Study of proximate composition, antioxidant activity and sensory evaluation of cooked rice with addition of cherry (Muntingia calabura) leaf extract.

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Abstract. The aim of this study is to analysis the composition of proximate, antioxidant activity and sensory test of rice cooked from rice that has been added with cherry leaf extract. The ingredients used are rice without addition (NF0) and that has been added cherry leaf extract with a concentration of 20% (NF20). Rice is added water in a ratio of 1:2, then cook using a rice cooker until cooked through. Parameters tested in this study are proximate composition, total phenolics, total flavonoids, antioxidant activity, and sensory tests (color, taste, aroma, texture, overall). Proximate composition of the cooked rice showed the presence of quantities of total ash (0.27 – 0.24%), carbohydrate (83.15 – 82.72%), protein (8.05% – 8.16%) and fat (0.43-0.46%). This result showed that NF20 have high quantities on protein and fat than NF0. Phenolic, flavonoid and antioxidant levels of NF20 were higher than NF0 (66.79 mg GAE/g extract; 38.10 mg Quersetin/g extract; 87.16%). Sensory evaluation results of color, aroma, and taste showed that NF20 and NF0, are in the “neutral” range.

1. Introduction

Nowadays, public concern about healthy food is increasing. This is due to the increasing cost of health that is increasing every day. Based on World Health Organization (WHO) data, Indonesia spends approximately US$ 27 billion, or about US$ 107 per capita on health costs (WHO, 2013). Therefore, there are now many food products developed that combine the functions of nutrition and health, namely functional food. One functional food that has physiological functions for the body is antioxidants. Daily intake of antioxidants can prevent the occurrence of degenerative diseases. One type of functional food that is widely developed is artificial rice, rice made with raw materials from sorghum flour, corn and tubers. Artificial rice developed has better nutritional value than real rice, but has a low level of consumer acceptance, so that consumers are only limited (Valencia & Purwanto, 2020).

In previous research developed rice made from rice flour with added cherry leaf extract, from the results of the study showed that the addition of cherry leaf extract 20% can increase the antioxidant
activity of rice processed using extruder machine (Bait et al. 2021). The next stage of the study was cooking rice from the best treatment.

Rice can be cooked in various ways, at home or on an industrial scale. Cooking the raw rice into the cooked rice could be done in various ways. Indonesian people used two ways to cook rice namely conventional and modern ways. The conventional ways consist of liwet method using stovetop and combination of boiling and steaming method. The modern ways were cooking rice using electrical rice cooker (Syafutri et al. 2016). The most widely used tool for cooking rice is a rice cooker. Zaupa et al (2015) suggested that waterbath cooking, defined as “risotto cooking”, is the cooking method that allows preserving, both its phenolics compounds and total antioxidant capacity. Cooking with the rice cooker led a lower and comparable losses of phenolics (Fracassetti et al. 2020). This study aimed to evaluate the composition of proximate (moisture, ash, crude fat, crude protein and carbohydrate by difference), content of phenolics and flavonoid as well as the antioxidant activity, and sensory test of cooked rice with cherry leaf extract.

2. Material and Methods

2.1 Materials

Raw rice with 0% (NF0) and 20% (NF20) cherry leaf extract (from previous research/Bait et al. 2020).

2.2 Sample preparation

2.2.1 Cooking the rice with rice cooker method (Syafutri et al. 2016)

Water as much as 200 mL is put in the rice cooker. Then thermostat was clicked and light “cooking” light up on the rice cooker to boil the water. After that put the rice into the boil water. The thermostat button will automatically move from the position of the lights “warmer” that shows rice cooked. The cooked rice dried into cabinet dryer in the temperature of 50 °C for 12 hours. Then dried cooked rice was homogeneously powdered in a laboratory grinder and stored at 4 °C until extraction and analysis.

2.2.2 Extraction Procedure for phenolic, flavonoid and DPPH assay

The sample was extracted using (Chakuton, et al., 2012) method, 28 grams of sample is added with 56 mL of absolute methanol, then macerated using shaking water bath in the temperature of 32 °C for 1 hour in the speed of 170 rpm. The filtrate is separated from the residue by using the whatmann paper no. 40. Next, the separated from filtrate residue was further extracted twice by using similar treatment to the first treatment. All the obtained filtrates were then mixed and evaporated with Rotary evaporator in the temperature of 50 °C and 200 bar pressure, thus, 3 mL dense extract was obtained. Further, this dense extract was analyzed of total phenolics, total flavonoid, and ability to scavenge free radical DPPH. Each sample receives 3 times of repetition.

