Improving meatball quality using different varieties of rice bran as natural antioxidant

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Abstract

Meatballs are very popular in every social class in many developing countries including Indonesia and are produced both under the cottage or commercial meat processing industries. Rice bran contains antioxidants and therefore the substitution of cassava starch (tapioca) in making meatball are expected to improve the functional quality of “bakso” into one of traditional healthy restructured meat product. Rice bran has antioxidant activity which different among varieties and agronomical environment. Therefore, the objective of this study was to determine the bioactive compounds such us antioxidant activity of crude extract of rice bran from Bramo, Serang and Menthik rice varieties, those were obtained from East Java, Indonesia and to study the efficacy of utilization the above rice bran to improve functional quality of meat ball. Results of the study showed that the best substitution preferred by panelists was the substitution of tapioca starch with 50% Serang rice bran with organoleptic scores of 4.98; 4.84; 4.93; 4.6; 4.5 and 4.9, respectively, for color, aroma, hardness, chewiness, taste, and flavor. This substitution treatment also increased (p<0.05) the antioxidant activity from 16.75% to 35.78% and total phenol of meatballs from 37.82 mg/100 g to 90.81 mg/100 g. The results also showed that substitution of tapioca starch with 50% Serang rice bran obviously improved the eating quality of the meatballs as a community healthy food.

1. Introduction

Meatballs are restructured meat products which can be produced from ground beef, pork, chicken or fish and beef meatballs being the most popular in Indonesia. This product is prepared by mixing finely ground beef with cassava starch (tapioca), cooking salt and sodium tripolyphosphate (STP) and the meatball batter are formed into marble of pingpong ball size or smaller/bigger, and boiled until well cook (Romans et al., 1994; Purnomo and Rahardiyan, 2008). According to Fischer (1996), beef meatballs are made of 53% lean beef, 17% fat and starch, STP, salt, monosodium glutamate (MSG) 30% ice cubes. While Rahardiyan (2002) reported that Indonesian meatball or better known as “bakso” are prepared using finely ground beef mixed with cooking salt, starch and garlic.

Beef meatballs are very popular in every social class in Indonesia being produced in cottage industries as well as by commercial meat processing manufacturers (Purnomo and Rahardiyan, 2008). Since meatball is a popular meat product, it would be ideal to improve its nutritive value and functional quality such as enhancing its antioxidant content to produce a healthy food. Antioxidants act as radical scavenger, can decrease oxidative stress, so it plays a vital role in prevention of various disease. The use of natural antioxidant in food is more preferable compared to the use of synthetic antioxidant for the consumers (Lobo et al., 2010). Therefore, substitution of cassava starch (low in antioxidant content) with rice bran as source of natural antioxidant, and it can be a viable option to develop meatball into one of traditional functional restructured meat product.

Rice bran is the cuticle between paddy husk and rice grain produced during rice milling and is mainly sold cheaply as animal feed (Kahlon, 2009). Rice bran is reported to contain fiber and also high in energy and protein and also contain zinc, iron, folic acid and other nutrients but no cholesterol (Rabbani and Ali, 2009). It also contains phytochemicals including phytosterols, vitamin B group and polyphenols, and polyphenols are commonly known as antioxidants (Rabbani and Ali, 2009). All these constituents contribute to the lowering blood serum cholesterol, have anti-cancer properties and
protect the body from free-radical damage (Amarasinghe et al., 2009).

The antioxidant activity in rice bran different among rice varieties and agronomical environment (Xu and Godber, 2001; Nam et al., 2006; Sompong et al., 2011). Although antioxidant of rice bran has been extensively studied, the potential antioxidant of local rice bran is still lack. Therefore, the objectives of this study were to determine the bioactive compounds such as antioxidant activity of crude extract rice bran from several varieties of rice, namely Bramo, Serang and Menthik and to study the efficacy of the above rice bran to improve functional quality of meatball as a community healthy food.

2. Materials and methods

2.1 Preparation of rice bran samples

Rice varieties used in this study were local rice such as Bramo, Serang, and Menthik. All dry paddy samples were dehulled and milled to obtain mainly white rice and rice bran flour as by-product. The freshly milled rice bran samples were collected immediately from the milling in polyethylene plastic bags. Stabilization of rice bran was carried out according to methods as reported by Amarasinghe et al. (2009) and Bagchi et al. (2014) with slight modification. A total of 100 g of fresh fine powder rice bran was exposed to microwave heating for 2.5 mins in polyethylene plastic bags and stored in refrigerator at 4°C until laboratory analysis.

