Indonesian Journal of Pharmacy

VOL 32 (2) 2022: 244–250 | RESEARCH ARTICLE

The Effects of Media and Blanching Time on the Antioxidative Properties of C*urcuma aeruginosa* Roxb.

Dwiyati Pujimulyani[1]*, Sulkhan Windrayahya[2,3] and Irnawati Irnawati[4]

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

Info Article

Submitted: 16-12-2021
Revised: 17-04-2022
Accepted: 18-04-2022

*Corresponding author
Dwiyati Pujimulyani

Email:
dwiyati2002@yahoo.com

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 distilled water) 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*., 2019a). Black saffron 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. Black saffron is also reported to have anti-inflammation (Andrina *et al*., 2017) and antibacterial activity (Akarchariya *et al*., 2017). Moreover, it contains several phytochemical compounds, such as essential oil (turmerone, zingiberene), curcuminoid, alkaloid, saponin, resin, flavonoid, and polyphenol (Nugrahaningtyas *et al*., 2005; Khumaida *et al*., 2019b). 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).

Black saffron 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.,* 2020a). The antioxidant compound is available in the human body as endogen 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 (Arvianasari *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 emphasizing the blanching and media concentration to get optimum responses. 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 local market in Yogyakarta. Butylated hydroxytoluene (BHT) and 2,2′-diphenyl-1-picrylhydrazil (DPPH) were purchased from Sigma (Aldrich, USA) while sodium carbonate ($NaCO_3$), sodium bicarbonate (Na_2CO_3), Folin-Ciocalteu Reagent (FCR), H_2SO_4, sodium hydroxide, ethanol, sodium nitrite ($NaNO_2$), and aluminium chloride hexahydrate ($AlCl_{3.6}H_2O$) 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 distilled water and citric acid solution at a temperature of 100°C with various times of 0-, 2.5-, 5-, 7.5-, and 10-min. The BS samples were drained, sliced ± 2 mm thickness, 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). The BS powders 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 H_2SO_4 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*. (2020b). Black saffron (1 g) was added with 10 volumes of ethanol (10 mL) and then was mixed with vortex (1 min), macerated 1 h at room temperature, and then 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

wavelength. 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 (%)

$$=\frac{(\mathrm{Abs}_{\mathrm{control}}-\mathrm{Abs}_{\mathrm{sample}})}{\mathrm{Abs}_{\mathrm{control}}}\mathrm{x}100$$

Determination of total phenolic content

The total phenolic content (TPC) of BS was determined spectrophotometrically using the FCR as employed by (Rohman *et al*. 2020b) with slight modification. A-200 µL of the ethanolic solution containing BS samples was mixed with 0.4 mL of FCR 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. In brief, 50 µl of BS extract was mixed with 4 mL of distilled water and 0.3 mL of $NaNO_2$ 10%. The mixture was left for 5 min. Afterward, 0.3 µL of $AlCl_3$ 10% was added and left for 5 min. The solution was added with 4 mL of NaOH 10% and distilled water 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 expressedasmgcatechinequivalent(CE)/g samples.

Data analysis

All analytical measurements were carried out in three replicates. The obtained data were subjected to statistical analysis using a one-way 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 distilled water and citric acid with different blanching times (Table I). The statistical results denote that longer blanching time generates 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 be 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 distilled water and citric acid with different blanching times (Table II). The crude fiber content of BS powders ranges from 17.29-20.12% in both distilled water 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%

Time (min)	Water content (%)	
	Blanching with aquadest	Blanching with citric acid 0.05%
0	7.95 ± 0.59 ^{ab}	8.66 ± 1.70 ^{b}
2.5	7.59 ± 0.65 ^{ab}	7.09 ± 0.58 ^{ab}
5	6.95 ± 0.54 ^{ab}	8.46 ± 1.23 ^{b}
7.5	7.89 ± 0.63 ^{ab}	7.93 ± 2.32 ^{ab}
10	6.25 ± 0.29 ^{a}	6.87 ± 1.04 ^{ab}

