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Chemical Characteristic of Kembang Loyang from Red, Black and White Rice.

Tuty Anggraini*, Riska Amelia, and Surini Siswardjono

Faculty of Agriculture Technology Kampus Limau Manis Andalas University, 25163 Padang West Sumatra Indonesia.

ABSTRACT

This research was aimed to characterized Kembang Loyang from red, black and white rice towards the antioxidant content, total polyphenol, anthocyanin, amylose and protein content. This research used explorative method with three replications. Thirtheen of coloured rice was processing to be a traditional food named Kembang Loyang. The result showed that, black rice kembang loyang from solok Selatan was the highest for antioxidant activity 43.4% at concentration 0.25 mg/mL, total polyphenol content 20.4 mg/mL, anthocyanin 83.11 mg/L, protein content 1.84% and amylose content 7.3%.
Keywords: colored rice; antioxidant; kembang loyang

**Corresponding author*

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INTRODUCTION

Industial development and life style give positive and negative impact to society. One of the negative impact was emerged some health issues regarding degenerative diseases in Indonesia. Food and nutrition researchers in Indonesia are concerned about to find the source of antioxidant in food to scavenge the radicals can caused degenerative diseases. They have been trying to examine antioxidant activity on some natural materials such as rice.

Whole grain of rice is a good source of antioxidant, including vitamin E homologues (including α-, β-, γ- and δ- tocopherols and α-, β-, γ- and δ- tocotrienols) and γ- oryzanol which have health beneficial potential (Kariwala, 2001). Rice has different colors that is arranged genetically caused by different gen of rice arranging aleurone colors, endospermia colors, and starch composition on endospermia. Different colors of rice also because of soluble pigmen in water is called anthocyanin. Anthocyanin is one of antioxidant compound that has capability to break chain reaction of free radical in human's body (Chung and Shin, 2007). Colored rice varieties have good quality protein, high fiber content and vitamin content that potential to promote human health because antioxidative compound (Adom and Liu, 2002).

West Sumatra is a potential area for growth of colored rice. From our previous research, colored rice from some area in West Sumatra showed high level of antioxidant. The research determined the antioxidant activity of red, black and white rice, polished and unpolished rice (Anggraini *et al*, 2015). The present study was to explore the product made from colored rice from West Sumatra, Indonesia.

Kembang Loyang is a traditional food , usually made for special event and as a trade mark from West Sumatra. Kembang Loyang has crispy character processed with deep fat frying usually from white rice flour. Rice which has a color consist of anthocyanin that has capability as antioxidant, therefore further research regarding other varieties of rice used to make kembang loyang has been intiate. Rice that were used for this research consist of several varieties of rice such as white rice flour, red rice flour, and black rice flour. Besides as antioxidant, the pigment in red and black rice could be use as colorant for Kembang Loyang.

MATERIALS AND METHODS

Plant Materials

Seven red (include three polished red rice cultivars), four black, and two white rice cultivars as control were used in this study (Figure 1). These materials were derived from different planting area in West Sumatra region : Red rice : Batu Sangkar (S) (1), Padang Panjang (2), Kubang Putih (3), Lembah Gumanti (S) (4) , Solok (5), Pasaman (6) and Sariak Alahan Tigo (7). Black rice : Batusangkar (8), Palembayan (9), Sariak Alahan Tigo (10), Solok Selatan (11). White rice : Kuriak Batusangkar (12) and Seratus Hari Kamang (13). (S) mean polished rice.

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), ethanol, methanol, gallic acid, Na_2CO_3, KCl, HCl, sodium acetate, CH_3CO_2Na, distilled water, sulphuric acid, ammonium sulphate, brucine reagent, sodium hydroxide.

Kembang Loyang Processing

First, rice flour was added into 150 mL coconut milk and also was added an egg. Stir dough equally. Then, dough which has already formed, fried into deep oil.

DPPH radical scavenging activity

DPPH radical scavenging activity was determined using the method originally developed by Blois (1958). A portion (0.1 ml) of the extract solution (1.0 mg/ml methanol) in a test tube was well mixed with 3.9 ml of methanol and 1.0 ml of a DPPH solution (1.0 mM in methanol). The mixture was kept at ambient temperature for 30 min prior to measurement of the absorbance at 517 nm (A517 nm). All measurements were done in triplicate.

$$\text{Antioxidant Activity} = [(\frac{\text{control absorbance} - \text{extract absorbance}}{\text{control absorbance}}) \times 100\ \%$$

Total Polyphenol

The total phenolic content of the extract was measured with the Folin–Ciocalteau method (Taga, Miller, and Pratt, 1984), using gallic acid as a standard. A 0.1 ml of the extract solution was sampled into 2 ml of 2% Na_2CO_3 and mixed for 3 min. After adding 0.1 ml of 50% Folin–Ciocalteau reagent, the final mixture was left for 30 min before reading the absorbance at 750 nm. All measurements were conducted in triplicate and the data were expressed as g gallic acid equivalent (GAE) per kg of the extract, based on the calibration curve of gallic acid.

