The Effects of Media and Blanching Time on the Antioxidative Properties of Curcuma aeruginosa Roxb.

Dwiyati Pujimulyani1*, Sulkhan Windrayahya2-3 and Irnawati Irnawati4

1. Faculty of Agroindustry, University of Mercu Buana Yogyakarta, Yogyakarta, 55753, Indonesia
2. Particle and Interfacial Technology Group (PaInT), Department of Green Chemistry and Technology, Ghent University, CouPure Links 653, 9000 Ghent, Belgium
3. Centre for Food and Microbial Technology, KU Leuven, Kasteelpark Arenberg 22, B-3001 Haverlee, Belgium
4. Kampus Hijau Bumi Tridharma, Anduonou, Kambu, Kendari, Kambu, Kota Kendari, Sulawesi Tenggara 93132

ABSTRACT

Black saffron (Curcuma aeruginosa Roxb.) belongs to the Zingiberaceae family and is one of rhizomes widely used as raw material in Indonesian traditional medicines. Black saffron (BS) contains some bioactive compounds, such as phenolics and flavonoid compounds, responsible for certain biological activities, including antioxidants. Blanching has been reported to increase the antioxidant activity of BS. This study aims to formulate BS containing high antioxidant activity along with the determination of total phenolic, total flavonoid, tannin, water, and crude fiber contents in dried BS. This research was conducted by varying blanching medium (citric acid and aquadest) and blanching times (0; 2.5; 5; 7.5 and 10 min). The fabrication stages of BS powder included peeling, cleaning, blanching, slicing drying, grinding, and sieving. After that, the treated BS was analyzed for the antioxidant activity, total phenolic, total flavonoid, tannin, crude fiber, and water contents. BS powder with the blanching process has shown better antioxidant activity than that without the blanching process. Blanching using citric acid media 0.05% for five minutes has the best antioxidant activities, as indicated by high contents of total phenolic, total flavonoid, and tannins. Powdered BS is potentially used as material to fortify agents in food products.

Keywords: Black saffron, Blanching, Antioxidant activity, Total phenolic content

INTRODUCTION

Black saffron (BS) belongs to the Zingiberaceae family and is widely distributed in South Asia and South-East Asia, including Indonesia and Malaysia (Khumaida et al., 2019). BS is one of the herbs commonly used as a mixed ingredient in Indonesian traditional medicine. The rhizome of BS has a bitter taste and can be used to increase the appetite, smooth the output of dirty blood after birth, and treat several diseases, such as skin illness (scabies and ulcers), stomachache (colic), sprue, cough, breathless, intestinal worms, rheumatism, and obesity. BS is also reported to have anti-inflammation (Andrina et al., 2017) and antibacterial activity (Akarchariya et al., 2017). Moreover, it contains more phytochemical compounds, such as essential oil (turmerone, zingiberene), curcuminoid, alkaloid, saponin, resin, flavonoid, and polyphenol (Nugrahaningtyas et al., 2005; Khumaida et al., 2019). The contents of curcumin and essential oil can be utilized to eradicate intestinal worms and increase body metabolism (Akarchariya et al., 2017). Curcumin in BS can reduce the formation of intracellular lipid droplets by inhibiting genes and adiponectin regulation. The formation of lipid droplets is influenced by several factors: the presence of active peroxisome proliferator receptors (PPAR) -γ and type 4 glucose transporter (GLUT4) as glucose transporters (Yen et al., 2015).

BS has been reported to have good antioxidant activities in vitro and in vivo (Simoh et al., 2018; Ghafoor et al., 2020). An antioxidant compound is a compound that could trap free...
radicals, which trigger several diseases due to oxidation reactions, such as cardiovascular and cancer (Rohman et al., 2020). Basically, the antioxidant compound is available in the human body as endogenous antioxidants, such as Super Oxide Dismutase (SOD) and Glutathione-S-Transferase (GST). Besides, the antioxidants contained in the food include phenolic compounds and flavonoids (Rohman et al., 2019). The deficiency of antioxidants inside the human body can decrease body protection against free radical attack (Arivazhagan et al., 2000).