2.3 Determination of proximate composition

2.3.1 Ash Content (Total Ash Method)

A known porcelain cup of fixed weight (A). Included samples that have been weighed as much as 5 g (B). Then the sample is charcoed on top of Bunsen with a small flame until the smoke is gone, then put in a furnace at a temperature of 500-600 °C until it becomes white ash. The cup containing ash is cooled in a depasitor and then weighed until a fixed weigh t is obtained (C). Ash content is calculated by the formula:

\[ \text{Ash Content (\%db)} = \frac{\text{ash content (\%bb)}}{(100 - \text{water content (\%bb)}} \times 100\% \]

\[ \text{Ash Content (\%wb)} = \frac{(C - A)}{(B - A)} \times 100\% \]

2.3.2 Protein Content (Micro-Kjeldahl Method)

A sample of 0.5-3.0 g is weighed (A) and put in a 30 ml Kjeldahl flask. Then added 1.9 ± 0.1 g K2SO4, 40 ± 10 mg HgO and 2.0 ± 0.1 ml H2SO4 then instructed by heating until the solution is clear. The solution of destructive result is diluted and distilled with the addition of NaOH-Na2S2O3 as much as 8-10 ml. Destilat is accommodated in 5 ml of H3BO3 solution and 2-4 drops of indicator (a mixture of 2
parts methyl red 0.2% in alcohol and 1 part 0.2% in methyl blue alcohol). Then distilled until accommodated approximately 50 ml of destilat in erlenmeyer, then titrated with HCl 0.02 N until there is a change in color to gray. From the titration results, total nitrogen can be known, and the sample protein content is calculated by multiplying the total nitrogen by conversion factor. Protein levels are calculated by the formula:

\[
\text{Nitrogen Total} \% = \frac{ml \text{ HCl} - ml \text{ blanko}}{A} \times N_{N\text{HCl}} \times 14.007 \times 100
\]

Protein (%wb) = nitrogen total (%) x correction factors (6.25)
protein (%db) = crude protein (%wb)/ (100-moisture content %wb) x 100%

2.3.3 Fat Content (Soxhlet Extraction Method)
A thimble is dried in the oven (110°C for 1 hour), cooled in a desikator and weighed to a fixed weight (A). The sample of 5 g (B) is wrapped in filter paper and then inserted in soxhlet flask and then installed condenser. The hexane solvent is poured into the fat flask to taste according to the size used. Reflux is carried out for a minimum of 5 hours until the solvent drops back into a clear-colored fat flask. The solvents inside the fat gourd are distilled and accommodated. Then the fat pumpkin containing the extraction results is heated in the oven at a temperature of 105°C, then cooled sidesikator and carried out weighing pumpkin and fat until obtained a fixed weight (C). Fat content is determined by the formula:

\[
\text{Fat Content} \% = \frac{C - A}{B} \times 100\%
\]

Fat content (%db) = crude fat content (%wb)/(100-moisture content %wb) x 100%

2.3.4 Carbohydrate Content (by difference)
Carbohydrates content was calculated by difference using the following formula; 100 % - (CP % + CF % + crude fat % + Ash %) as described in AOAC (2006).

2.4 Total Phenolic Analysis
Total phenolic analysis is carried out using the method developed by Sahreen, et al. (2017), where 0.2 mL of functional rice extract is added with 1 mL of 10% FolinCiocalteu in the 10 mL volumetric flask. The mixture is mixed evenly and let it stand for 3 – 5 minutes. Further 2 mL of 7.5% of Na2CO is added and aquades to make it 10 mL and mixed evenly. The sample absorbance is measured in λ=760 nm after 30 minutes exposure in a dark room. The total phenol is stated in mg equivalent to /100 gram of dried-based gallic acid (GAE) sample.

2.5 Total Flavonoid Analysis
Total flavonoid was analyzed based on (Das, et al., 2019) method. A 0.1 mL of functional rice extract is poured into a 10 mL volumetric flask and 0.15 mL of 5% NaNO and 4 mL of aquades are added into the mixture. Let the mixture stands for 5 minutes. Then it was added 0.3 mL of 5% AlCl2 5% and let the mixture stands for 6 minutes. Further, 2 mL of 0.1M NaOH is added with aquades to the marker point. The absorbance is measured in λ=510 nm. Total flavonoid is stated in mg equivalent to (+) Quercetin/100 gram of dried-based sample.

