic formulation (F1) is made into a formulation of a combination of red fruit yogurt that is tested for pH, water content, total solids, ash content, and total titrated acid.

In food products such as yogurt the pH value is one of the important factors in determining the level of resistance to the growth of spoilage microorganisms during processing, distribution and storage. The greater the concentration of dissolved hydrogen ions in a food product, the higher the acidity (lower pH) and vice versa. Yogurt formulations based on pH values, included in the category of high acid food products (high acid food) which naturally has a pH <4.6 (Oberman, 1985). PH measurements carried out indoors are measured using a pH meter.

The results of pH measurements on the formulation of a combination of red fruit yogurt showed an average pH of 3.91. In the yogurt formulation, there is a decrease in pH due to increased storage time, the lactic acid bacteria will continue to overhaul lactose and sucrose for its metabolic activity to run so as to produce relatively much lactic acid and cause a significant decrease in pH.
Moisture formulation of red fruit combination yogurt with 3x replication was 38.97%. Water content is obtained from soybean juice, a starter culture which is partly composed of water. In addition, the water content is also obtained from skim milk, arabic gum and sugar which is added during the process of making the formulation of yogurt combination of red fruit that affects the appearance of yogurt. The standard water content for yogurt products is not determined by SNI 01-2981 of 2009 so that the formulation of a combination of red fruit yogurt has the form of a semi-solid viscous liquid.

Total solids in food products such as milk and yogurt consist of protein, fat, carbohydrates, vitamins, minerals that are not soluble in water and a small portion of water. The standard value of total solids in yogurt products by SNI 01-2981 in 2009 is at least 8.2%. The results of testing the total solid formulation of yogurt combination red fruit that is equal to 61.02%, the increase in total solids in yogurt formulations allegedly due to lactic acid plays a role in the process of milk casein coagulation using the lactase enzyme which causes an increase in total solids (Susanti, 2005), and the effect of composition fat in red fruit paste which ranges from 62.14% which makes the formulation of yogurt combination of red fruit has a fairly high viscosity (Murtiningrum, 2009).

The presence of mineral content in a food is the reason for testing the total ash content must be done. According to (Irawati, 2008) testing the total ash content used aims to determine whether or not a process of processing, and as a safe limit parameters of ash material in food. The results of the analysis of the ash content of the formulation of yogurt combination of red fruit by 0.71%. The standard set by SNI in 2009 is ash content for yogurt products up to 1% (w / w). Thus the formulation of a combination of red fruit yogurt still meets the standard composition of ash content in the yogurt formulation.

Total titrated acid is the total percentage of lactic acid found in yogurt products. The results of fermentation are lactic acid (C3H6O3) which is formed from L. bulgaricus and S. thermophilus bacteria. According to SNI 01-2981 of 2009, the quality requirements of yogurt products that are suitable for consumption have a total lactic acid level of 0.5-2.0%. Based on the measurement results, the total titrated acid contained in the formulation of a combination of red fruit yogurt is 1.01% so that the soybean acid content produced is included in the SNI standard.

**Antioxidant Activity**

Antioxidant compounds are useful in stabilizing free radicals by completing the lack of electrons, also inhibits the chain reaction of the formation of free radicals so that the oxidation process is not sustainable in the body of Selawa et al, (2013).

In determining the percentage of inhibition, only selected yogurt formulations were used, namely red soybean milk combination, where the preliminary test was made in several concentrations, then the mother solution was made in a 50 mL volumetric flask, each test solution reacted with DPPH, incubated in a dark room for up to 30 minutes, then DPPH will change color from purple to yellow, which means that between antioxidant compounds and DPPH has been successfully paired so that free radicals can be neutralized.

The preliminary test results were then analyzed with the help of UV / Vis spectrophotometer to then calculate the...
value of % inhibition and found the results in
Figure 1. as follows:

![Graph of antioxidant activity vs. concentration](image)

**Figure 1. Antioxidant graph of a red fruit combination yogurt sample**

Based on the graph in Figure 1, a comparison of concentration with percent inhibition will get a linear regression equation. Based on the results of the percentage inhibition graph shows the higher the concentration of the sample is directly proportional to the value of % inhibition, where the inhibition value indicates the inhibitory activity of the sample against DPPH radical expressed in percent. The more amount of antioxidants in the test solution that is reacted with DPPH, causing the number of electrons given to DPPH to increase so that the formation of non-radical DPPH will increase and make the potential for free radicals to form. Then the linear regression equation is used to determine the IC50 value.

The following is a table of linear regression equations and IC50 values for each sample:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Linear Regression Equation</th>
<th>IC50 (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formulation of soy yoghurt combination of red fruit</td>
<td>$y = 0.1657x + 46.466$</td>
<td>21.32 ppm</td>
</tr>
<tr>
<td></td>
<td>$R^2 = 0.9906$</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. IC50 calculation results for a combination of red fruit yogurt

The linear regression equation in table 2 is used to determine the IC50 value, which is the concentration value of the sample which can reduce 50% of free radicals. According to (Murtiningrum et al, 2009) Red fruit is one of the natural ingredients that can function as an antioxidant because it
Soy Yoghurt Product Combined with Red Fruit contains compounds such as flavonoids, β-carotene, tocopherol (vitamin E).

From the results of research Murtiningrum et al, (2009) stated that the chemical composition of red fruit paste can be seen from the total tocopherol which is equal to 21.84 ppm, where the active ingredients in red fruit juice that are not included with red fruit oil will usually be left in the red fruit paste that is considered as a waste, then some of the active ingredients present in red fruit paste have the potential to contain higher antioxidant compounds.

Exogenous antioxidants (derived from food consumed) are needed by the body with the aim of enhancing the antioxidant status in the body so that antioxidant nutrients in greater amounts can eliminate and neutralize the effects of free radicals. From the results of inhibition values obtained showed that the formulation of red fruit yogurt combination has a very strong antioxidant activity of 21.32 ppm. According to Jun et al, (2003) a compound is stated to have very strong antioxidant activity if the IC₅₀ value is less than 50 ppm, strong if IC₅₀ is 50-100 ppm, while IC₅₀ is 100-150 ppm and weak when IC₅₀ is between 150-200 ppm.

CONCLUSION
The results of this study indicate the results of antioxidant activity of red fruit combination yogurt formulation with IC₅₀ value of 21.32 ppm where antioxidants are classified as very strong.

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