Some treatments have been proposed to enhance its antioxidant activities, including blanching used to inactivate the polyphenol oxidase enzyme (Pujimulyani et al., 2020). The correct treatment of blanching could prevent the unwanted color change, decrease microbial values, soften the tissue, and help the excretion of cellular gasses in the tissue; thus, the corrosion could be prevented and the texture of the dry food products could be repaired (Noreña and Rigon, 2018). Several studies have proved that the blanching process of agricultural products can increase antioxidant activities. Cabbage’s antioxidant activity with blanching has increased by 9% unlike that without blanching (Puupponen-Pimiä et al., 2003). Besides, the blanched wheat at 100°C has shown an increase in total phenolic content (Cheng et al., 2006). The blanching of BS can also increase the antioxidant activities using the Ferric Reducing Antioxidant Power (FRAP) method (Pujimulyani et al., 2012) and the DPPH radical scavenging assay (Arviansari et al., 2020). However, the effect of the blanching process on black saffron has not been previously studied. Therefore, it is necessary to evaluate the effects of blanching processes in dried BS in this study. The objective of this study is to evaluate the antioxidant activity, total phenolic, total flavonoid, tannin, water, and crude fiber contents in dried BS subjected to different blanching processes.

MATERIALS AND METHODS

A fresh rhizome of black saffron was obtained from the black saffron farms in Yogyakarta. Butylated hydroxytoluene (BHT) and 2,2’-diphenyl-1-picrylhydrazil (DPPH) were purchased from Sigma (Aldrich, USA) while sodium carbonate (NaCO₃), sodium bicarbonate (Na₂CO₃), Folín-Ciocalteu Reagent (FCR), H₂SO₄, sodium hydroxide, ethanol, sodium nitrite (NaNO₂), and aluminum chloride hexahydrate (AlCl₃·6H₂O) were purchased from E. Merck (Darmstadt Germany).

Sample preparation

Black saffron (BS) was collected from the local market with a size of approximately 8-10 cm. After that, the BS was sorted, cleaned, and blanched in aquadest and citric acid solution at a temperature of 100°C with various times of 0, 2.5, 5, 7.5, and 10 min. BS samples were drained, sliced ± 2 mm, and dried using a cabinet dryer with a temperature of 55°C for 8 h. Furthermore, the dried BS was evaluated for the antioxidant activity, total phenolic, total flavonoid, tannin, water, and crude fiber contents. The experimental design used in this research was the random complete block design (RCBD) with two factorials.

Determination of moisture content

The moisture content was determined using the method of Muhammad et al. (2020) while the crude fiber was determined using the AOAC method (Thiex, 2009). BS powder was dried in an oven at a temperature of 105 ± 1°C until a constant weight was achieved. The difference in weight was determined, and the moisture was calculated in the percentage of BS powder weight.

Determination of crude fiber

The crude fiber was determined by referring to the theory of Busuttil-Griffin et al. (2015). A-5 g of dried BS samples were subjected to defatting using Soxhlet with petroleum ether as extracting solvent. The defatted BS sample was digested using H₂SO₄ 1.25% and NaOH 1.25% solutions. Afterward, the BS samples were dried in an oven at a temperature of 130°C ± 1°C for two hours and then ignited at a temperature of 600°C for 30 min. The loss in sample weight on the ignition and the weight of the ground sample before defatting were utilized to determine the % crude fiber content.

