

The Phenolic Substances and Antioxidant Activity of White Saffron (*Curcuma mangga* Val.) as Affected by Blanching Methods

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Abstract—Background and objectives: Most of the agricultural products are processed by blanching. Blanching can increase antioxidant activity in white saffron products. The objective of this research were to determine antioxidant activity, to identify, and to measure changes in phenolic substances of fresh and blanched white saffron rhizomes (*Curcuma mangga* Val.). Methods: White saffron rhizomes were peeled, washed and blanched in boiling water containing 0% or 0.05% citric acid solution for 5 and 10 minutes. Samples were extracted using methanol, rotaevaporated, and freeze-dried. Dried extract was determined antioxidant activity by DPPH method, identified and quantified for the phenolic substances by High Performance Liquid Chromatography (HPLC) equipped with column C₁₈ and Photodiode-array detector (PAD). Result: This research showed that the quantity of the 6 phenolic substances identified in blanched white saffron in citric acid solution increased significantly compared to that of the non-blanched. Blanching white saffron in 0.05% citric acid media for 5 minutes increased its antioxidant activity, and total phenolic content. Conclusions: The identified phenolic substances of white saffron were Gallic Acid (GA), Catechin (C), Epicatechin (EC), Epigallocatechin (EGC), Epigallocatechingallat (EGCG) and Gallocatechingallat (GCG). The blanched white saffron contained C and EGCG significantly higher than that of fresh rhizomes.

Keywords—White saffron, antioxidant activity, blanching, phenolic.

I. INTRODUCTION

THE main purpose of blanching is to inactivate enzymes [1]. Blanching can decrease or increase antioxidant in agricultural products. Most of the agricultural products are processed by blanching. Blanching is one of the most important preparation steps in processing various frozen vegetables. The blanching of red turi (*Sesbania grandiflora* L. (Pers) flower decreased anthocyanin and vitamin C content, due to the fact that antioxidant substances leached into the blanching media, and vitamin C was damaged by heating [2]. The blanching of red cabbage at 94-96°C for 3 minutes decreased 42% of FRAP value [3]. The blanching of lentil

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beans at 5 psi pressure for 5 minutes decreased RSA by 29.5% and ORAC by 11.1% [4]. The decrease of antioxidant activity was probably due to the leaching of antioxidant compounds in the blanching media.

Other studies showed that blanching increased antioxidant activity. The blanching of wheat with pressure at 100 °C after harvesting increased the total phenol of wheat powder [5]. Blanching of corn in autoclave increased its total phenol [6]. The antioxidant activity of beans, corn, and tomato using DPPH method increased after blanching [7]. Brussel sprouts (*Brassica oleracea* L.), after water blanching at 100°C for 2 and 3 minutes, have higher antioxidant activity compared to fresh brussel sprouts [8], [9]. Bilberry extract which was heated at 100°C for 10 minutes had a higher antioxidant activity compared to the fresh extract, because anthocyanin was hydrolysed into anthocyanidin and sugar [10]. References [11], [12] found that hydrolysis of anthocyanin glycoside into anthocyanidin occurred during heating in acidic condition.

Processed white saffron, in the form of wet and dry sweets, was well accepted by panelists and had antioxidant activity of Radical Scavenging Activity (RSA) 42.94% [13] and 40.68% [14] respectively. These products were prepared by heating (boiling) and showing antioxidant activity. It is probably because some of antioxidant compounds within the white saffron are heat resistant and some other compounds may change into different compounds with higher antioxidant activity.

The purposes of this research were to determine antioxidant activity, to identify and to quantify of phenolic compound in fresh and blanched white saffron.

II. METHODOLOGY

A. Materials

White saffron rhizomes (*Curcuma mangga* Val.) were harvested from a local farm in Yogyakarta. This study used GA, C, EC, EGC, EGCG, GCG, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) were obtained from Sigma Chemical Co. (St. Louis, Mo, U.S.A). Methanol, HCl, acetic buffer, H₃PO₄, N₂, CH₃CN, citric acid, acetic ethyl were from E. Merck and aquabidestilata was from Ika Pharmindo.

B. Methods

1. Extraction of White Saffron Samples

The white saffron rhizomes were peeled, washed, and dripped. 500g of white saffron was blanched at 100°C with 0.05% and 0% citric acid solution blanching media, for 0, 5, and 10 minutes respectively. Fresh and blanched white saffron was extracted using methanol. White saffron was crushed for 5 minutes in methanol. White saffron paste was macerated at room temperature, in methanol 1:1 (w/v). The white saffron maceration was conducted for 12 hours, homogenized using homogenizer for 15 minutes, then filtered in vacuum condition to get extract I. A 500ml methanol was added to the residue, then macerated for 12 hours and homogenized using homogenizer for 15 minutes. The homogenized residue was then filtered in vacuum condition to get extract II. Extract I and II were mixed and then filtered again and evaporated using rotary evaporator to get concentrated extract. The concentrated extract was dried using freeze drier to obtain the dried extract. The antioxidant activity of the dried extract was examined using FRAP method. The identification and quantification of phenolic compound was carried out by HPLC [15]. The total extraction process was done in triplicate.

