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**15th National Student Conference on
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Integrating Innovative Food Product
Development and Consumer
Preferences through Sensory
Evaluation**

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On Food Science and Technology

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Page i

THE STABILITY OF BETALAINS IN RED BEET (*Beta vulgaris* L.) AS A NATURAL FOOD COLORANT DURING STEAMING OF GLUTINOUS RICE FLOUR BATTER

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ABSTRACT

Red beet (*Beta vulgaris* L.) powder can be added to food as a natural food colorant that gives purplish red color. Betalains are pigment contained in red beet that responsible for purplish red color. It also has high antioxidant activity which gives health benefits. Red beet powder is added to glutinous rice flour batter for enhancing the color appearance. One kind of glutinous rice flour heat processing is by steaming, which can affect the stability of betalains pigment in red beet powder. Change of betalains content contribute to the change of color intensity and antioxidant activity, so the aim of this research is to know the effect of steaming process and red beet powder addition on the betalains content, antioxidant activity and color intensity of glutinous rice flour batter. In this research, the amount of red beet powder added to glutinous rice flour batter are 0%, 10%, and 20% based on glutinous rice flour weight. The batter were steamed for 15 minutes. Before and after steaming process, betalains (betacyanins and betaxanthins) content and antioxidant activity of batter were analyzed. While, the color intensity of batter was measured at 0', 3', 6', 9', 12', and 15'. In this research, the addition of red beet powder significantly increase betalains content and a* value of sample. Sample with concentration of 20% red beet powder before steaming had the highest betacyanins and betaxanthins (33.15±2.07 mg/L and 16.84±3.30 mg/L), antioxidant activity (6,24±0,90%) and highest a* value which was 22.73±3.72. Betacyanins and betaxanthins content as well as antioxidant activity significantly decreased after steaming. The a* value of batter decreased as long as steaming process.

Keywords: red beet, betalains, color, antioxidant, glutinous rice flour

INTRODUCTION

People begins to have concern¹ about the risk of using synthetic colorants, so the needs of natural colorants being increasing. Red beet is one of the natural colorant that has functional value. The color comes from betalains pigment that contribute to give red-purp color. Betalains content in red beet are around 1000mg/100g of total solids or 120mg/100g of wet basis (Nemzer *et al.*, 2011). Betalains are synthesized into two structural groups, i.e. the red-violet betacyanins and the yellow-orange betaxanthins (Azeredo, 2009a). Furthermore from Agrawal (2013), betacyanins content in red beet was higher more than betaxanthin that amount about 0.04-0.21% and betaxanthins amount about 0.02-0.14% and depends on the type of it roots. Beside of the colorant properties of red beet, it has functional value as antioxidant source that can reduce the risk of chronic diseases such as cancer and tumor (Ravichandran *et al.*, 2013).

¹ As natural colorant, red beet can be applied in the form of powder on many kinds bakery product for improving the appearance. Red beet powder has advantage when compare to liquid form because their higher stability (Nemzer *et al.*, 2011). Red beet powder that be produced by freeze or spray drying have contains 0,3%-1% of pigment (Azeredo, 2009a). Glutinous rice flour is one of the basic ingredients for bakery products that having stickiness when its processing and also having the white color and turbid (Codex, 1995). However, during heating process (steaming process) the glutinous rice flour had physicochemical changes.

Heating process also can affect the changes of betalains content that contribute to the change of color intensity and antioxidant activity (Ravichandran *et al.*, 2013).¹ So the aim of this research is to know the effect of steaming process on the betalains content, antioxidant activity and color intensity of glutinous rice flour batter.

¹⁰ MATERIALS AND METHODS

Material

Red beet powder was made from red beet roots (*Beta vulgaris* L.) which were obtained from local farmer in Kopeng, aquades, ascorbic acid, maltodextrin DE 10. Glutinous rice flour batter was made from glutinous rice flour (Rose Brand), water, and red beet powder. DPPH (2,2-diphenyl-1-picrylhydrazyl) and methanol was used for antioxidant activity analysis, while betalains content analysis used ethanol 50%.