Anthocyanin content (Lee , 2005)

1.86 g KCl into a beaker and add distilled water to ca 980 mL. Measure the pH, and adjust pH to 1.0 (±0.05) with HCl (ca 6.3 mL). Transfer to a 1 L volumetric flask, and dilute to volume with distilled water. (**b**) *pH 4.5 buffer (sodium acetate, 0.4M).*—Weigh 54.43 g $CH_3CO_2Na{\cdot}3H_2O$ in a beaker, and add distilled water to ca 960 mL. Measure the pH, and adjust pH to 4.5 (±0.05) with HCl (ca 20 mL). Transfer to a 1 L volumetric flask, and dilute to volume with distilled water.

Calculate anthocyanin pigment concentration, expressed as cyanidin-3-glucoside equivalents, as follows:

Anthocyanin pigment (cyanidin-3-glucoside equivalents, mg/L) =

where A = (*A*520nm – *A* 700nm)pH 1.0 – (*A*520nm – *A*700nm)pH 4.5;

$$(\text{mg L}^{-1}) = \frac{(A\,x\,MW\,x\,DF\,x\,1000)}{(\varepsilon\,x\,d)}$$

Where,

MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside (cyd-3-glu); DF = dilution factor established in **D**; l = pathlength in cm; = 26 900 molar extinction coefficient, in L & mol–1 & cm–1, for cyd-3-glu; and 103 = factor for conversion from g to mg.

Protein Content

The crude protein was determined by the Kjeldahl method with slight modification. 0.5 g of the powdery form of each colored rice variety was digested with 5 ml of concentrated sulphuric acid in the presence of Kjeldahl catalyst. The nitrogen from the protein in the sample was converted to ammonium sulphate that reacted with 2.5 ml of 2.5 % Brucine reagent, 5 ml of 98 % sulphuric acid to give a coloured derivative and the absorbance read at 470 nm. The percentage nitrogen was calculated and multiplied by 6.25 to obtain the value of the crude protein (A.O.A.C., 1990).

Amylose content

To 100 mg of flour, 1 ml of 95% ethanol and 9 ml of 1N sodium hydroxide were added. After mixing, the samples were heated for 10 min in a boiling water bath to gelatinize the starch. Samples were cooled down and transferred to a 100-ml volumetric flask and 5 ml of starch solution and 1 ml of 1N acetic acid were added. After the addition of 2 ml of iodine solution, the volume was adjusted to 100 ml with distilled water, mixed, and allowed to stand for 20 min. The absorbance was measured at 620 nm using a spectrophotometer (UV mini 1240 UV-VIS, Shimadzu, Kyoto, Japan). The amylose content was determined from a previous standard curve of rice.

RESULT AND DISCUSSION

Antioxidant Activity of Kembang Loyang

Different color of rice were used affecting to antioxidant activity of each kembang Loyang. The result showed that color/pigmens in each rice affected to antioxidant activity of kembang Loyang. Color rice has natural pigmen called anthocyanin. The presense of anthocyanin in rice followed by the presence of antioxidant in Kembang Loyang. Results of antioxidant activity of kembang Loyang are shown at Figure 1.

