ANTIOXIDANT ACTIVITY, DEXTROSE EQUIVALENT, TOTAL DISSOLVED SOLIDS, AND VISCOSITY OF MALTED RED RICE MILK AT DIFFERENT ENZYME CONCENTRATIONS

AKTIVITAS ANTIOKSIDAN, DEXTROSE EQUIVALENT, TOTAL PADATAN TERLARUT DAN VISKOSITAS SUSU MALT BERAS MERAH DENGAN TAMBAHAN KONSENTRASI ENZIM YANG BERBEDA-BEDA

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ABSTRACT

Rice milk is a plant-based milk-like product that can be an alternative for people who have an allergy to bovine milk and lactose intolerance. Malting and enzymatic hydrolysis can bring out the natural sweetness of the rice milk product and thus minimize the use of sweeteners. The current research aimed to determine the total value of dissolved solids, viscosity, dextrose equivalent, and antioxidant activity in malted red rice milk with the addition of glucoamylase at different concentrations. The red rice was malted for 48 hours before drying and milling. The resulted malt powder was then diluted and added with glucoamylase enzyme at the concentration of 0%, 1%, 2%, and 3%. Enzymatic hydrolysis occurred at 60°C for 3 hours. Completely Randomized Design was used for the parameters of total soluble solids, viscosity, and dextrose equivalent while the antioxidant activity parameter was analyzed descriptively. The result showed that the addition of glucoamylase significantly ($p<0.05$) increased the total soluble solids, viscosity, and dextrose equivalent. The highest values were 9.960 °Brix, 1620 cP, and 1.872 for total dissolved solids, viscosity, and dextrose equivalent, respectively. Increasing antioxidant activity was also observed from 6.094% at 0% glucoamylase to 10.762% at 3% glucoamylase addition.

Keywords: antioxidant, glucoamylase, malt, red rice, rice milk

ABSTRAK

Yoga Pratama*, Dina Yulia Anggraeni, Yasmin Aulia Rachma, Lita Lusiana Surja, Siti Susanti, 2021. Aktivitas Antioksidan, Dextrose Equivalent, Total Padatan Terlarut dan Viskositas Susu Malt Beras Merah dengan Tambahan Konsentrasi Enzim Yang Berbeda-Beda.

Susu beras adalah produk minuman menyerupai susu yang berbahan dasar nabati. Produk ini dapat menjadi alternatif bagi konsumen yang memiliki alergi terhadap susu atau intoleran terhadap laktosa. Proses malting dan hidrolisis menggunakan enzim dapat menghasilkan rasa manis alami dari produk susu beras sehingga dapat meminimalkan tambahan bahan pemanis. Tujuan dari penelitian ini adalah untuk menguji total padatan terlarut, viskositas, dextrose equivalent dan aktivitas antioksidan pada susu malt beras merah dengan tambahan enzim glukoamilase pada konsentrasi yang berbeda-beda. Proses malting beras merah dilakukan selama 48 jam sebelum dikeringkan dan dihaluskan. Tepung malt yang dihasilkan kemudian dilarutkan dan ditambah glukoamilase dengan konsentrasi 0%, 1%, 2% dan 3%. Hidrolisis enzimatis dilakukan pada suhu 60°C selama 3 jam. Rancangan acak lengkap diterapkan pada parameter total padatan terlarut, viskositas dan dextrose equivalent sedangkan aktivitas antioksidan dianalisa secara deskriptif. Hasil penelitian menunjukkan bahwa penambahan glukoamilasi secara signifikan ($p<0,05$) meningkatkan total padatan terlarut, viskositas dan dextrose equivalent. Nilai tertinggi didapatkan pada penambahan 3% glukoamilase dengan total padatan terlarut 9.960 °Brix, viskositas 1620 cP dan dextrose equivalent 1,872. Aktivitas antioksidan meningkat akibat penambahan glukoamilase dari 6.094% pada konsentrasi 0% menjadi 10.762% pada konsentrasi 3%.