2.6 Free-Radical Scavenging Assay (DPPH)
Ability to scavenge free radical DPPH is analyzed based on (Das et al., 2019) method, a 3 mL of DPPH solution (4 mg/100 mL) is inserted into reaction flask and added 1 mL of aqueous rice extract, further, the sample is made homogenous and the absorbance is measured in λ=517 nm after 30 minutes of incubation. The free radical absorbance DPPH is measured using the following formula:
2.7 Sensory Evaluation
Organoleptic testing is carried out using the preferred (hedonic) test method. Hedonic method is to test the level of preference for taste, texture, smell and color. Coded examples are randomly presented to panelists, and panelists (30 people) are asked to grade according to their favorite level. The number of scales used is 5 scales, consisting of:
1. Very disliked
2. Dislike
3. Neutral
4. Likes
5. Very Like

3. Result and Discussion
3.1. Proximate composition
Proximate composition of the cooked rice showed the presence of quantities of total ash (0.27 – 0.24%), carbohydrate (83.15 – 82.72%), protein (8.05% - 8.16%) and fat (0.43-0.46%). This result showed that NF20 have high quantities on protein and fat than NF0 (Table 1). Ash content plays an important role to reflect the mineral elements of a food sample, and gives an idea to determine the levels of essential minerals present in the food. In this research ash content is lower than ash content in aromatic rice those reported by Verma & Srivastav (2017). The differences of ash content in rice may due to the differences in mineral content of the soils and the water used for irrigation (Shayo et al. 2009). Rice carbohydrates are mainly starch which is composed of amyllose and amylopectin. The similar range of the carbohydrate content of different rice accessions has also been reported by (Shayo et al. 2009). Mbatchou & Dawda (2013) reported that a high level of starch makes the individual grains sticked to each other while low starch content prevents well from the sticking of the grains together after cooking.

The results obtained from the proximate analysis of cooked rice showed that they are good sources of nutrients such as protein therefore can be ranked as protein rich food due to their relatively high protein content in the range of 8.05% (NF0) and 8.16% (NF20). This value is higher than the values reported in previous studies on artificial rice from sorghum flour and sago with 1% spices (onion, garlic, bay leaves, ginger, and lemongrass) (5.78%) and lower than white (paddy) rice (10.85%) (Valencia et al. 2020). Fat content influences the taste of cooked rice because rice with high fat content tends to be tastier and have less starch. Valencia et al. (2020) reported that artificial rice and white (paddy) rice have higher value of fat content (6.22% and 0.58%) than cooked rice NF0 and NF20 (0.43 – 0.46%).

| Sample | Ash (%db) | Carbohydrate (%db) | Protein (%db) | Fat (%db) |
|--------|-----------|---------------------|---------------|-----------|
| NF0    | 0.27±0.01 | 83.15±0.01         | 8.05±0.02     | 0.43±0.01 |
| NF20   | 0.24±0.01 | 82.72±0.03         | 8.16±0.03     | 0.46±0.00 |

Values are mean ± standard deviation (n = 3); sample code: cooked rice without addition (NF0) and that has been added cherry leaf extract with a concentration of 20% (NF20).

3.2. Phenolic content
Phenolic content of the cooked rice showed that NF20 have high quantities than NF0 (Table 2). The average content of phenolic in the NF0 are 44.31 mg GAE/g extract while NF20 are 66.79 mg GAE/g extract. This is due to the addition of cherry leaf extract in rice. Buhian et al. (2017) suggest that cherry leaf contains phenolic content detected using longwave UV irradiation. Substances containing aromatic moieties nominally absorb at 254 nm, and are easily detected when illuminated at this wavelength.
Phenolic compounds, which include tannins and flavonoids, may be visualized at this wavelength. According to Le Bourvellec & Renard (2012), non-covalent and covalent associations of polyphenols with food macromolecules are two of the most fundamental factors affecting the quality of polyphenol-rich food products.

### 3.3. Flavonoid content
Flavonoid content of the cooked rice showed that NF20 have high quantities than NF0 (Table 2). The average content of flavonoid in the NF0 are 29.15 mg Quersetin/g extract while NF20 are 38.10 mg Quersetin/g extract. Same as phenolic content, flavonoid content that higher on NF20 caused by adding cherry leaf extract. Chen et al. (2005) showed that that cherry leaf contains flavonoid content. The flavonoids are an important group among the cherry leaf, and this plant is rich in flavones, flavans, flavans, and biflavans. Yang et al. (2014) reported that four flavonoid compounds from Phyllostachys edulis leaf extract bind with starch.

