The effect of brown rice flour on the quality of dangke nuggets during cold storage

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Abstract. Brown rice contains anthocyanin, which has an antioxidant activity that can inhibit the oxidation process in food during storage. This research aimed to determine the effect of brown rice flour levels substituting dangke on the quality of dangke nuggets for 14 days of cold storage. This research used a completely randomized design factorial pattern with two factors, i.e. the levels of brown rice flour (0%, 10%, and 20%) and the storage duration in the refrigerator (0 days, 7 days, and 14 days). The parameters analyzed included antioxidant activity with free radical scavenging activity DPPH method, pH and TBA values of dangke nuggets. The results showed that the levels of brown rice flour and storage duration had a very significant effect (P<0.01) on antioxidant activity, pH and TBA values of the product. The interaction between the two factors had a very significant effect (P<0.01) on the antioxidant activity and the TBA value of the product. The increased level of brown rice flour increased antioxidant activity and pH value; however, it decreased TBA value. Meanwhile, the increased storage duration increased antioxidant activity but decreased pH and TBA values of the product. Dangke nuggets with 10% and 20% brown rice flour levels had the same antioxidant activity on the 7th and 14th day of storage, and the same TBA value on the 14th day of storage. Dangke nuggets with 10% brown rice flour level could effectively inhibit the oxidation process of the product during 14 days of storage at cold temperatures.

1. Introduction
Dangke is a dairy product and a kind of fresh cheese made traditionally by the community in Enrekang Regency, South Sulawesi, Indonesia. The relatively high water content causes dangke to have a short shelf life, especially if stored at room temperature. This condition resulted in a limited number of dangke consumers in Enrekang region and its surrounding areas. Currently, there is a tendency of the local community to further process dangke into products that can last longer, so that the market can reach areas outside Enrekang. One form of product diversifications that has the potential to be developed is processing dangke into nuggets.

The quality of nuggets as an emulsion product is largely determined by the ability of the main ingredients used in binding water and fat to stabilize the emulsion. The binding capacity of water and fat from dangke protein may have decreased due to heating during the making process; therefore, it is necessary to add other ingredients that have the adequate water-binding ability. In chicken nuggets, the addition of appropriate non-meat ingredients can improve the shape and stability of the meat emulsion [1]. One ingredient that can be used as a binder in meat emulsion product is rice. Rice flour can be used in low-fat or gluten-free chicken nuggets without reducing their quality, and it adds values for people who need a low-fat diet and suffers from the celiac disease [2]. Chicken nuggets formulated
with rice flour have the same quality and shelf life as nuggets that use flour [3]. The use of rice flour in chicken-based emulsion products, such as nuggets and patties, can improve yield and better sensory quality [4].

Frying treatment on nuggets during the making process makes the nugget susceptible to lipid oxidation during storage, which will reduce the quality and shelf life. Lipid oxidation can be slowed by adding antioxidants to the formulation. Various synthetic antioxidants can be used to prevent lipid oxidation; however, in recent years, the use of natural antioxidants has been more desirable as they are safer for health [5]. Brown and black rice are valuable because of their natural antioxidant contents [6]. Bioactive components that provide rice with high antioxidant activity are cyanidin-3-O-glucoside, peonidin-3-O-glucoside and phenolic acids [7]. The use of black rice can slow the oxidation rate of fish sticks stored for three months in the freezer. The fish sticks produced also have better sensory quality than those using red sorghum [8]. The substitution of flour with purple rice in flour making shows that the antioxidant activity of the product increases as the substitution level increases [9]. Scientific information regarding the use of brown rice in nugget products is still limited. The aim of this research was to examine the use of brown rice flour in the formulation of dangke nuggets to slow the decrease in product quality during storage in the refrigerator.

2. Materials and methods

2.1. Making of brown rice flour
Brown rice was obtained from a supermarket in Makassar. The brown rice was washed with clean water to remove dirt attached to it and then soaked for 12 hours to undermine the rice texture. Then, the brown rice was dried to reduce the water content by leaving it at room temperature for 24 hours. Dried brown rice was pounded with a pestle and then sieved with a size of 80 mesh. Brown rice flour was stored in a refrigerator until used to make dangke nuggets.

2.2. Making of dangke nuggets
Dangke was purchased from a dangke producer in Enrekang district. The main ingredients to make nuggets, in addition to dangke and brown rice flour, were egg yolks, wheat flour, and ice cubes. The levels of all key ingredients were set to reach 100% formulation. Additional ingredients used included salt, powdered garlic and pepper, and flavoring. The levels of all additives were calculated based on the total weight of the main ingredients. All main and additional ingredients were purchased from a supermarket in Makassar.