Kata kunci: anti oksidan, beras merah, glukoamilase, malt, susu beras

INTRODUCTION

For several reasons, consumers need plant-based milk products and demand for milk alternative products have increased in the last few years. Consumers are avoiding milk consumption for several reasons. Firstly, milk has been linked to health problems such as milk allergy, lactose intolerance, calorie concern, and prevalence of hypercholesterolemia[1]. Secondly, milk is also avoided by veganism, which is gaining popularity in the western world. The new consumption practice aims to exclude all forms of animal exploitation[2]. People who embrace this movement may base their action from the perspective of animal welfare, health, or environment sustainability[3]. Nevertheless, milk and its derivatives are blacklisted by vegan.

The avoidance of milk consumption has driven the development of milk alternatives such as milk-like products made from plant-based materials. Plant-based milk alternatives are fluids that come from the breakdown (size reduction) of plant material (cereals, pseudo-cereals, legumes oilseeds, nuts) which is followed by water extraction. Further homogenization of such fluids and the addition of suitable food additives imitate milk in appearance and consistency[4].

Nowadays, many plant-based milk products made from soybean, almond, coconut, oats, rice, are available commercially. Rice milk as one of the alternatives, has been consumed and even produced on a large scale in Japan, America, and Australia. However, like many other commercial plant-based milks, commercial rice milk products contain sweetening ingredients, natural or artificial, to increase consumer acceptance.

Techniques to improve the sweetness properties without additional sugar are available. One possible approach is to convert the starch naturally contained in the rice into simple sugars. Our previous research has investigated the effect of the malting process on the degradation of red rice starch[5]. This technique not only increased dextrose equivalent as a result of starch degradation, but also improved the antioxidant level and the physical characteristic of malted red rice flour.

The use of the malting process is a standard in beer processing industry; where it is followed by yeast fermentation to further hydrolyze starch into sugar and alcohol. However, there is lack of literature in malting technique incorporation in the processing of plant-based milk[4]. One possible reason, the resulting sweetness is not satisfactory. We have also observed this in our preliminary study with malted red rice flour. Note, the yeast fermentation cannot be employed because alcohol production is avoided for plant-based milk. In replacement of yeast, further starch degradation can be achieved with the help of enzyme[4]. Therefore, it is the objective of the current study to combine both malting and enzymatic methods to produce rice milk. The malted red rice flour was further processed into ready to drink malted red rice milk (henceforth called MRRM) with the addition of a glucoamylase enzyme. The evaluated parameters include the total value of dissolved solids, viscosity, dextrose equivalent, and antioxidant activity.

MATERIALS AND METHODS

Materials

Red rice grain was obtained from Bionic Farm, Bogor. Commercial glucoamylase enzyme was purchased from Toko Sagu, Indonesia. Mineral water was purchased in Semarang.

Methods

Red Rice Malt Flour

The flour was produced according to our previous research[5]. The red rice malting process included immersion, germination, drying, and powdering. Immersion of red rice began with red rice being washed twice and then soaked with clean water at a ratio of 1:2 for 2 hours and drained. Germination of red rice was carried out by spreading red rice in plastic trays, with a stack height maximum of 0.5 cm for 48 hours at room temperature with sufficient light exposure. Malting conditions must be controlled every 6 hours by spraying the grain with clean water and stirring to maintain air circulation and humidity. The drying process was performed in an oven at 50 °C for 3 hours. Powdering was carried out by grinder (Herb Grinder Maksindo, Indonesia) for one minute and then sifting with a standard sieve.

Malted Red Rice Milk (MRRM) Production

Red rice malt flour was dissolved in mineral water with a ratio of 1:4. The suspension was then gelatinized at 80 °C for 30 minutes and cooled down to 60 °C before the addition of glucoamylase enzyme for the saccharification process. The enzyme was added in 3 concentration, i.e. 1%, 2%, 3% (v/v) and a control without enzyme addition (0%). The saccharification process lasted for 3 hours at 60 °C.

Dextrose Equivalent (DE) Analysis

Dextrose equivalent was determined by the Lane-Eenon method conducted by Oktafiani and Tjahjani[6].

Total Dissolved Solids Analysis

Total dissolved solids were measured by a hand refractometer.

Viscosity Analysis

Viscosity testing was carried out using a cup and bob viscometer (Rion VT-06, Japan) referring to the Apriliyan *et al*[5] methods.

Antioxidant Activity Analysis

Antioxidant activity was evaluated according to the method used by Zuhra *et al*[8] by using DPPH (2,2-diphenyl-1 picrylhydrazyl) free radical method.