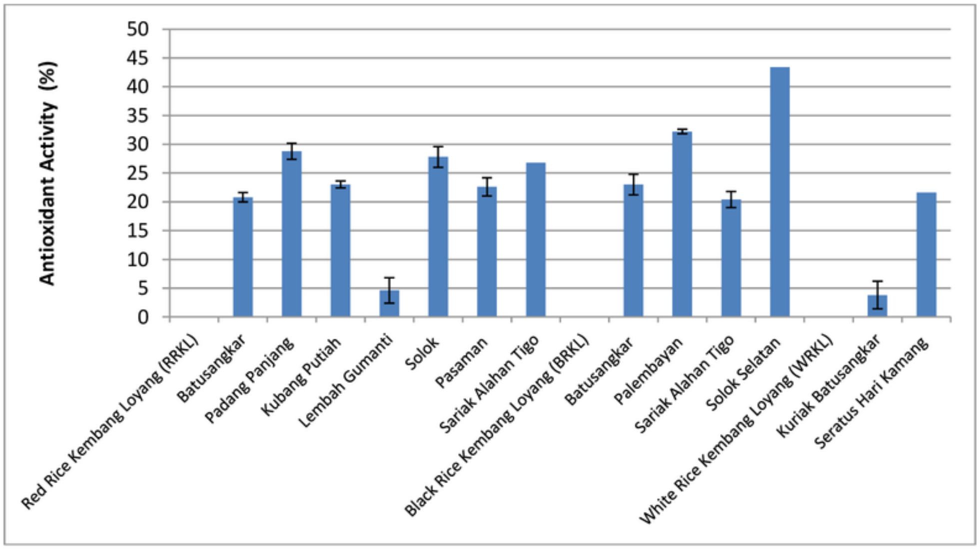

Figure 1. Antioxidant Activity of Kembang Loyang

Based on Fig 1, antioxidant activity of black rice kembang Loyang (BRKL), Solok Selatan cultivar was the highest among others. Red rice kembang loyang (RRKL), antioxidant activity ranged from 4.30 – 28.80%. The result showed that the level anthocyanin in rice effected to antioxidant activity for Kembang Loyang. The rice sample were vary in level of anthocyanin, some of the rice cultivar were polished rice in some level. Red rice Kembang Loyang, Lembah Gumanti cultivar have the lowest antioxidant activity among others, because Lembah Gumanti is a polised rice.

BRKL Solok Selatan had the highest antioxidant activity because, from the rice color, it have the darkest color compared with another black rice cultivar. White rice had low antioxidant activity because of lack source of antioxidant. The same results showed from previous research that red rice cultivar from Solok Selatan was the best cultivar among other with antioxidant acitivity 54.2% at concentration 0.25 mg/ml, total polyphenol content 31.3mg/ml and protein content 7.9% (Anggraini *et al*, 2015).

Compared with raw material, the antioxidant activity of Kembang Loyang was decreased because of high temperature on processing. Heating were affecting to antioxidant activity of rice caused by oxidation that affected to degredation. Colored rice varieties with high antioxidant activities become a good source of antioxidant and a genetic resource to develop new health (Nam *et al*, 2006).

Total Polyphenols

Total polyphenols RRKL, BRKL and white rice kembang Loyang (WRKL) showed on Figure 2. From previous research (Anggraini, 2015) from red, black and white rice have high level of total poyphenols. This present study showed that the processing of red, black and white rice to make Kembang Loyang with deep frying method still have functional compound.