DPPH radical scavenging assay

DPPH radical scavenging assay was performed according to Rohman et al. (2020). BS (1 g) was added with 10 volumes of ethanol (10 mL) and then was mixed with vortex (1 min), maceration 1 h at room temperature, and filtered. The supernatant obtained was determined using DPPH as follows: a 0.2 mL sample (supernatant) added with 3.8 mL DPPH 0.01 mMol, incubation at room temperature for 30 min, then the absorbance was measured at 517 nm wave length. The control was ethanol without extract. The capacity of free...
radical scavenging activity (RSA) was calculated according to the following equation: was added to 1.0 mL of ethanolic DPPH solution (0.4 mM) and 3.9 mL of ethanol. The reduced absorbance values were analyzed using a spectrophotometer (Shimadzu UV 1240, Japan) at 515 nm after 30 min of incubation in darkness at ambient temperature. The absorbance measurement was corrected with blank absorbance containing ethanol (without sample) and the studied BS sample. The percentages of DPPH radical scavenging activity were determined using the following equation: DPPH radical scavenging activity (%)\[ \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100 \]

**Determination of total phenolic content**

The total phenolic content (TPC) of BS was determined spectrophotometrically using the Folin-Ciocalteu reagent as employed by (Rohman et al. 2020) with slight modifications. A-200 μL of the ethanolic solution containing BS samples was mixed with 0.4 mL of Folin-Ciocalteu reagent and 4.0 mL sodium carbonate solution in a 10 mL volumetric flask and was adjusted to volume with deionized water. The absorbance was evaluated at 750 nm after 60 min of incubation at ambient temperature. TPC was expressed as mg gallic acid equivalent (GAE)/g samples.

**Determination of total flavonoid contents**

Total flavonoid content (TFC) was determined using a visible spectrophotometric method by Yang et al. (2015) with several modifications. BS extract (50 μL) was mixed with 4 mL of aquadest and 0.3 mL of NaNO2 10%. The mixture was left for 5 min. Afterward, 0.3 μL of AlCl3 10% was added and left for 5 min. The solution was added with 4 mL of NaOH 10% and aquadest until 10.0 mL. The final solution was incubated for 22 min at room temperature, and the absorbance was measured at wavelength 510 nm. TFC was expressed as mg Quercetin equivalents (QE)/g samples.

**Determination of total tannin**

Total tannin was determined by referring to the theory of Kassim et al. (2013). Approximately 100.0 mg of BS samples were dissolved in 10.0 mL of distilled water. After that, 2.0 mL of 5 M HCl and 2.0 mL of formaldehyde solution 37% were added, and the mixture was heated under reflux for an hour. The mixture was filtered while hot through vacuum suction. The reddish precipitate was washed with 10.0 mL of hot water five times. The precipitate was then dried in desiccators of silica gel and weighted. Finally, the total tannin was expressed as mg catechin equivalent (CE)/g samples.

**Data analysis**

All analytical measurements were carried out in three replicates. The obtained data were subjected to the statistical analysis using a one-way analysis of variance (ANOVA) and post-hoc procedure. The statistical software used SPSS Version 22. The results were expressed as means ± SD.

**RESULTS AND DISCUSSION**

In this study, black saffron was subjected to blanching treatment. Afterward, the physico-chemical properties, such as water and crude fiber contents, and antioxidative properties were determined. The results of water content in BS powder with a blanching process in the aquadest and citric acid with different blanching times (Table I). The statistical results denote that the longer blanching time generates the lower water content. The pretreatment of the blanching process in a citric acid solution can accelerate water transfer (Ananingsih et al., 2017). The water content of food products is influenced by the cooking process because the water content will decrease during the cooking process. The cooking process creates heat that is transferred to the samples, heating the water inside the samples, changing the water into steam, and coming out from the samples. However, a longer blanching time generates a higher temperature. The most important thing is that the temperature used should not too high because it would result in undesirable changes in foodstuffs. The SNI 01-3709-1995 has determined the quality requirements of powdered spices with a maximum water content of 12%. Therefore, the water contents of the BS samples have met the required standard, below 12%.