2.2 Preparation of Indonesian meatball

The preparation of meatball followed the method of Rahardiyan (2002) with slight modification. The formula was as follows: finely ground shank beef (1000 g), ice cubes (288 g), cassava starch (144 g where in this study this starch was substituted with 50 (72 g) and 100% (144 g) rice bran, and control or sample without substitution of starch was made), cooking salt (24 g), fried garlic (16 g), fried red onion (16 g) and STP (4 g). These ingredients were mixed thoroughly using meat cutter to make the meatball batter and then it was formed into balls and subsequently boiled for 15 minutes. Well cook meatballs were cooled to ambient temperature, and then packed in nylon vacuum bag, vacuumed and stored at 4°C before laboratory analysis was conducted.

2.3 Experimental design

This study was conducted in a two-stage laboratory experiment. Stage 1 of the experiment was conducted to study the antioxidant activity, anthocyanin content and total phenolic content from three different rice bran varieties and the second stage of the experiment was to study the effect of substitution of cassava starch with rice bran in meatball production having antioxidant activity, high phenolic content and acceptable for consumers. Experimental design of this study was randomized completely experimental design. All experiments were carried out with three replications of analysis and the data obtained will be processed by mathematical and statistical methods. Data were subjected using one-way analysis of variance (ANOVA) using the statistical analysis Microsoft Excel for Mac, significance at 5% (p<0.05) (Gedrovica and Karklina, 2013). The means were separated by Tukey’s honestly significant difference (HSD) to Realstat-2011 that was added to Microsoft Excel for Mac (Ott and Longnecker, 2001).

2.4 DPPH radical scavenging assay

Antioxidant activity test of crude extract of rice bran was determined by the free radical-scavenging 1,1-diphenyl-2- picrylhydrazyn (Lee et al., 2006). The extraction of antioxidant compound was conducted by mixing 200 mg sample with 5 mL of methanol and shaken for 1 hr. 5 mL supernatant extract was reacted with 1 mL DPPH 0.1 mM working solution. The mixture was shaken and incubated in the dark condition at room temperature for 30 mins (Hatano et al., 1988; Yen and Chen, 1995). The absorbance was measured by UV-Vis spectrophotometer at 517 nm. The inhibition was calculated using following formula:

\[
\text{Scavenging ability} = \left( 1 - \frac{\text{Absorbance sample at } 517 \text{ nm}}{\text{Absorbance control at } 517 \text{ nm}} \right) \times 100\%
\]

2.5 Total phenolic content

Total phenolic content of crude extract of rice bran was determined by spectrophotometric method using the Follin-Ciocalteu reagent (Singleton and Rossii, 1965). Rice bran was extracted by maceration with ethanol 60% for one night and repeated for three times. A total of one hundred microliters of the crude extract of rice bran solution (5 mg/mL) was mixed with 1.5 mL sodium carbonate solution 10% and then 3 mL of Follin-Ciocalteu 10% reagent was added. The final mixture was kept in the dark condition at ambient temperature for 2 hrs to complete the reaction. The absorbance was measured by spectrophotometer at 765 nm. All measurements were determined triplicate and the data will be expressed as mg Gallic Acid Equivalent (GAE) per 100 g of crude extract of rice bran.

2.6 Total anthocyanin content

Determination of the total amount of anthocyanins was carried out using spectrophotometric method as reported by Markakis (1982) and Moko et al. (2014). A total of 100 g sample was macerated with 100 mL mixture of ethanol 95% and HCl 1.5 N (85:15, v/v) and stored at 4°C for overnight. The mixture was decanted
and the pomace was washed repeatedly with acidic ethanol solvent and made up to volume 500 mL. Anthocyanins were measured using spectrophotometer at wavelength 535 nm against a blank reagent. Cyanidin 3 glucoside chloride was used as standard pigment, and total anthocyanin content will be expressed as mg cyanidin 3 glucose equivalent per 100 g sample.