Data Analysis

The study used a Completely Randomized Design with 4 treatments and 5 replications. Data were presented as mean and standard deviation. Statistical significances were measured by Analysis of Variance (ANOVA) using SPSS program 19.0 series. Antioxidant activity was analyzed descriptively using Microsoft Excel 13 for Windows.

RESULTS AND DISCUSSION

Malted red rice flour was chosen as raw material in this study because it has higher solubility and antioxidant compared to normal red rice flour[5]. Malting is biological process that generates hydrolytic enzyme thus it potentially reduces the amount of enzyme addition to obtain the expected sweetness level.

Dextrose Equivalent

Dextrose equivalent analysis aims to determine the simple sugar produced from the enzymatic hydrolysis process. According to Anwar[9], the definition of dextrose equivalent is the total amount of reducing sugars resulting from hydrolysis of starch, both chemically and enzymatically. Determination of dextrose equivalent value by using Lane Eynon method shows how much reducing sugar is formed in the sample. According to Sofyan *et al*[10], the basic principle of the Lane Eynon method is redox titration which is the reduction of Fehling solution by reducing sugars contained in the sample.

Table 1. Dextrose equivalent of MRRM in different glucoamylase concentrations

Glucoamylase Concentration	Dextrose Equivalent
0 %	1.012 ± 0.076^{a}
1 %	$1.312 \pm 0.124^{a,b}$
2 %	$1.640 \pm 0.055^{b,c}$
3 %	1.872 ± 0.652^{c}

**different superscripts show a significant difference (P<0.05)*

The result of dextrose equivalent analysis on MRRM (Table 1) showed a significant increase (P <0.05), where the higher enzyme concentration resulted in higher dextrose equivalent values. The dextrose equivalent values in MRRM were 1.012; 1.312; 1.640; and 1.872 for 0, 1, 2 and 3% glucoamylase concentration, respectively. The highest dextrose equivalent value on rice milk red rice was found at an enzyme concentration of 3%.

This result in line with Irzam and Harijono[11] who explained that the amount of reducing sugar is closely related to hydrolytic enzyme activity, where higher reducing sugar is to be expected in a system with the higher enzyme concentration or activity. Glucoamylase cleaves the starch molecules resulting in shorter and simpler carbohydrate molecules. This includes monosaccharides such as glucose and fructose which have reducing effect. According to Susmiati *et al*[12] the higher the dextrose equivalent value, the better the hydrolysis process occurs.

Despite the significant difference among treatments, the DE value of less than 2 shows that the current enzymatic process was not satisfactory with regards to increasing the sweetness properties of the product. Hydrolyzed starch with DE value up to 20 is regarded as maltodextrin. The product is known for its solubility but not for its sweetness. Meanwhile, further hydrolyzation will result in syrup products that are recognized for the sweetness and characterized by DE value of >20[13]. Therefore, improvements are needed to increase the hydrolyzation rate such as sourcing a purer or more effective enzyme and the optimization of the processing condition. Plant-based milks are often marketed as healthier option than bovine milk, with claims such as bioactive compounds, non-cholesterols and non-allergenic[4]. Elimination of sugar addition by employing the hydrolysis process aligns well with positioning of the plant based milk as a healthy product.

Total Dissolved Solids

In the beverage industry, total dissolved solids measurements are often employed as a ‘quick test’ to measure the sucrose concentration, thus representing the

sweetness of the products. Test results of total dissolved solids in MRRM were shown in Table 2. A significant effect of the addition of glucoamylase enzyme (P <0.05) was observed, where the higher the glucoamylase enzyme was added, the higher the total dissolved solids value. The total values of dissolved solids in MRRM obtained by 0, 1, 2, and 3% glucoamylase addition were 1.720; 8.000; 9.040; and 9.960 °Brix, respectively. The highest total dissolved solids were with 3% glucoamylase enzyme addition treatment.

Table 2. Total dissolved solids of MRRM in different glucoamylase concentrations

Glucoamylase Concentration	Total Dissolved Solids (°Brix)
0 %	1.720 ± 0.109[a]
1 %	8.000 ± 0.616[b]
2 %	9.040 ± 0.589[c]
3 %	9.960 ± 0.623[d]

*different superscripts show a significant difference (P<0.05)

The increase in the total value of dissolved solids was due to the process of changing the starch to shorter saccharide by the natural malting enzyme alpha amylase[5] and additional glucoamylase enzymes. This is consistent with the statement from Sintasari *et al*[14] which explains that total dissolved solids can be obtained from the breakdown of carbohydrates, protein breakdown, and fat breakdown.

