Bioactive compound content and antioxidant activities of analog rice enriched with the germinated of white corn (zea mays) and the germinated of mung bean (vigna radiata)

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Abstract. The research aimed to know bio-active compounds' content and antioxidant activity of rice analog based on germinated white corn (Zea mays), germinated mung bean (Vigna radiata) and white sweet potatoes. There were several stages: first, the formulation of analog rice—the second analyzes of total phenol and flavonoids content. The third is the analysis of antioxidant activity done by DPPH, FTC, and TBA methods. Results showed that formulation I TPC levels are 385.00 ± 0.04 (mgGAE/100g), TFC 267.60 ± 0.42 (mgQCE/100g). Formulation II TPC levels are 379.20 ± 0.85 (mgGAE/100g), TFC 235.40 ± 0.92 (mgQCE/100g). Formulation III TPC levels are 346.50 ± 0.85 (mgGAE/100g), TFC 227.00 ± 0.42 (mgQCE/100g). Antioxidant activity formulation I with DPPH method: 67.71 ± 0.20%; The percentage inhibitory with the FTC 69.10 ± 1.01%, TBA 65.28 ± 0.28%. Antioxidant activity formulation II with DPPH method: 66.57 ± 0.20%; The percentage inhibitory with the FTC 60.63 ± 0.81%, TBA method 64.42 ± 0.58%. Antioxidant activity formulation III with DPPH method: 63.57 ± 0.20%; The percentage inhibitory with the FTC 45.41 ± 0.30%, TBA 63.21 ± 0.58%. There is a significant and positive correlation between bioactive compounds with three methods of antioxidant activity used.

1. Introduction

Rice is still the main component of energy in the diet of Indonesian society, so that lack of rice will cause food insecurity and malnutrition. Domination of dependence on certain types of food should be reduced by the diversification of food-based local Carbohydrate (Non-rice), namely by developing analog rice that has good nutritional quality and antioxidant capabilities, thus having the function of minimizing the occurrence of degenerative diseases and can support the resilience and independence of Indonesia food. This developed analog rice has three requirements, first: having proper nutrients, secondly, is sensory accepted by the community; Third, it has functional properties that can prevent and manage degenerative diseases.

The tuber is a significant component of carbohydrate sources in analog rice, so the use of cereals and nuts is necessary to increase the protein and fiber content of tuber composite flour. However, appropriate modifications, formulations, and processing processes are required to produce analog rice. Cereal and legumes germination is a modification to minimize its deficiencies and improve nutrients that are lowering carbohydrates and improving antioxidant efficacy. The process of germination on the legumes and cereal can enhance its functional properties to provide a positive effect on the increase of
bioactive compounds [1]; Increase antioxidant activity [2] and the prevention of colon cancer [3]. Research shows that the extrusion process can increase antioxidant activity [4].

Dependence on rice causes Indonesia to be vulnerable to food insecurity. Need to diversify food based on local potentials by developing analog rice. The development of analog rice can be directed into a functional diet. It contains not only high-quality nutrients, is sensory accepted by the community, but also has specific useful properties. The local food-based analog rice formulation cereals, legumes, and tuber are expected to possess antioxidant activity and have a low glycemic index that has a positive impact on health.

This research aims to determine the content of bioactive compounds (total phenol and flavonoids content) and antioxidant activities of rice analog with DPPH, FTC, and TBA methods.

2. Methods

2.1. Formulation of Analog rice

The ingredients used for the manufacture of analog rice are white sweet potato flour, modified white sweet potato flour, the germinated of mung bean (Vigna radiata), the germinated of white corn (Zea mays), Sago Starch, hydrocolloid, GMS, coconut oil, and water. The percentage for germinated of mung bean (Vigna radiata) 20%, germinated of white corn (Zea mays) 20%, and a different percentage is white sweet potato flour (25-35%) and Sago starch (20-30%). Three analog rice formulations are coded 35.20, 30.25, and 25.20. Code used with explanation: the first is the percentage of white sweet potato flour, while the second digit is the percentage of sago starch. The process of making is done by mixing all dry material until homogeneous, then mixing with hydrocolloid, GMS, coconut oil, and water. It is then printed using an extruder, so it is shaped like rice.

2.2. Extraction process

The analog rice extraction by the maceration method using solvents methanol. The ratio of analog rice: solvent was 1:5 (w/v). The process of maceration was carried out for seven days. After seven days, the solution was filtered with Whatman No.1 Filter paper and evaporated using a rotary evaporator to remove the solvent. The extracts were then stored at at -22°C.