2.7 Sensory evaluation

Preference of the meatballs with rice bran was evaluated by thirty untrained panelists for each replication with hedonic test (Beinner et al., 2010). Each panelist was served with seven samples (without rice bran (0) as control, substitution with Bramo rice bran 50% (B50), substitution with Bramo rice 100% (B100), substitution with Serang rice bran 50% (S50), substitution with Serang rice bran 100% (S100), substitution with Menthik rice bran 50% (M50), substitution with Menthik rice bran 100 % (M100)). Meatballs were offered to panelists for sensory evaluation using a 7-point hedonic scale to determine the panelists expression in the degree of liking or disliking for each kind of sample (Beinner et al., 2010) whereby the scale was described as 7 – like very much, 6 - like moderately, 5 – like slightly, 4 – neither like nor dislike, 3 – disliked slightly, 2– disliked moderately and 1 – disliked very much. Seven coded samples were served and water was provided for rinsing the panelists mouth between taste the samples. The organoleptic parameters including color, aroma, hardness, chewiness, taste, and flavor. Panelists were asked to choose the best and the worst with comment for better understanding.

3. Results and discussion

3.1 Determination of antioxidant properties and phytochemical contents of rice bran from Jombang and Kediri

The overall results of the antioxidant properties and phytochemical contents of rice bran are shown in Table 1. The antioxidant activity was determined with DPPH free radical scavenging for each variety of rice bran and the average radical scavenging activity varied between Bramo, Serang and Menthik (p≤0.05) which were 15.25±0.07, 25.37±0.07, and 28.15±0.19%, respectively. Phytochemical contents were studied from total anthocyanin content and total phenolic content. Total anthocyanin content (p≤0.05) was ranged from 2.23±0.07 to 3.89±0.05 mg/100 g and total phenolic content was not significantly different (P>0.05) between varieties which ranged from 266.05±19.19 to 286.72±1.37 mg/100 g. From this study, Menthik rice bran was the best for the antioxidant activity, total anthocyanin content and total phenolic content. Saenkod et al. (2013) also proved different varieties of brown rice from China had different antioxidant activity and total phenolic compound. Hartati et al. (2015) also reported that a variety of rice influencing the antioxidant activity and phytochemical content and between IR-64, Sintanur, Rajalele, and Menthik. Menthik was observed as the best for total phenolic content and antioxidant activity. Furthermore, Huang and Ng (2012) and Hartati et al. (2015) also showed that higher total phenolic content of rice bran also give a higher antioxidant activity.

Table 1. Antioxidant properties and phytochemical contents of Rice Bran from Jombang and Kediri

<table>
<thead>
<tr>
<th>Rice Varieties</th>
<th>DPPH (%)</th>
<th>Total Anthocyanin Content (mg/100g)</th>
<th>Total Phenolic Content (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bramo</td>
<td>15.25±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.90±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>266.05±19.19&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Serang</td>
<td>25.37±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.23±0.07&lt;sup&gt;c&lt;/sup&gt;</td>
<td>276.92±1.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Menthik</td>
<td>28.15±0.19&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.89±0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>286.72±1.37&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means ± standard deviations with different alphabet superscripts within a column are significantly different at p<0.05.
total vitamin E homologue content in rice bran increased when exposed to heat treatment, but then decreased following longer heating times. Vitamin E was suggested strongly linked to insoluble cellular component and could be released by heating rice bran. Same with vitamin E, total phenolic inbound fractions also increased with hydrothermal processes. Ferulic acid is known as the major phenolic compounds bound to the cell wall in grains. Heat treatment causes the instability of cell wall structure and binding properties, that made an increase in extractability of bound phenolic, that would increase antioxidant activity. However, if further heat treatment is applied, antioxidant activity and phenolic content will be decreased due to the degradation of antioxidant (Min et al., 2014).

Table 2. Antioxidant activity and total phenolic content from meatball with rice bran substitutions

<table>
<thead>
<tr>
<th>Substitution of Rice Bran</th>
<th>DPPH (%)</th>
<th>Total Phenolic Content (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>16.75±0.18&lt;sub&gt;b&lt;/sub&gt;</td>
<td>37.82±0.44&lt;sub&gt;c&lt;/sub&gt;</td>
</tr>
<tr>
<td>B50</td>
<td>19.71±0.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>84.06±0.68&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>B100</td>
<td>39.53±0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>92.31±0.94&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>S50</td>
<td>35.78±0.31&lt;sup&gt;d&lt;/sup&gt;</td>
<td>90.81±1.24&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
<tr>
<td>S100</td>
<td>55.21±0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100.76±0.99&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>M50</td>
<td>30.49±0.07&lt;sup&gt;d&lt;/sup&gt;</td>
<td>89.56±0.06&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>M100</td>
<td>42.77±0.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>95.31±0.68&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means ± standard deviations with different alphabet superscripts within a column are significantly different at p<0.05.