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%

Time (min)	Crude fiber (%)	
	Blanching with aquadest	Blanching with citric acid 0.05%
0	20.03 ± 0.43 ^{a}	20.03 ± 0.43 ^{a}
2.5	18.91 ± 3.10 ^{a}	19.96 ± 0.55 ^{a}
5	20.12 ± 0.60 ^{a}	19.92 ± 0.78 ^{a}
7.5	19.07 ± 1.60 ^{a}	19.65 ± 5.11 ^{a}
10	17.29 ± 3.81 ^{a}	17.94 ± 0.96 ^{a}

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

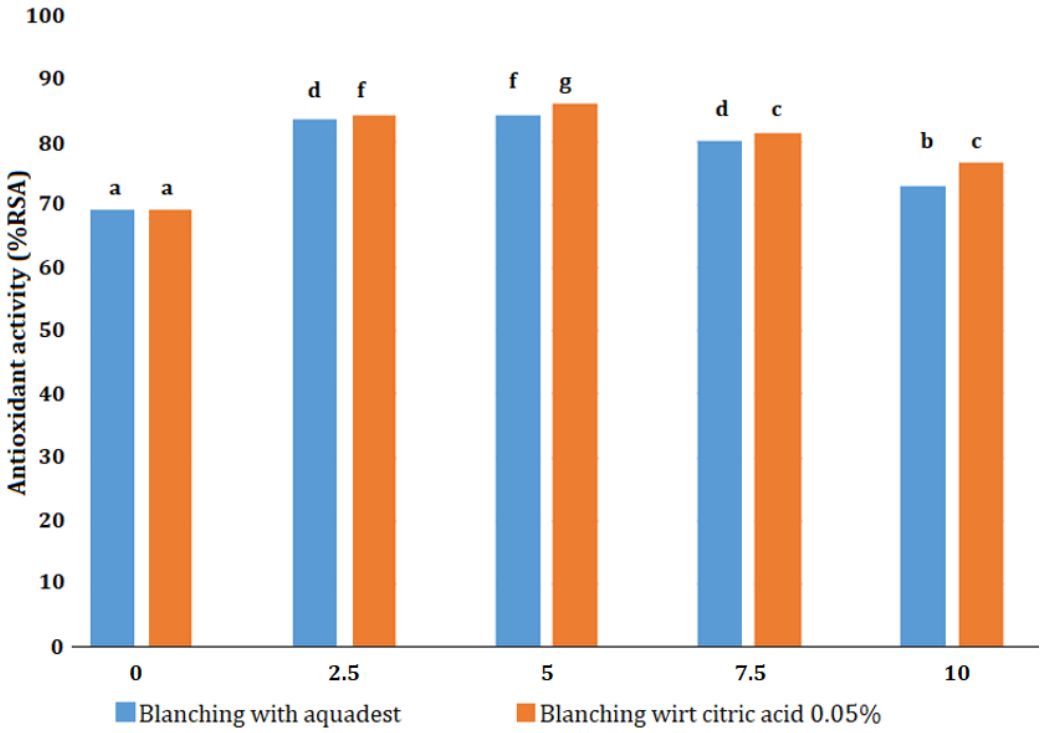

Figure 1. Antioxidant activity of black saffron powder due to blanching treatment with aquadest and citric acid 0.05%

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

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

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%

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

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%

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

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

The antioxidant activities of the blanched BS with distilled water 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 distilled water for 5 min shows a higher antioxidant activity of 84.11% than a process without blanching (0 min). Blanching with distilled water 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 of white saffron. The blanching treatment has resulted in white saffron having higher free radicals than fresh white saffron. The blanching process 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 distilled water because the citric acid could chelate the metal.

The total phenolic content (TPC) of BS powder with blanching in the distilled water and citric acid with the different blanching times (Table III). The TPC shows significant differences between the samples and the blanching in the distilled water and citric acid 0.05%. Total phenolic content in the sample with blanching medium of distilled water 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 İzli (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 distilled water 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 blanching methods using media of distilled water 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 medium 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, 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 distilled water 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 method using citric acid 0.05% for 5 min has the best antioxidant properties, total phenolic, flavonoid, and tannin contents.