The total soluble solids show the presence of dissolved substances in a solution. According to Yulianti *et al*[15] components that might be measured into total dissolved solids include sucrose, reducing sugars, organic acids, and proteins. In the current study, the high °Brix value does not represent a high concentration of sucrose as has been revealed in DE value measurements. Instead, it shows higher soluble substance or solubility of the products.

Viscosity

Viscosity analysis showed an increase in MRRM viscosity along with the addition of a glucoamylase enzyme (Table 3). The viscosity values of were 600; 660; 1025; and 1620 cP for 0, 1, 2 and 3% glucoamylase concentration, respectively. Based on the results of statistical analysis it can be seen that the addition of glucoamylase enzyme to rice milk malt red rice has a significant effect (P <0.05) on the product viscosity. However, there was no significant difference between MRRM with 1% enzyme addition and the control.

Table 3. The viscosity of MRRM in different glucoamylase concentrations

Glucoamylase concentration	Viscosity (cP)
0 %	600 ± 70.71[a]
1 %	660 ± 89.44[a]
2 %	1025 ± 95.74[b]
3 %	1620 ± 148.32[c]

*different superscripts show a significant difference (P<0.05)

The addition of glucoamylase enzymes to rice milk red rice caused the formation of sugar components, which causes an increase in the value of product viscosity. In line with the opinion of Winarno[16] which explains that increasing viscosity is influenced by the addition of sugar and the presence of high sugar concentrations.

Viscosity has a linear relationship with total dissolved solids. The higher the total value of dissolved solids, the higher the viscosity value. This is in agreement with Pratama *et al*[17] that the more components dissolved the more dissolved substances, the higher the value of dissolved solids, and the higher the viscosity value.

Antioxidant Activity

Antioxidant has been widely acknowledged to be an important constituent in food. It helps to reduce the risk of chronic disease such as cancer and cardiovascular disease. The presence of antioxidant in rice has been reported[5,18]. Red rice contains anthocyanin which exerts antioxidant activity. In a malting process, this activity was found to be increasing[5]. Interestingly, the same pattern is observed with the addition of the enzyme in MRRM as can be seen in Figure 1. It is shown that antioxidant activity tends to increase along with the increase in glucoamylase enzyme concentration. One possible explanation was the enzymatic activity of glucoamylase resulted in various complex substances being degraded which allowed the release of various phenolic compounds and amino acids which have the antioxidant activity potential[19]. The released antioxidant compounds resulted from hydrolytic process in rice include γ-oryzanol and γ-aminobutyric acid[18]. Astawan *et al*[20] stated that an increase in antioxidant activity is related to the ability or strength of the formation of new phenolic compounds which play a role in increasing antioxidant activity.

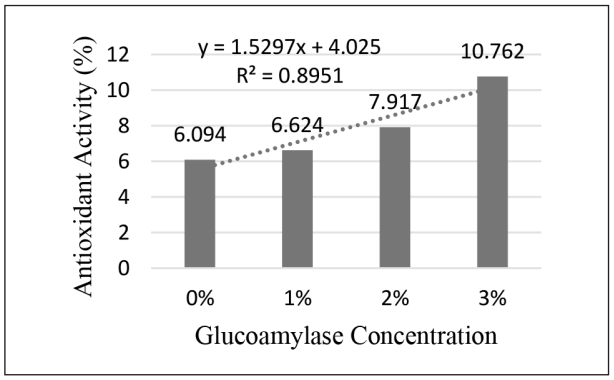

Figure 1. Antioxidant activity of MRRM in different glucoamylase concentrations

CONCLUSION

The result showed that the addition of glucoamylase significantly ($p<0.05$) increased the total soluble solids, viscosity, and dextrose equivalent. The highest values were 9.960 °Brix, 1620 cP, and 1.872 for total dissolved solids, viscosity, and dextrose equivalent, respectively. Increasing antioxidant activity was also observed from 6.094% at 0% glucoamylase to 10.762% at 3% glucoamylase addition. However, future improvements are needed to obtain satisfactory sweetness properties in the product.

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