Three experiments of nugget formulations were carried out i.e. the dough without the addition of brown rice flour as a control (0%), while the other treatments of 10% and 20% brown rice flour added to replace dangke in the formulation. Dangke, brown rice flour, wheat flour, egg yolks, half ice cubes, and salt were ground together in a food processor. After that, half of the ice cubes, powdered garlic and pepper, and flavoring were added to the mixture and ground to form a dough mixture. The mixture was poured into a baking sheet (20×15×3 cm³) and steamed until cooked. The cooked samples were cut in a square shape (4×4 cm²), dipped in egg white, covered with panir flour and stored in the freezer. The frozen sample was then fried in 170°C boiling oil until cooked and then packed with polyethylene (PE) plastics and stored in the refrigerator (4 ± 1°C) until the quality assessment carried out (0, 7, and 14 days). Product quality testing was conducted immediately after frying for 0-day storage treatment.

2.3. Measurement of quality parameters
The antioxidant activity of dangke nuggets was measured using 1.1-diphenyl-2-picryl-hydrazyl (DPPH) radicals by Blois method [10]. A sample of 1 mg was added with 3.9 mL of DPPH solution in methanol (0.02 g/L), then shaken and stored at room temperature for 30 minutes. A spectrophotometer measured absorbance at 517 nm wavelength. Blanko used DPPH solution in methanol without sample through the same measurement procedure as the sample.
The pH value was measured using a pH meter at 10 g of dangke nuggets that had been homogenized with 100 mL of aqua dest. The pH meter was previously calibrated with buffer solutions of pH 4 and pH 7.

Lipid oxidation in dangke nuggets was expressed by the numbers of Thiobarbituric acid (TBA). TBA numbers (mg malonaldehyde/kg) in dangke nuggets were determined by using Tarladgis Method [11]. 2-Thiobarbituric acid reacted with malonaldehyde to form red color whose intensity was measured with a spectrophotometer.

2.4. Statistical analysis
The treatments were arranged in a completely randomized factorial design with two factors: brown rice flour levels (0, 10, and 20%) and storage duration in the refrigerator (0, 7, and 14 days). Each treatment was repeated three times. Data measured for antioxidant activity, pH and TBA values of dangke nuggets were interpreted with analysis of variance (ANOVA). The least significant difference test followed the statistical significance of treatment at a 5% or 1% level. The data processing utilized SPSS-16.0 software.

3. Results and discussions

3.1. The effect of brown rice flour level
Table 1 presents the effect of brown rice flour level on antioxidant activity, pH, and TBA (Thiobarbituric Acid) values of dangke nuggets. The antioxidant activity of dangke nuggets increased linearly from 57.64% to 71.86% (P<0.01) as the brown rice flour level in nugget formulation increased. This was caused by the presence of anthocyanin, phenol and flavonoid components in brown rice which has antioxidant activity. Molecules in rice with antioxidant activity included phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, \( \epsilon \)-oryzanol, and phytic acids [12]. An increase in biscuits' antioxidant activity as the substitution level of wheat flour with purple rice flour in biscuit formulation increased [13].

The pH value of dangke nuggets added with 10% and 20% brown rice flour was slightly higher (P<0.01) compared to controls (without the addition of brown rice flour). The use of brown rice flour to substitute some dangke in nugget formulation will increase the pH value of the nuggets. This could be caused by the degradation of anthocyanin in brown rice which releases OH\(^{-}\) ions due to an increase in temperature during the dough steaming. Anthocyanin thermal degradation caused the conversion of flavilium cations to carbonyl bases [14].

| Levels of brown rice flour (%) | Antioxidant activity (%) | pH         | TBA (mg of malonaldehyde/kg) |
|--------------------------------|--------------------------|------------|-----------------------------|
| 0                             | 57.64±1.16\(^a\)        | 6.95±0.03\(^a\) | 0.53±0.01\(^a\)            |
| 10                            | 65.48±1.46\(^b\)        | 7.07±0.05\(^b\) | 0.43±0.01\(^b\)            |
| 20                            | 71.86±0.59\(^c\)        | 7.12±0.05\(^c\) | 0.38±0.02\(^c\)            |

\(^{abc}\)Different letter notations in the same column show significant differences (P<0.01)

TBA (Thiobarbituric Acid) value of dangke nuggets decreased as the brown rice flour level increased (P<0.01). This indicated that the addition of brown rice flour in dangke nugget formulation can reduce the product oxidation level. Brown rice contains anthocyanin and various other compounds that have an antioxidant activity so that it can slow down the chain reaction of free radical formation from the oxidation process. Black and brown rice grains contain anthocyanin (maximum: 5,045.6 µg/g) and proanthocyanidin (maximum: 3,060.6 µg/g) as the main pigments so that it has high potential as a source of antioxidants [15].
3.2. The effect of storage duration