ACKNOWLEDGEMENT

The authors are grateful for the CV. Windra Mekar and the Ministry of Education, Culture, Research, and Technology of the Republic of Indonesia for providing the financial support to conduct this research in 2021.

REFERENCES

Akarchariya N, Sasithorn S, Jakaphun J, Sunee C. 2017. Chemical Profiling and Antimicrobial Activity of Essential Oil from *Curcuma Aeruginosa* Roxb., Curcuma Glans K. Larsen & J. Mood and Curcuma Cf. Xanthorrhiza Roxb. Collected in Thailand. *Asian Pac J Trop Biomed* 7(10):881–85.

Ananingsih, Victoria K, Gracia A, Robertus PYN. 2017. The Impact of Pretreatment on the Quality of Turmeric Dried by Solar Tunnel Dryer. *Jurnal Ilmu Pertanian Indonesia* 22(2):79–86.

Andrina S, Churiyah C, Nuralih N. 2017. Anti-Inflammatory Effect of Ethanolic Extract of *Curcuma aeruginosa* Roxb Rhizome, *Morinda citrifolia* Fruit and *Apium graveolens* Leaf on Lipopplysaccharide-Induce RAW 264.7 Cell Lines. *Indonesian J Cancer Chemop* 6(3):84.

Arivazhagan P, Thangaswamy T, Chinnakkannu P. 2000. Antioxidant Lipoate and Tissue Antioxidants in Aged Rats. *J Nutr Biochem* 11(3):122–27.

Arvianasari E, Rosalia AS, Dwiyati P. 2020. The Effect of Concentration of Citric Acid and Blanching Time on the Antioxidation Properties of White Turmeric. *Int J Adv Sci Technol* 29(7):1647–53.

Busuttil-Griffin F, Shoemake C, Attard E, Azzopardi LM. 2015. Crude Fibre Determination of *Malva sylvestris* L. and Evaluation of its Faecal Bulking and Laxative Properties in Rats. *Int J Biology* 7(4): 1-8

Cheng Z, Lan S, Jeffrey M, Kequan Z, Marla L, Jun JY, Liangli Y. 2006. Effects of Postharvest Treatment and Heat Stress on Availability of Wheat Antioxidants. *J Agric Food Chem* 54(15):5623–29.

Ghafoor K, Fahad AJ, Mehmet MO, Nurhan U, Elfadıl EB, Isam AMA. 2020. Total Phenolics, Total Carotenoids, Individual Phenolics and Antioxidant Activity of Ginger (*Zingiber officinale*) Rhizome as Affected by Drying Methods. *LWT Food Sci technol*. 126:109354.

İzli G. 2017. Total Phenolics, Antioxidant Capacity, Colour and Drying Characteristics of Date Fruit Dried with Different Methods. *Food Sci Technol* 37(1):139–47.

Khumaida N, Sintho WA, Adi S, Latifa NA. 2019a. In Vitro Multiplication and Acclimatization of Black Galingale (*Curcuma aeruginosa* Roxb.). *J Appl Pharm Sci* 9(4):110–16.

Khumaida N, Muhamad S, Maria B, Waras N. 2019b. Phenolic and Flavonoid Content in Ethanol Extract and Agro-Morphological Diversity of *Curcuma aeruginosa* Accessions Growing in West Java, Indonesia. Biodiversitas 20(3):656–63.

Kassim MJ, Hussin MH, Achmad A, Dahon NH, Suan TK, Hamdan HS. 2013. Determination of Total Phenol, Condensed Tannin and Flavonoid Contents and Antioxidant Activity of *Uncaria Gambir* Extracts. *J Chem Inform Modeling* 53(1):1689–99.

Kedare, Sagar B, Singh RP. 2011. Genesis and Development of DPPH Method of Antioxidant Assay. *Journal of food science and technology* 48(4): 412-22.