Methods

Red beet powder preparation

⁷ Red beet roots were washed, peeled, and sliced into small pieces. For extraction of red beet was done by juicer. Red beet extract was added aquades (half-part), then filtered through filter cloths. The pH of solution was set at 5 by addition of ascorbic acid (Cai and Corke, 2000). Maltodextrin DE 10 was used as microencapsulation agents. 60% of maltodextrin were added to red beet extract and homogenized using mixer. Spray drying process was done using spray dryer with 150°C inlet and 90°C outlet temperature. Red beet powder which was obtained was filtered.

Glutinous rice flour batter preparation

Sample was prepared by mixing of glutinous rice flour, water, and red beet powder. Additional of red beet powder was variegated to 0%, 10% and 20% of powder based of flour weight. Material mixing was done by mixer at first speed level for 25 seconds. 30 gram of batter were put on container 9 x 4 cm and steamed for 15 minutes until gelatinized.

Physicochemical Analysis

Betalain Content Analysis (Ravichandran *et al.*, 2013)

Extraction of Betalains

0,1 g of freeze dried sample were dissolved in 10 ml of 50% ethanol and agitated for 10 second, then centrifuged at 6000 rpm for 10 minutes. The supernatant was collected after centrifugation and the same was repeated for 2 more times to get better extracted betalains.

Determination of Betalains Compound with Spectrophotometric Analysis

The betacyanins and betaxanthins content in extracted betalains were determined spectrophotometrically at 538 nm and 480 nm by using UV-Vis spectrophotometer. The betalain content (BC) was calculated as :

$$BC = [(A \times DF \times MW \times 1000) / (e \times l)]$$

The A is absorption, DF the dilution factor and l the path length (1 cm) of the cuvette. For betacyanins and betaxanthins, the molecular weights (MW) and molar extinction coefficients (e) were applied. (MW betacyanins= 550 g/mol; e=60,000 L/mol cm in H₂O) and (MW betaxanthins= 308 g/mol; e=48,000 L/mol cm in H₂O).

Antioxidant Activity Analysis (Brand-William *et al.*, 1995)

0.5 g of freeze dried sample was extracted in 5 ml methanol for 2 hours in dark room. 0.1 ml of extract react with 3.9 ml of DPPH solution (2,2-diphenyl-1-picrylhydrazyl) 6×10^{-5} mol/L (2.9 mg DPPH in 100 ml methanol) for 30 minutes. The antioxidant activity analysis used spectrophotometer at 515 nm. The DPPH solution with no added extract was analyzed as At=0.

The calculation of antioxidant activity is determined in %inhibition:

$$\% inhibition = \left[1 - \left(\frac{A_{t=30}}{A_{t=0}} \right) \right] \times 100$$

Color Intensity Analysis (Lebesi & Constantina, 2009).

Color intensity was measured by Chromameter. The chromameter was calibrated before used. The sample was put in a transparent plastic and shot by chromameter. The result will be shown as L* (lightness) for showing brightness, a* (redness) for showing red or green color, and b* (yellowness) for showing yellow or blue color.

Data Analysis

The obtained data from the analysis was analyzed using Statistical Package for The Social Science (SPSS) for Windows version 16.0 with 95% level of confidence. The analysis type that was used was One Way Anova with Duncan method.

RESULT AND DISCUSSION

Betalains content analysis

According to Vargas *et al.* (2000), betalains are limited and found in

Caryophyllales, for example red beet root. Betalains are water soluble pigment that can be analyzed by UV-visible spectroscopy. Betacyanins absorb the light at 540 nm wavelength, whereas the betaxanthins at 480 nm wavelength (Azeredo, 2009). The spectrophotometry method were used in this research. Based on the research, red beet powder addition contributes the betacyanins and betaxanthins significantly in sample. Betacyanins and betaxanthins in sample increases as higher concentration of red beet powder added. Sample that added by 20% red beet powder contain the highest betacyanins ($33,15 \pm 2,07$ mg/L) and betaxanthins ($16,84 \pm 3,30$ mg/L) before steaming process. It is caused by the addition of red beet powder, therefore the betalains content increased.