2.3. Determination of total phenol content

Determination of total phenol content using the spectrophotometric method [5]. A total of 0.2 mL of analog rice methanol extracts formulation I, II, and III with a concentration of 100 mg/L, plus as much as 2.5 mL of 10% Folin-Ciocaltelu reagent and 2 mL of 7.5% Na₂CO₃. The obtained mixture is allowed for 15 minutes at a temperature of 45°C. The absorbancy of the solution is measured using a spectrophotometer at a wavelength of 765 nm. The content of total phenolics measured expressed in milligrams (mg) of gallic acid equivalent per 100 grams of the dried extract (mg of GAE/100g extract).

2.4. Determination of total flavonoids content

Determination of total flavonoids content using spectrophotometric methods [6]. At concentrations of 1000 mg/L, as much as 1 mL of methanol extract was added at 1 mL 2% AlCl₃ dissolved with ethanol 50%. Incubation is done for 20 minutes, the mixture is homogenized using vortex and then measured in the wavelength 415nm. The total content of flavonoids expressed as quercetin equivalents (mg of quercetin/100g of extract).

2.5. Antioxidant activity evaluation with DPPH method

Evaluation of antioxidant activity conducted by Method DPPH [7]. The extracts were aliquoted (40 μg/mL) and mixed with 2 mL of DPPH (0.1 mM in methanol solution). The solution homogenized with Vortex, which allowed for 30 minutes at room temperature, protected from light. The absorbance
of the solution measured at a wavelength of 517 nm. The radical scavenging activity was calculated using the formulation:

\[
\frac{A_0 - A_1}{A_0} \times 100\%
\]

(1)

Where \(A_0\) is the absorbance of the control (without the extract), and \(A_1\) is the absorbance of the extract.

2.6. Antioxidant activity evaluation with FTC method
Method of evaluation of antioxidant activity by the FTC method [8]. A total of 2 ml phosphate buffer 0.1 M pH 7, as much as 2 ml linoleic acid 50 mM in ethanol 99.8% and 1 Mliom-free water mixed with 1 ml 2000 ppm of analog rice extract, included in the screw capped vial and placed in a dark room condition at a temperature of 37\(^\circ\)C. The measurement of absorbency was performed 2 days before the control linoleic acid peroxide reached maximum. Then, a number of 50 µl of a mixture of samples incubated added 2.35 mL of ethanol 75% and 50 µl ammonium thiocyanate 30%, then added with 50 ML 0.02 M FeCl2 in 3.5% of the HCl solution for 3 minutes and measured its absorption at a wavelength of 500 nm. Percent of inhibitory peroxide calculated in the following way:

\[
\text{Percentage of inhibition (\%)} = \frac{\text{Absorption of control} - \text{absorbance of sample}}{\text{absorbans control}} \times 100\%
\]

(2)

2.7. Antioxidant activity evaluation with thiobarbituric acid (TBA) method
The method to the analysis of antioxidant activity with TBA [9]. A total of 1 ml of a sample solution containing antioxidant extracts that have incubated at 37 °c plus 2 ml trichloracetic acid (TCA) 20% and 2 ml of thiobarbituric acid (TBA) 1% in the 50% acetic acid solvent. This mixture placed in a boiling water bath for 10 minutes. After the cold condition, centrifuged at 3000 rpm for 20 minutes. The sample absorbance measured at a wavelength of 532 nm. Percent inhibition (\%) = 100-(absorption of sample: absorbansi blanko) x 100%.

2.8. Statistical Analysis:
The obtained results are presented in the form of average and standard deviation. One-Way ANOVA was used to analyze differences in means between the samples followed by the least significant difference test at \(p < 0.05\). The correlation between the bioactive compounds with the antioxidant activity tested with Pearson correlation bivariate Using SPSS version 16.0.