According to Kumar et al. (2015), one of the majority of natural antioxidants from plant origin is tocopherols besides flavonoid and phenolic acids. Antioxidant inhibits lipid oxidation by reacting to free radicals which is happened in the initiation phase, propagation phase or termination phase. Lipid and their derivatives are present in muscles. When meat ages, myoglobin is converted to metmyoglobin, which acts as a catalyst for lipid oxidation. When rate of lipid oxidation increases, deterioration of product color and flavor occurs. It is assumed that antioxidant components in rice bran were g-oryzanol and tocopherol (Pestana-Bauer et al., 2012). Gamma oryzanol has potential effect to stabilize free radical which is caused by organical radicals (Juliano et al., 2005). Tocopherol can act as donor or acceptor of chain breaking electron and to stabilize singlet oxygen scavenging (Shahidi, 1997).

3.3 Effect of substitution with rice bran in meatballs to acceptance of consumer

The acceptance test for rice bran meatball has been done by a 7-point hedonic scale (Table 3). Color acceptance was not significantly (p≥0.05) different between meatball without rice bran (control) and 50% substitution of tapioca, but significantly (p<0.05) higher to 100% substitution. Since rice bran is brown, substitution of rice bran made the meatball’s color darker. Wanyo et al. (2009) reported that L* value decreased and a* and b* value increased as the increase of rice bran substitution on rice flake, which means its color became darker. Meatball with 100% substitution with rice bran was too dark in its color and cause a decrease in the consumer likeness to the color of meatball.

Aroma of rice bran was different between each variety, some rice bran gave a pleasant sweet or nutty flavor that improve aroma of food, but other rice bran gave unpleasant odor (Hu et al., 2009; Gracia et al., 2012). The best aroma was performed by sample substituted with 50% of Menthik rice bran but not significantly (p≥0.05) different with other rice bran varieties and without substitution. However, substitution of 50 to 100% rice bran gave significant differences in aroma (p<0.05).

Increase of rice bran substitution have decreased the hardness, chewiness and likeness of meatball. Substitution with 50% rice bran was not significantly different with the one without substitution (p≥0.05), but 100% substitution of tapioca with rice bran was significantly different (p<0.05). Panelists found that meatball with 100% substitution of rice bran was too hard. Substitution with 50% Bramo rice bran had a significantly lower score of taste than without substitution. Panelist noted that meatball samples substituted with Bramo rice bran had a strange taste. However, different with Bramo rice bran substituted samples, that the taste of meatballs substituted with 50% of Menthik rice bran and Serang rice bran were better than without substitution even it was not significantly different....
different (p≥0.05). Panelists who chose meatballs with 50% substitution of Menthik and Serang rice bran as the best samples, stated that the taste of these meatballs was the tastiest. Delahaye et al. (2004) also showed that enrichment 5% of rice bran to pizza dough was higher in likeness score than without rice bran. Therefore, rice bran in a certain amount could give a nice taste making them more attractive. Substitution with 100% rice bran was significantly (p≤0.05) decreased taste score, and make meatballs were not preferable to panelists.

Flavor is the integration of taste odors and oral somatosensory quality. Flavor seems to be important attribute of food that influence our motivation to consume (Prescott, 2015). Same with color, aroma, hardness, and chewiness, substitution with 50% rice bran was not significantly different with the samples without rice bran, but 100% rice bran substitution significantly decreased the flavor score of meatball. From all of the results, the best substitution was 50% substitution with Serang rice bran since it was not significantly different with highest score in organoleptic result (p≥0.05) but had better antioxidant activity which is significantly different from 50% substitution with Menthik rice bran (p≤0.05).

4. Conclusion
Substitution of tapioca with rice bran in the production of meatballs could increase the antioxidant activity and total phenolic content of meatballs. Although rice bran from Menthik rice variety had the highest antioxidant activity, anthocyanin, and phenolic contents, when used for meatball production, meatballs with substitution of starch with Serang rice bran had highest antioxidant activity and phenolic content. Results from this study showed that substitution of tapioca with 50% Serang rice bran was most appropriate to improve the functional quality of meatball as a healthy food.

Conflict of Interests
The authors declare that there is no conflict of interests regarding the publication of this paper.

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