The levels of crude fiber in the black saffron powder with blanching in the aquadest and citric acid with different blanching times (Table II). The crude fiber content of BS powders ranges from 17.29-20.12% in both aquadest and citric acid 0.05% blanching media. The statistical tests have found that there was no significant difference between each treatment. This finding was supported by the content of BS consisting of starch, resin, essential oil, starch, tannins, and minerals. Crude fiber is a part of food that cannot be hydrolyzed by chemicals.
Table I. Water content of black saffron powder due to blanching treatment with aquadest and citric acid 0.05%

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Water content (%)</th>
<th>Blanching with aquadest</th>
<th>Blanching with citric acid 0.05%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.95 ± 0.59 ab</td>
<td>8.66 ± 1.70 b</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>7.59 ± 0.65 ab</td>
<td>7.09 ± 0.58 ab</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>6.95 ± 0.54 ab</td>
<td>8.46 ± 1.23 b</td>
<td></td>
</tr>
<tr>
<td>7.5</td>
<td>7.89 ± 0.63 ab</td>
<td>7.93 ± 2.32 ab</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>6.25 ± 0.29 a</td>
<td>6.87 ± 1.04 ab</td>
<td></td>
</tr>
</tbody>
</table>

The different letter in the same column indicated significant difference (P < 0.05). The data were presented as mean ± standard deviation from 3 replicates.

Table II. Crude fiber of black saffron powder due to blanching treatment with aquadest and citric acid 0.05%

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Crude fiber (%)</th>
<th>Blanching with aquadest</th>
<th>Blanching with citric acid 0.05%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>20.03 ± 0.43 a</td>
<td>20.03 ± 0.43 a</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>18.91 ± 3.10 a</td>
<td>19.96 ± 0.55 a</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>20.12 ± 0.60 a</td>
<td>19.92 ± 0.78 a</td>
<td></td>
</tr>
<tr>
<td>7.5</td>
<td>19.07 ± 1.60 a</td>
<td>19.65 ± 5.11 a</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>17.29 ± 3.81 a</td>
<td>17.94 ± 0.96 a</td>
<td></td>
</tr>
</tbody>
</table>

The different letter in the same column indicated significant difference (P < 0.05). The data was presented as mean ± standard deviation from 3 replicates.

Figure 1. Antioxidant activity of black saffron powder due to blanching treatment with aquadest and citric acid 0.05%
The antioxidant activities of the blanched BS with aquadest and citric acid using the variation of blanching time as determined by the DPPH radical assay (Figure 1). DPPH radical assay was developed by Blois in 1958 based on the capability of samples to scavenge DPPH radical. The odd electron of a nitrogen atom in DPPH is reduced by receiving a hydrogen atom from antioxidants to the corresponding hydrazine (Kedare et al., 2011). The percentages of DPPH radical scavenging activities of BS powder with the variation of the media and blanching time showed a significant difference (P < 0.05). A process with blanching and aquadest for 5 min shows a higher antioxidant activity of 84.11% than a process without blanching (0 min). Blanching with aquadest for 10 min shows a significantly higher antioxidant activity than fresh BS. Similarly, BS blanched with citric acid 0.05% shows a higher antioxidant activity than fresh BS. Blanching with citric acid for 5 min has revealed the highest antioxidant activity in BS. These results agree with those of Pujimulyani et al. (2010) who report that the blanching method can increase the antioxidant activity.

### Table III. Total phenolic content of black saffron due to blanching treatment with aquadest and citric acid 0.05%

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Total phenolic content (mg GAE/g)</th>
<th>Blanching with aquadest</th>
<th>Blanching with citric acid 0.05%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.04 ± 0.30 a</td>
<td>6.72 ± 0.00 a</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>6.43 ± 0.35 a</td>
<td>7.96 ± 0.00 ab</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>7.20 ± 0.28 ab</td>
<td>9.21 ± 0.00 b</td>
<td></td>
</tr>
<tr>
<td>7.5</td>
<td>7.19 ± 0.00 ab</td>
<td>9.60 ± 1.86 b</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>7.28 ± 2.46 ab</td>
<td>8.37 ± 0.34 ab</td>
<td></td>
</tr>
</tbody>
</table>

The different letter in the same column indicated significant difference (P < 0.05). The data was presented as mean ± standard deviation from 3 replicates.