### 3.4. Antioxidant activity
Antioxidant activity of the cooked rice showed that NF20 have high quantities than NF0 (Table 2). The antioxidant activity (%RSA) in the NF0 are 26.26 % while NF20 are 87.16 %. The high antioxidant activity on NF20 directly proportional to the content of phenolic and flavonoid. Finocchiaro et al. (2007) reported that Cooking caused a further loss of antioxidants, but when there was a full uptake of cooking water by the grains (“risotto”) this loss was limited.

| Sample | Phenolic (mg GAE/g) | Flavonoid (mg Q/g) | Antioxidant Activity (%RSA) |
|--------|---------------------|--------------------|-----------------------------|
| NF0    | 44.31±5.11          | 29.15±1.46         | 26.28±1.35                  |
| NF20   | 66.79±3.09          | 38.10±4.92         | 87.16±0.70                  |

Values are mean ± standard deviation (n = 3); sample code: cooked rice without addition (NF0) and that has been added cherry leaf extract with a concentration of 20% (NF20).

### 3.5 Sensory Evaluation

#### 3.5.1 Color
The score of color from NF20 are lower than NF0. Cooked rice NF20, have a color darker compared to the NF0 (Fig. 1). The change in color caused by phenolic and flavonoid content in cherry leaf extract. The score of color NF20 was 3.20, while NF20 2.50 (Table 3), this score belongs to the neutral category that is between likes and dislikes. Hidayati et al. (2016) reported that instant rice enriched with Centella asiatica leaf extract, have a yellow color due to phenolic content.

![NF0 and NF20](image)

**Figure 1.** Cooked rice without addition (NF0) and that has been added cherry leaf extract with a concentration of 20% (NF20).
3.5.2 Aroma
The result of sensory evaluation of aroma from cooked rice with cherry leaf extract showed that NF0 was higher score (2.83) than NF20 (2.43) (Table 3). The active compounds suspected to contribute aroma effects are tannin and flavonoid compounds classified as phenol compounds. Phenol compounds have aromatic rings containing hydroxy group, carboxyls, metoxy and also non-aromatic ring structures (Hidayati et al. 2016). Results from earlier studies in food fortification hold that consumers favorably embrace the incorporation of new and innovative nutrient rich supplements into food (Eyenga et al. 2020).

3.5.3 Taste
The result of sensory evaluation of aroma from cooked rice with cherry leaf extract showed that NF0 was have higher score (3.47) than NF20 (3.03) (Table 3). The low score of taste in NF20 samples is due to the bitter taste of the sample. Bitter taste is caused by the presence of alkaloids in cherry leaf extract. Sindhe et al (2013) reported by preliminary phytochemical screening of M. calabura leaves extracts contain alkaloid. The same result with Hidayati et al. (2016) research, that enrichment of Centella asiatica leaf extract to instant rice caused bitter taste.

| Table 3. Score of sensory evaluation of cooked rice with cherry leaf extract |
|-----------------------------|-----------------------------|-----------------------------|
| Sample         | Color | aroma | Taste |
| NF0            | 3.30±0.70 | 2.83±0.73 | 3.47±0.92 |
| NF20           | 2.50±1.06 | 2.43±0.76 | 3.03±0.84 |

Values are mean ± standard deviation (n = 3); sample code: cooked rice without addition (NF0) and that has been added cherry leaf extract with a concentration of 20% (NF20).

4. Conclusion
Proximate composition of the cooked rice showed the presence of quantities of total ash (0.27 – 0.24%), carbohydrate (83.15 – 82.72%), protein (8.05% - 8.16%) and fat (0.43-0.46%). This result showed that NF20 have high quantities on protein and fat than NF0. Phenolic, flavonoid and antioxidant levels of NF20 were higher than NF0 (66.79 mg GAE/g extract; 38.10 mg Quersetin/g extract; 87.16% respe). Sensory evaluation results of color, aroma, and taste showed that NF20 and NF0, are in the “neutral” range.

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References
[1] Aruna Sindhe, M., Bodke, Y. D., & Chandrashekar, A. (2013). Antioxidant and in vivo anti-hyperglycemic activity of muntingia calabura leaves extracts. Der Pharmacia Lettre, 5(3), 427–435.
[2] Buhian, W. P. C., Rubio, R. O., & Martin-Puzon, J. J. (2017). Chromatographic fingerprinting and free-radical scavenging activity of ethanol extracts of Muntingia calabura L. leaves and stems. Asian Pacific Journal of Tropical Biomedicine, 7(2), 139–143. https://doi.org/10.1016/j.apjtb.2016.11.016
[3] Chakuton, Puangpranpitag, D., & Nakornriab, M. (2012). Asian Journal of Plant Sciences. Asian Journal of Plant Sciences, 11(6), 285–293.
[4] Chen, J. J., Lee, H. H., Duh, C. Y., & Chen, I. S. (2005). Cytotoxic chalcones and flavonoids from the leaves of Muntingia calabura. Planta Medica, 71(10), 970–973. https://doi.org/10.1055/s-2005-871223
[5] Das, A. B., Goud, V. V., & Das, C. (2019). Microencapsulation of anthocyanin extract from
purple rice bran using modified rice starch and its effect on rice dough rheology. International Journal of Biological Macromolecules, 124, 573–581. https://doi.org/10.1016/j.ijbiomac.2018.11.247