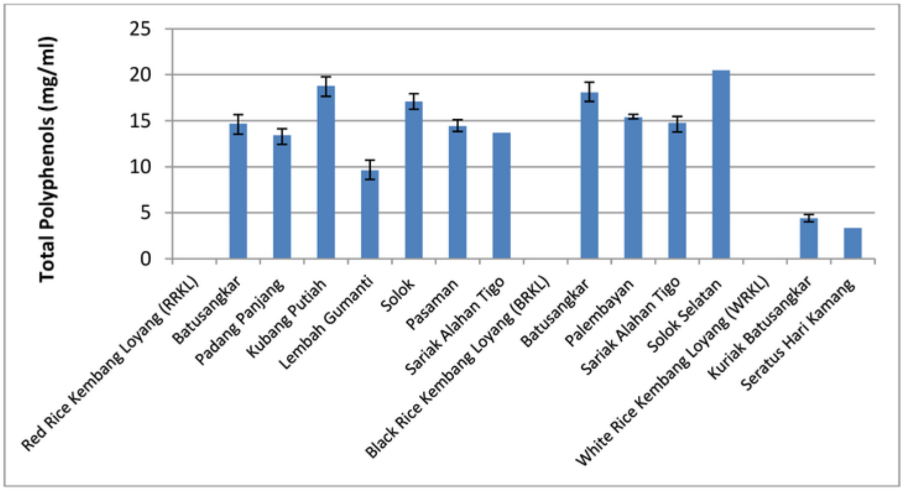

Figure 2. Polyphenols of Kembang Loyang

The highest polyphenols of kembang Loyang was BRKL from Solok Selatan, same result with antioxidant activity. WRKL have the lowest content of polyphenol compared with RRKL and BRKL. Kembang Loyang processed with deep frying method, which mean processed with high temperature. Fortunately Kembang Loyang still have 3.30-20.75 mg/ml of polyphenols for BRKL, RRKL and WRKL. BRKL ranged from 14.40-20.75 mg/ml, RRKL ranged from 9.74-19.06 mg/ml and WRKL ranged from 3.30-3.64 mg/ml. Rice is a rich source of many bioactive compounds, including phenolic antioxidants that have the potential ability to reduce the risk of disease by inhibiting platelet aggregation (Daniel *et al.*, 1999). The bran of colored rice varieties has greater antioxidant and free radical scavenging activities than white rice (Nam et al, 2016). Hydrothermal processing of purple bran rice, the retention of extractable anthocyanins was low, but was high for simple phenolics (Min *et al*, 2014).

Anthocyanin

Anthocyanins are water soluble pigments exhibit red, purple and blue colors. Anthocyanins possess great potential as food colorants, which have increased interested to replace synthetic colorants (Brouillard, 1982).

Anthocyanin was only examined to black rice because its high anthocyanin. Red rice had less anthocyanin, whereas white rice had no anthocyanin. The highest anthocyanin showed from BRKL from Solok Selatan and the lowest anthocyanin was at BRKL from Painan. Result of the study suggest that anthocyanin in black rice could be an alternative way to produce functional traditional food like Kembang Loyang . Same result with Sui *et al* (2016), that fortification of anthocyanin to bread with lower digestion rate and extra health benefit. After hydrothermal processing of purple bran rice,the retention of extractable anthocyanins was low, but was high for simple phenolics. For proanthocyani-dins found in red bran rice, there was a significant decrease that was positively correlated with the degree of polymerization and the temperature of the processing methods (Min, McClung, Chen 2014).

A significant decrease correlated with degree of anthocyanin polymerization and the temperature of the processing (Min *et al*, 2014). According to Hoa *et al* (2015), The anthocyanin-rich black rice extract remarkably reduced the levels of serum creatine. The protective effect of anthocyanins were due to the free radical scavenging capacity.

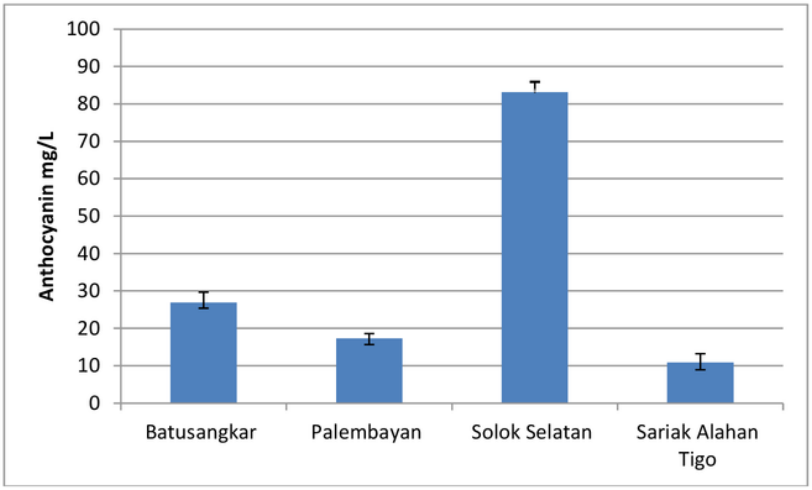

Figure 3. Anthocyanin of Black Rice Kembang Loyang

Amylose

The most abundance component in rice is starch. Starch consists of amylose and amylopectin (Hizukuri, Takeda, Yasuda and Suzuki, 1981). Figure 4 showed the amylose content of Kembang Loyang From Colored Rice.