Betalains content in each sample significantly decreased after steaming process of 0%, 10% and 20% red beet powder concentration. According to Stinzing & Carle (2004), temperature is the most affecting factor that decomposed betalains compared to pH, water activity, oxygen, light exposure, metal and enzyme activity. Higher temperature of steaming would decreases the betalains in sample (Ravichandran et al., 2013). Reduction of betalains content in sample after heating was caused of betanins degradation from betacyanins group through isomerisation process and decarboxilation or cleavage because of heat and indicaxanthin from betaxanthins group isomerisation (Stinzing et al., 2007).

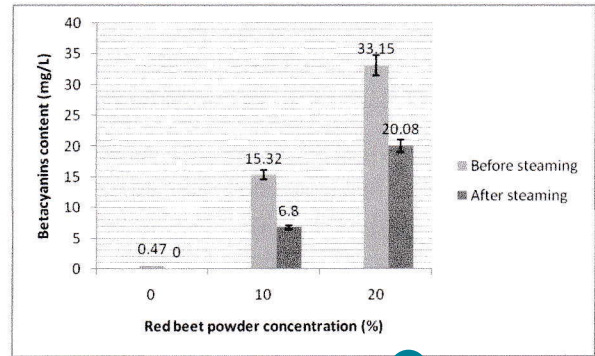


Figure 1. Betacyanins changes affected by the difference addition of red beet powder and steaming period

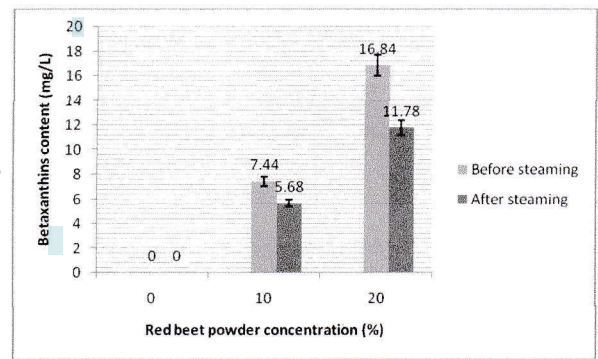


Figure 2. Betaxanthins content in glutinous rice flour batter with addition of red beet powder variation before and after steaming

Antioxidant activity

Betalains pigment contained in red beet root has major contribution on antioxidant activity of red beet (Nemzer, et al., 2011). Different addition of red beet powder affects on antioxidant activity of glutinous rice flour batter. This research showed that the addition of red beet result in high antioxidant activity. In figure 3, there was increasing on antioxidant activity of sample as increasing of red beet concentration on the same heat treatment. It is caused by the present of hydroxyl group on betacyanins structure. The hydroxyl group acts as hydrogen donor of betanin (Azeredo, 2009a). The addition of red beet powder in sample result in

increasing of betacyanins content, therefore antioxidant activity was higher.

Figure 3 shows there are decreasing on antioxidant activity of sample after heat treatment by steaming. Steaming process of batter decreases antioxidant activity up to 43.27%. It is significantly lower than antioxidant activity of sample before steaming. Heat is one of factors that affect stability of betalains. Heating process causes decreasing of betalains content by degradation process. Decreasing of betalains contribute to decreasing of antioxidant activity (Ravichandran *et al.*, 2013).