The effects of storage duration on antioxidant activity, pH and TBA values of dangke nuggets are presented in Table 2. The antioxidant activity of dangke nuggets significantly increased (P<0.01) during storage, i.e., from 41.67% on day 0 to 82.50% on day 14. Increased β-carotene levels of brown rice caused an increase in antioxidant activity during storage. Levels of anthocyanin and colored rice polyphenols decreased during storage; however, their antioxidant activity increased due to the increased levels of β-carotene during storage [16].

| Storage duration (days) | Antioxidant activity (%) | pH           | TBA (mg of malonaldehyde/kg) |
|-------------------------|--------------------------|--------------|-----------------------------|
| 0                       | 41.67±0.66<sup>a</sup>   | 7.31±0.05<sup>a</sup> | 0.71±0.01<sup>a</sup>       |
| 7                       | 70.81±1.75<sup>b</sup>   | 7.06±0.03<sup>b</sup> | 0.44±0.01<sup>b</sup>       |
| 14                      | 82.50±0.79<sup>c</sup>   | 6.77±0.03<sup>c</sup> | 0.19±0.01<sup>c</sup>       |

<sup>abc</sup>Different letter notations in the same column show significant differences (P<0.01)

The pH value of dangke nuggets decreased as the storage duration increased (P<0.01) i.e. from 7.31 on the 0<sup>th</sup> day to 6.77 on the 14<sup>th</sup> day. This is probably due to the natural fermentation of carbohydrates from brown rice by lactic acid bacteria that produce lactic acid during storage. 

*Leuconostoc, Lactobacillus, Streptococcus, Pediococcus, Micrococcus*, and *Bacillus* are common types of fermentative bacteria in cereals [17]. A decrease in the pH value of the product can contribute to an increase in shelf life as the activity of spoilage bacteria will be inhibited.

The oxidation level of dangke nuggets decreased as the storage duration increased. This can be seen from the TBA value of the product which significantly decreased (P<0.01) as the storage duration increased. This condition can be attributed to the increased antioxidant activity of dangke nuggets as the storage duration increased. Antioxidants are components that can inhibit the chain reaction of free radical formation, especially those that occur during fat oxidation process of the product.

3.3. The effect of interaction between brown rice flour level and storage duration

The effects of interaction between brown rice flour level and storage duration on antioxidant activity, pH and TBA values of dangke nugget values are presented in Table 3. There was an interaction between brown rice flour level and storage duration on antioxidant activity and TBA value (P<0.01), yet there was no interaction between the two treatments on the pH value of dangke nuggets.

| Brown rice flour levels (%) | Storage duration (days) | Antioxidant activity (%) | pH           | TBA (mg of malonaldehyde/kg) |
|-----------------------------|-------------------------|--------------------------|--------------|-----------------------------|
| 0                           | 0                       | 30.01±0.17<sup>a</sup>   | 7.23±0.03    | 0.78±0.01<sup>a</sup>       |
| 10                          | 0                       | 40.31±1.31<sup>bc</sup>  | 7.35±0.05    | 0.68±0.01<sup>bc</sup>      |
| 20                          | 0                       | 54.69±0.51<sup>bc</sup>  | 7.36±0.06    | 0.66±0.02<sup>bc</sup>      |
| 0                           | 7                       | 65.09±2.83<sup>bc</sup>  | 6.95±0.04    | 0.59±0.01<sup>bc</sup>      |
| 10                          | 7                       | 72.96±1.37<sup>bc</sup>  | 7.07±0.08    | 0.42±0.01<sup>bc</sup>      |
| 20                          | 7                       | 74.38±1.05<sup>bc</sup>  | 7.15±0.04    | 0.32±0.02<sup>bc</sup>      |
| 0                           | 14                      | 77.83±0.49<sup>bc</sup>  | 6.68±0.01    | 0.21±0.00<sup>bc</sup>      |
| 10                          | 14                      | 83.17±1.69<sup>bc</sup>  | 6.79±0.01    | 0.18±0.01<sup>bc</sup>      |
| 20                          | 14                      | 86.51±0.20<sup>bc</sup>  | 6.85±0.06    | 0.17±0.01<sup>bc</sup>      |

<sup>abc</sup>Different letter notations in the same column show significant differences (P<0.01)
The use of brown rice flour at the level of 10% and 20% produces dangke nuggets that have higher antioxidant activity compared to the controls (without brown rice flour) on all storage days. Dangke nuggets added with 10% and 20% brown rice flour have the same antioxidant activity after 7th and 14th days of storage.

TBA value of dangke nugget without the addition of brown rice flour is higher than that added with brown rice flour at both 10% and 20% levels on all storage days. Dangke nuggets added with 10% and 20% brown rice flour have the same TBA value after the 14th day of storage. This can be related to the same antioxidant activity between the two levels of brown rice flour added after the 14th day of storage.

4. Conclusion
The addition of brown rice flour at 10% level in dangke nugget formulation has effectively been able to inhibit the product oxidation process during 14 days of storage in the refrigerator.

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