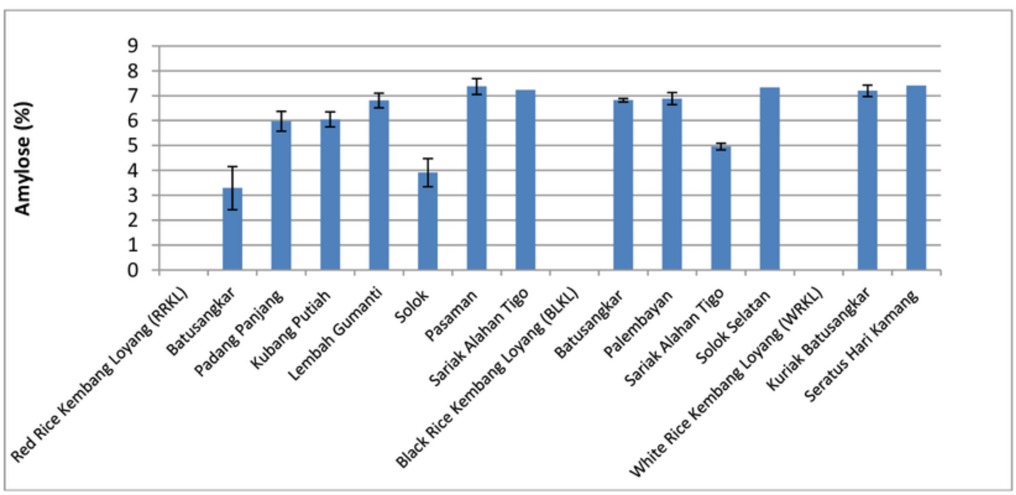

Figure 4. Amylose on Kembang Loyang

Figure 4 showed that there is no correlation between amylose and colored rice in Kembang Loyang. Polished rice have lack amylose content compared with non polished rice, because while polishing, will remove the amylose content in rice grain.

Amylose of kembang Loyang were examined to determine effect of processing toward amylose content on kembang Loyang. There were decreasing of amylose content after processing. Measurement of amylose content were measured by iodine principle which is amylose bond with iodine at low pH (4,5-4,8) at

620 nm wavelength, resulting blue color. Intensity of blue color were measure by using spectrophotometer. Higher color intensity measured, amylose content were higher. Highest amylose content was 15,88, which was black rice kembang Loyang from palembayan, whereas lowest amylose content was red rice kembang Loyang from lembah gumanti.

Amylose is a linear molecule containing glucose unit linked by α-1,4, linkages with a few branches (Hizukuri, Takeda, Yasuda and Suzuki, 1981).

Protein

Figure 5 showed the result of protein content in Kembang Loyang from colored rice. Protein is important for body metabolism. While tissue cells formed, protein has a role as macronutrient to biomolecule formed.

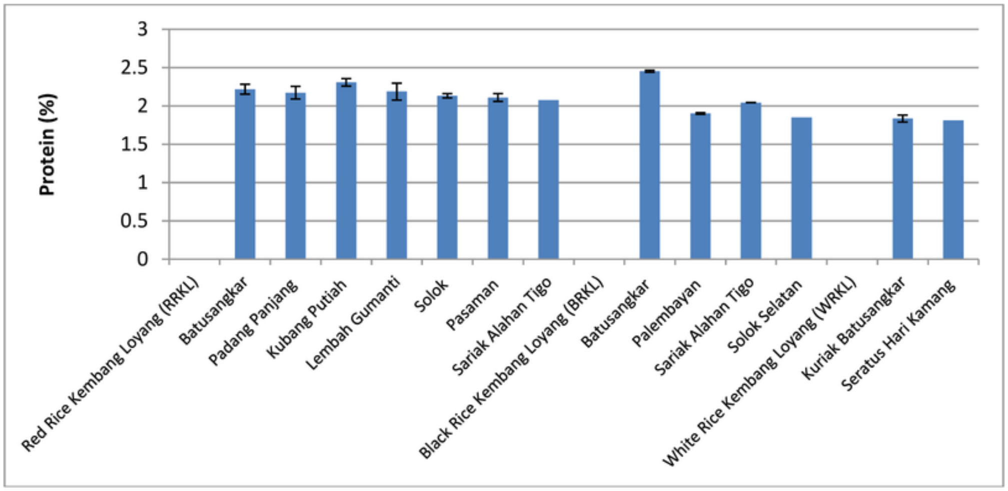

Figure 5. Protein of kembang loyang

The highest protein content was BRKL from Batu Sangkar, whereas the lowest were WRKL Seratus Hari Kamang. Protein on kembang Loyang has been decreasing after processing cause by heating at 160°C. Rice bran protein reported compose of high amount of lysine, an essensial amino acid and a good source of hypoallergenic proteins (Wang *et al*, 2009). Heating affected to decrease of protein. Generally, high protein food processing can be used physical, chemical, or biological treatments.

CONCLUSION

Kembang Loyang from solok selatan determined highest antioxidant activity 43,3 % at concentration 0,25 mg/ml, polyphenols 20, 4 %, anthocyanin 83,11 %, and amylose 7,32 %. Colored rice as a source of antioxidant and other nutritional value, can produced as functional food, such as Kembang Loyang.

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