Muhammad NWF, Nurrulhidayah AF, Hamzah MS, Rashidi O, Rohman A. 2020. Physicochemical Properties of Dragon Fruit Peel Pectin and Citrus Peel Pectin: A Comparison. *Food Res* 4:266–73.

Noreña CZ, Renata TR. 2018. Effect of Blanching on Enzyme Activity and Bioactive Compounds of Blackberry. Brz Arch Biol Technol 61(0):1–13.

Nugrahaningtyas KD, Sabirin M, Tutik DW. 2005. Isolasi dan Identifikasi Flavonoid dalam Rimpang Temu. *Biofarmasi* 3(1):32–38.

Pujimulyani D, Raharjo S, Marsono Y, Santoso U. 2012. The Effect of Blanching on Antioxidant Activity and Glycosides of White Saffron *(Curcuma mangga Val.)*. *Int Food Res J* 19(2): 617-621.

Pujimulyani D, Raharjo S, Marsono Y, Santoso U. 2010. Aktivitas Antioksidan dan Kadar Senyawa Fenolik Pada Kunir Putih (*Curcuma mangga* Val.) Segar Dan Setelah Blanching . *Agritech* 30(2).

Pujimulyani D, Santoso U, Luwihana SD, Maruf A. 2020. Orally Administered Pressure-Blanched White Saffron (*Curcuma mangga* Val.) Improves Antioxidative Properties and Lipid Profiles *in Vivo*. *Heliyon* 6(6): e04219.

Pujimulyani D, Yulianto WA, Astuti S, Arumwardana S, Rizal R. 2018. Antidiabetic and Antioxidant Potential of *Curcuma mangga* Val Extract and Fractions. *Asian J Agri Biol* 6(2):162-168.

Puupponen-Pimiä R, Häkkinen ST, Aarni M, Suortti T, Lampi A-M, Eurola M, Piironen V, Nuutila AM, Oksman-Caldentey KM. 2003. Blanching and Long-Term Freezing Affect Various Bioactive Compounds of Vegetables in Different Ways. *J Sci Food Agric* 83(14):1389–1402.

Rohman A, Rafi M, Alam G, Muchtaridi M, Windarsih A. 2019. Chemical Composition and Antioxidant Studies of Underutilized Part of Mangosteen (*Garcinia Mangostana* L.) Fruit. *J Appl Pharm Sci* 9(8): 47-52.

Rohman A, Can AT, Irnawati, Rafi M, Lukitaningsih E, Fadzilah NA. 2020a. Principal Component Analysis of Antioxidant Activities, Total Phenolic Contents, and Total Flavonoid Contents of Turmeric (*Curcuma Longa* L.). Int J Pharm Res 12: 2966-2972.

Rohman A, Widodo H, Lukitaningsih E, Windarsih A, Rafi M, Nurrulhidayah AF. 2020b. Review on *In vitro* Antioxidant Activities of Curcuma Species Commonly Used as Herbal Components in Indonesia. *Food Res* 4(2): 286–93.

Simoh S, Shin SY, Abd Rahim F, Ahmad MA, Zainal A. 2018. Comparative Analysis of Metabolites and Antioxidant Potentials from Different Plant Parts of *Curcuma aeruginosa* Roxb. *Sains Malaysiana* 47(12):3031–41.

Thiex N. 2009. Evaluation of Analytical Methods for the Determination of Moisture, Crude Protein, Crude Fat, and Crude Fibre in Distillers Dried Grains with Soluble. *J AOAC* Int 92(1): 61-73.

Yang M, Qing S, Li LQ, Huang YQ, Cheung HY. 2015. Phytochemical Profiles, Antioxidant Activities of Functional Herb Abrus Cantoniensis and Abrus Mollis. *Food Chem* 177: 304–12.

Yen HF, Hsieh CT, Hsieh TJ, Chang FR, Wang CK. 2015. In Vitro Anti-Diabetic Effect and Chemical Component Analysis of 29 Essential Oils Products. *J Food Drug Anal* 23(1):124–29.