[6] Eyenga, E. F., Tang, E. N., Achu, M. B. L., Boulanger, R., Mbacham, W. F., & Ndindeng, S. A. (2020). Physical, nutritional, and sensory quality of rice-based biscuits fortified with safou (Dacryodes edulis) fruit powder. Food Science and Nutrition, 8(7), 3413–3424. https://doi.org/10.1002/fsn3.1622

[7] Finocchiaro, F., Ferrari, B., Gianinetti, A., Dall’Asta, C., Galaverna, G., Scazzina, F., & Pellegrini, N. (2007). Characterization of antioxidant compounds of red and white rice and changes in total antioxidant capacity during processing. Molecular Nutrition and Food Research, 51(8), 1006–1019. https://doi.org/10.1002/mnfr.200700011

[8] Fracassetti, D., Pozzoli, C., Vitalini, S., Tirelli, A., & Iriti, M. (2020). Impact of cooking on bioactive compounds and antioxidant activity of pigmented rice cultivars. Foods, 9(8), 1–12. https://doi.org/10.3390/foods9080967

[9] Hidayati, S., Nurdin, S. udayana, & Ryan Ajie Nugroho. (2016). Aktivitas antioksidan dan sifat sensori nasi instan hasil hidrolisis pati yang diperkaya dengan ekstrak pegagan (Centella asiatica). 21(2), 77–88.

[10] Le Bourvellec, C., & Renard, C. M. G. C. (2012). Interactions between polyphenols and macromolecules: Quantification methods and mechanisms. Critical Reviews in Food Science and Nutrition, 52(3), 213–248. https://doi.org/10.1080/10408398.2010.499808

[11] Mbatchou, V. C., & Dawda, S. (2013). The Nutritional Composition of Four Rice Varieties Grown and Used in Different Food Preparations in Kassena-Nankana District, Ghana. Int. J. Res. Chem. Environ. International Journal of Research in Chemistry and Environment, 3(1).

[12] Sahreen, S., Khan, M. R., & Khan, R. A. (2017). Evaluation of antioxidant profile of various solvent extracts of Carissa opaca leaves: An edible plant. Chemistry Central Journal, 11(1), 1–7. https://doi.org/10.1186/s13065-017-0300-6

[13] Shayo, N., Mamiro, P., Nyaruhucha, C., & Mamboleo, T. (2009). Physico-chemical and grain cooking characteristics of selected rice cultivars grown in Morogoro. Tanzania Journal of Science, 32(1). https://doi.org/10.4314/tjs.v32i1.18427

[14] Syafutri, M. I., Pratama, F., Syaiful, F., & Faizal, A. (2016). Effects of Varieties and Cooking Methods on Physical and Chemical Characteristics of Cooked Rice. Rice Science, 23(5), 282–286. https://doi.org/10.1016/j.rsci.2016.08.006

[15] Valencia, E., & Goretti Marianti Purwanto, M. (2020). Artificial Rice As an Alternative Functional Food to Support Food Diversification Program. KnE Life Sciences, 2020, 177–186. https://doi.org/10.18502/kls.v5i2.6449

[16] Verma, D. K., & Srivastav, P. P. (2017). Proximate Composition, Mineral Content and Fatty Acids Analyses of Aromatic and Non-Aromatic Indian Rice. Rice Science, 24(1), 21–31. https://doi.org/10.1016/j.rsci.2016.05.005

[17] Yang, J. P., He, H., & Lu, Y. H. (2014). Four flavonoid compounds from phyllostachys edulis leaf extract retard the digestion of starch and its working mechanisms. Journal of Agricultural and Food Chemistry, 62(31), 7760–7770. https://doi.org/10.1021/jf501931m

[18] Zaupa, M., Calani, L., Del Rio, D., Brighenti, F., & Pellegrini, N. (2015). Characterization of total antioxidant capacity and (poly)phenolic compounds of differently pigmented rice varieties and their changes during domestic cooking. Food Chemistry, 187, 338–347. https://doi.org/10.1016/j.foodchem.2015.04.055