3. Result and Discussion

3.1. Bioactive compound content
The analog rice developed three formulations that analyzed the levels of its bio-active compounds, namely the phenol and the total levels of flavonoids. Based on Table 1 shows that all formulas contain TPC and TFC. The content of bioactive compounds (Total phenol and total flavonoids) comes from the composition of the constituent material, namely white sweet potato, germinated of white corn (Zea mays), and germinated of mung bean (Vigna radiata). Some research suggests that the germination process of mung bean (Vigna radiata) can increase the levels of bio-active compounds, which has an impact on increasing antioxidant activity [2]. The germination of white corn (Zea mays) can increase the antioxidant and bioactive compounds, including the total phenolic content [10]. White sweet potato flour is one of the constituent rice of analog that has antioxidant activity. White sweet potato flour contains phenolic [11].
Table 1. Total phenol and flavonoid content of rice analog

| Parameter | Total Phenol Content (mg GAE/100g) | Total Flavonoid Content (mg QUE/100 g) |
|-----------|-----------------------------------|--------------------------------------|
| Formula I | 385.00±0.4c                       | 267.6±0.42c                          |
| Formula II| 379.2±0.85b                       | 235.40±0.92b                         |
| Formula III| 346.50±0.85a                      | 227.00±0.42a                         |

Different Letters (A-f) within the same column indicate significant differences at P < 0.05.

3.2. Antioxidant activity

Table 2 shows that all of the analog rice formulations have an antioxidant activity evaluated using both the DPPH, FTC, and TBA methods. The magnitude of this free radical capture capability is caused by total levels of phenol, and total flavonoids in analog rice formula I are higher than rice analog formula II and III (Table 1). Phenol and flavonoid levels are essential antioxidant components and are responsible for deactivating free radicals based on their ability to donate hydrogen. The total phenol and flavonoid content become compounds that play a role in determining antioxidant activity. The compound flavonoids have antioxidant activity, and their oxidant potential depends on the number and position of the group OH free. Some research suggests that phenol compounds are compounds that have a strong relationship/correlation to antioxidant activity [12].

Table 2. Antioxidant activity of rice analog

| Parameter | DPPH (% RSA) | FTC (% inhibitory) | TBA (% inhibitory) |
|-----------|--------------|--------------------|--------------------|
| Formulation I | 67.71±0.01c  | 69.10±1.01c        | 65.28±0.28b        |
| Formulation II | 66.57±0.20b  | 60.63±0.81b        | 64.42±0.58ab       |
| Formulation III | 63.57±0.20a  | 45.41±0.30a        | 63.21±0.58a        |

Different Letters (a-c) within the same column indicate significant differences at P < 0.05.

The FTC and TBA evaluation were measurement of antioxidant activity based on inhibition of fat peroxidation reaction events. The FTC method is used to determine the level of lipid hydroperoxide in a biological system. Table 2. This shows that the extract of analog rice formulation I, II, and II can inhibit lipid peroxidation, which is evaluated by the FTC method. Formulation I Analog Rice can inhibit the largest lipid peroxidation than Formula II and III. The ability of antioxidants is associated with the ability of phenol and flavonoids in inhibiting the formation of peroxide from linoleic acid.

The TBA method is used to measure the number of peroxide in the second stage of lipid peroxidation and measure the free radicals that exist after the peroxide oxidation. In the second stage formed MDA as the final stage of lipid peroxidation. The principle of this method is absorption measurement with a spectrophotometer from the reaction of MDA with a pink thiobarbituric acid at a wavelength of 532 nm. The addition of an analog rice extract formulation I, II, and III can decrease the formation of MDA. Table 2 shows the Formulation I analog rice has the most significant ability to inhibit the formation of MDA than the Formulation II and III analog rice. It is associated with the presence of bioactive compounds, TPC, and TFC, which have the ability to inhibit lipid peroxidation.

Several studies have shown that the mechanisms of TPC and TFC as antioxidants are iron-chelating the transition [13], scavenging free radicals that can initiate oxidation of lipids or take part in the propagation of free radical chains [14].

3.3. Correlation bioactive compounds and antioxidant activity

There is a significant and positive correlation between phenol and flavonoid levels with antioxidant activity evaluated using DPPH, FTC, and TBA (Table 3). This indicates that the content of TPC and TFC on analog rice plays a role in determining the magnitude of antioxidant activity. Different literature suggests that there is a linear correlation between phenol levels and flavonoids with antioxidant activities of both DPPH, FTC, and TBA [15].
Table 3. Correlation of bioactive compounds and antioxidant activity

| Correlation | TPC   | TFC   | DPPH  | FTC   | TBA   |
|-------------|-------|-------|-------|-------|-------|
| TPC         | 1     | 0.756** | 0.945** | 0.973 | 0.863 |
| TFC         | 0.756 | 1     | 0.818 | 0.878 | 0.819 |
| DPPH        | 0.945 | 0.818 | 1     | 0.944 | 0.992 |
| FTC         | 0.973 | 0.878 | 0.944 | 1     | 0.869 |
| TBA         | 0.863 | 0.819 | 0.892 | 0.869 | 1     |

** means significant correlation at 0.01 level

4. Conclusion

Analog rice developed either formulation I, II, or III, has phenol and flavonoids content. Analog rice also has antioxidant activity evaluated using the methods of DPPH, FTC, and TBA. There is a positive and significant correlation between the content of bioactive compounds and antioxidant activity in analog rice. The resulting analog rice can be continued to be developed into functional food.