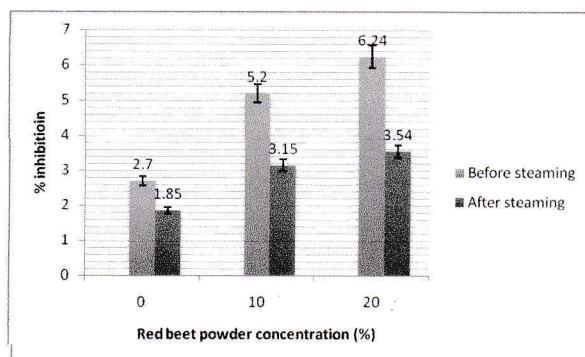


Figure 3. Antioxidant activity of glutinous rice flour batter with different concentration of red beet powder before and after steaming process

Color intensity of glutinous rice flour batter

Red color of sample was determined by a* value of chromametry test result. Figure 4 show there were different of a* value sample with different concentration of red beet powder. Addition of red beet powder increases the red color of sample significantly. The highest concentration of red beet powder has the highest a* value was 22.73 ± 3.72 after 15 minutes of steaming process. The red color was affected by the present of betacyanins in

red beet. Betacyanins are group of betalains that contained in red beet. Betacyanins content of peeled raw red beet was 84.26 mg/L (Azeredo, 2009b). The addition of red beet powder increases a* value of sample. It is caused by increase presence of betacyanins. It is proved by the strong linear correlation of betacyanins and a* (Table. 1).

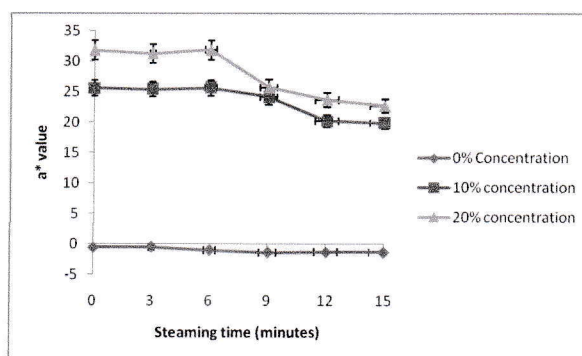


Figure 4. A* value of glutinous rice flour batter with different concentration of red beet powder during steaming process

Table 1. Correlation of a* value and betacyanins content of glutinous rice

Correlated factors	Correlation coefficient	Sign
a* value vs. Betacyanins content	0.871**	0.000

flour batter

**show significance interaction at 0.01 level (99%)

Red color of glutinous rice flour batter with addition of red beet powder decrease as long as steaming process (Fig 4). Heat process such as steaming, effects the stability of betalains pigment. High temperature process degraded the betalains pigment, as well as the red color (Ravichandran *et al.*, 2013). Betacyanins

degraded by isomerisation, decarboxylation, or cleavage mechanism. Degradation of betalains results in degradation of red color and appearance of light brown color as formation of neobetanin (Azeredo, 2009a). Therefore, the longest the steaming time, a* color decrease and form dark color. In figure 5, there was decrease on lightness sample during steaming process.

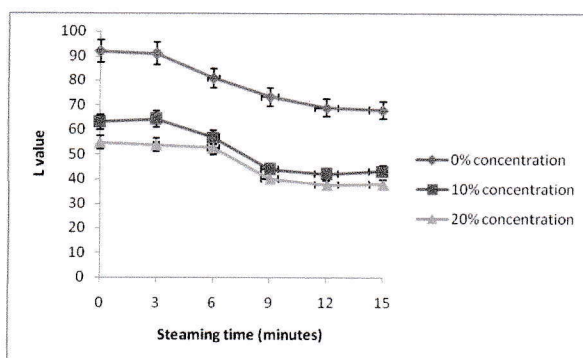


Figure 5. L value of glutinous rice flour batter during steaming process

CONCLUSION

Steaming process of glutinous rice flour batter with addition of red beet powder decrease in a* value (red color intensity), antioxidant activity, and betalains content. Glutinous rice flour batter with addition of 20% red beet powder had the highest betalains content, antioxidant activity, and a* value.

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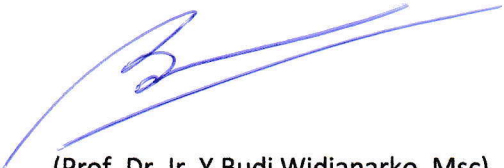
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