### Table IV. Total flavonoid content of black saffron powder due to blanching treatment with aquadest and citric acid 0.05%

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Total flavonoid content (mg QE/g)</th>
<th>Blanching with aquadest</th>
<th>Blanching with citric acid 0.05%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.52 ± 0.27 a</td>
<td>1.47 ± 0.27 a</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>1.61 ± 0.03 ab</td>
<td>1.72 ± 0.78 b</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1.76 ± 0.04 ab</td>
<td>1.92 ± 0.17 b</td>
<td></td>
</tr>
<tr>
<td>7.5</td>
<td>1.63 ± 0.06 ab</td>
<td>1.87 ± 0.09 ab</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1.74 ± 0.02 ab</td>
<td>1.63 ± 0.16 ab</td>
<td></td>
</tr>
</tbody>
</table>

The different letter in the same column indicated significant difference (P < 0.05). The data was presented as mean ± standard deviation from 3 replicates.

### Table V. The tannin contents of black saffron powder due to blanching treatment with aquadest and citric acid 0.05%

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Tannin content (mg CE/g)</th>
<th>Blanching with aquadest</th>
<th>Blanching with citric acid 0.05%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.66 ± 0.15 a</td>
<td>1.66 ± 0.02 a</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>2.04 ± 0.00 ab</td>
<td>2.64 ± 0.00 ab</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2.07 ± 0.56 ab</td>
<td>2.87 ± 0.65 b</td>
<td></td>
</tr>
<tr>
<td>7.5</td>
<td>2.13 ± 0.51 ab</td>
<td>2.67 ± 0.06 b</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2.17 ± 0.38 ab</td>
<td>2.20 ± 0.00 ab</td>
<td></td>
</tr>
</tbody>
</table>

The different letter in the same column indicated significant difference (P < 0.05). The data was presented as mean ± standard deviation from 3 replicates.
activity of white saffron. The blanching treatment has resulted in white saffron having higher free radicals than fresh white saffron. The blanching at 100 °C for 5 minutes can inactivate the polyphenol oxidase enzyme. The blanched BS with citric acid exhibited a higher % of RSA than the blanching treatment with aquadest because the citric acid could chelate the metal.

The total phenolic content (TPC) of BS powder with blanching in the aquadest and citric acid with the different blanching times (Table III). The TPC shows significant differences between the samples and the blanching in the aquadest and citric acid 0.05%. Total phenolic content in the sample with blanching media aquadest for 10 min is nearly similar to that in the fresh BS (without blanching). The blanching treatment with boiling in citric acid 0.05% for 5 min has discovered a more significant TPC than the sample without blanching. This finding is similar to that of Izli (2017), who has revealed the highest phenolic content of dates due to the implementation of a treatment with high temperatures.

The total flavonoid content (TFC) of BS, expressed by mg quercetin equivalent (QE)/g sample, with blanching using aquadest and citric acid with different blanching times (Table IV). The TFC in BS powder ranges from 1.47-1.92 mg QE/g and is resulted from both blanchings using a medium of aquadest and citric acid 0.05%. This result denotes that the differences in TFC are caused by various blanching media and blanching times (P < 0.01). It proves that the blanching treatment of white saffron in a media of citric acid 0.05% using a temperature of 100°C for 5 min can increase the levels of condensed tannin, total phenolic, and flavonoid. However, the high heat treatment can accelerate the oxidation of antioxidants contained in the natural material system (Pujimulyani et al., 2018).

The levels of tannin, expressed by catechin equivalent (CE), in BS powder blanched with aquadest and citric acid with the different blanching times (Table V). The tannin content of BS powder ranged between 1.66 to 2.87 mg CE/g. Since tannin is soluble in water, more tannin is dissolved and comes out, resulting in a low level of tannin during the blanching process. The blanching of BS treated using a medium of citric acid 0.05% at the temperature of 100°C for 5 min can increase the content of condensed tannins. The heating of the tannin showed a more significant increase in the antioxidant activity than the sample processed without heating.

CONCLUSION

The black saffron powder treated with a blanching process has shown higher antioxidative properties than that without a blanching process. The blanching using citric acid 0.05% for 5 min has the best antioxidant properties, total phenolic, flavonoid, and tannin contents.

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REFERENCES