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5. References

[1] Gan RY Lui WY Wu K Chan CL Dai SH Sui ZQ and Corke H 2017 Bioactive compounds and bioactivities of germinated edible seeds and sprouts An updated review Trends in Food Science & Technology 59: 1-14
[2] Xue Z Wang C Zhai L Yu W Chang H 2016, Bioactive Compounds and Antioxidant Activity of Mung Bean (Vigna radiata L.), Soybean (Glycine max L.) and Black Bean (Phaseolus vulgaris L.) during the Germination Process. Czech J. Food Sci., 34, 68–78.
[3] Shin A Lee J Lee J Park MS Park SC Oh JH and Kim J 2015. Isoflavone and Soyfood Intake and Colorectal Cancer Risk: A Case-Control Study in Korea. PLOS ONE, DOI:10.1371/journal.pone.0143228, 1-17.
[4] Hegazy HS El-Bedawey AEFA Rahma ESH Gaafar AM 2017 Effect of Extrusion Processes on Nutritional,Functional Properties and Antioxidant Activity of Germinated Chickpea Incorporated Corn Extrudates. American Journal of Food Science and Nutrition Research 4(1): 59-66.
[5] Singleton VL Orthofer R and Lamuela-Raventos RM 1999 Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods in Enzymology, 299, 152-178.
[6] Quettier-Deleu C Gressier B Vasseur J Dine T Brunet J Luyck M Cazin M Cazin JC Bailleul F and Trotin F 2000. Phenolic compounds and antioxidant activities of buckwheat (Fagopyrum esculentum Moench) hulls and flour. Journal of Ethnopharmacology 72(1-2) 35-40.
[7] Singh BN Singh BR Singh RL Prakash D Singh DP Sarma BK Upadhyay G Singh HB. 2009 Polyphenolics from various extracts/fraction of red onion (Allium cepa) peel with potential antioxidant and antimutagenic activities. Food Chem Toxicol. 47:1161–1167.
[8] Chen, H.M., Muramoto, K., Yamauchi, F. and Nokihara, K. 1996. Antioxidant activity of designed peptides based on the antioxidative peptides isolated from digests of a soybean protein. Journal of Agriculture Food Chemistry 44: 2619.
[9] Kikuzaki H Nakatani N 1993. Antioxidant effects of some ginger constituents. J. of Food Sci 58(6): 1407-1410.
[10] Žilić S, Delić N, Basić Z and Vančetović J 2015 Effects of alkaline cooking and sprouting on bioactive compounds, their bioavailability and relation to antioxidant capacity of maize flour *Journal of food and nutrition research* 54(2):155-164.

[11] Kim MY, Lee BW, Le HU, Le YY, Kim MH, Lee JY, Lee BK, Woo KS, and Kim HJ 2019 Phenolic compounds and antioxidant activity in sweet potato after heat treatment *Journal of the Science and Agriculture* 99 (15).

[12] Piluza G Bullitta S 2011 Correlations Between Phenolic Content and Antioxidant Properties in Twenty-Four Plant Species of Traditional Ethnoveterinary Use in the Mediterranean Area. *Pharm Biol* 49(3): 240-247.

[13] Hider RC, Liu ZD and Khodr H 2001 Metal Chelation of Polyphenols *Methods in Enzymology* 335:190-203.

[14] Mustafa RA, Abdul Hamid A, Mohamed S Bakar FA 2010 Total Phenolic Compounds, Flavonoids, and Radical Scavenging Activity of 21 Selected Tropical Plants *Journal of Food Science* 75(1):C28-35.

[15] Nugraheni M Santoso U Windarwati Phytochemical compounds and antioxidant activity of Coleus tuberosus flesh and peel on different solvent *Food Research* 2 (5) : 460 -467.