2. DPPH Free Radical Scavenging Activity

DPPH free radical scavenging capacity of white saffron extracts was determined according to [4] with slight modifications. Basically, 0.2mL of fresh and blanched white saffron extract was added to 3.8mL ethanol solution of DPPH radical (0.05mM). The mixture was shaken vigorously for 1 minute by vortexing and was left to stand in the dark for 30 minutes at room temperature. Thereafter, the absorbance for the sample was measured using spectrophotometer at 517 nm against ethanol blank. The free radical scavenging activity of white saffron extract was expressed as mean mg trolox equivalent/g using calibration curve of trolox.

3. Identification and Quantification of Phenolic Substances by HPLC

The phenolic substances in the dried extracts were identified and quantified using HPLC completed with column C₁₈ [15]. We used HPLC KNAUER with a 3880 auto sampler smartline, a Photodiode Array Detector (PAD) spectra system UV6000LP, and software chromgate 3. 1. 6 data module. The preparation of samples before injecting to HPLC was as follows: 0.5mL extract (from 100mg dried extract in 3mL methanol:HCl, ratio 1000:1) was evaporated using N₂ and was added with 1mL H₃PO₃, filtered using millex filter 0.45µm, then injected to HPLC system. The column used was C₁₈ (4.6 x 250mm, dp 5µm) and at 50°C, using λ 256nm for quercetin-3-rutinoside. The eluent used was H₃PO₃:CH₃CN:acetic ethyl (ratio 84:12:4). This procedure was applied to all samples and glycoside value was calculated in mg/g extract compared with standard glycoside used was quercetin-3-rutinoside and the standard aglycone was quercetin. The gradient of eluent used was 1.2mL/minute at 10 minutes. The volume of injection was 20µL using auto sampler smartline. This procedure was applied to all samples and to find the amount of standard GA,

EGC, EC, C, EGCG and GCG, the gradient of eluent used was 1 ml/minute at 0-4 minutes and 1.5 ml/minute at 4-20 minutes, using PAD λ 275nm.

4. Statistical Analysis

The experiment was designed a complete block. The variables were two different media (0.05% and 0% citric acid) and three different blanching times. Duncan's multiple range test was carried out to test any significant differences between media and blanching time (0.5 and 10 minutes). Significant levels were defined using $p \leq 0.05$. Statistical analysis was performed with the SPSS software package.

III. RESULT AND DISCUSSION

A. The Effect of Blanching on the Phenolic Compounds of Blanched White Saffron

Total Phenolic Content (TPC) of white saffron extracts are presented in Fig. 1. Significant differences ($P < 0.05$) in TPC were observed in both blanching in 0.05% citric acid and 0% (distilled water) compared with raw material. After blanching in 0.05% citric acid treatment, the TPC of white saffron was slightly higher compared to blanching in 0% citric acid (distilled water). It was probably because phenolic compounds could breakdown during the blanching. The same study on the blanching of corn [6] and the blanching of wheat [5] could increase their total phenol.

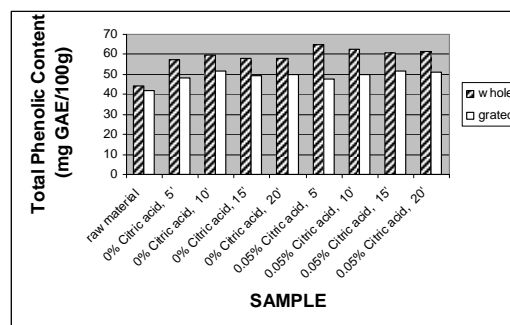


Fig. 1 Total phenolic content of white saffron

Boiled frozen broccoli increased the amounts of phenolic compounds from 0.964 to 2.50mg/g of fresh mass. This finding was explained based on the difference in extraction efficiency. The disruption of the structure of the cell walls or release of phenolics from insoluble complexes due to the blanching has made them more accessible for extraction [16].

The identified phenolic compounds in white saffron were GA, C, EC, EGC, EGCG, and GCG. The content of phenolic compound of blanched white saffron in 0.05% citric acid for 5 and 10 minutes showed significant increase compared to that of the non-blanched. The result of this research showed that the quantity of the 6 phenolic substances identified in blanched white saffron in citric acid solution increased significantly compared to that of the non-blanched. Changes in the content of individual phenolics after blanching of white saffron (GA, C, EC, EGC, EGCG, GCG) by HPLC method (mg/g) is shown in Table I.

TABLE I

CHANGES IN THE CONTENT OF INDIVIDUAL PHENOLICS AFTER BLANCHING OF WHITE SAFFRON (GA, C, EC, EGC, EGCG, GCG) BY HPLC METHOD (MG/G)

Treatment	GA	EGC	C	EGCG	EGC	GCG	Total
Fresh	0.124±0.008 ^b	0.442±0.024	0.134±0.049 ^a	0.037±0.016 ^a	0.113±0.017 ^{ab}	0.159±0.014 ^b	1.01 ± 0.04 ^a
Blanching Citric acid 5 min	0.107±0.015 ^{ab}	0.490±0.045	0.243±0.051 ^b	0.178±0.054 ^b	0.148±0.045 ^b	0.164±0.024 ^b	1.33 ± 0.15 ^b
Blanching Citric acid 10 min	0.095±0.011 ^a	0.490±0.058	0.147±0.012 ^a	0.334±0.040 ^c	0.102±0.037 ^{ab}	0.135±0.021 ^{ab}	1.30 ± 0.14 ^b
Blanching aquadest 5 min	0.128±0.009 ^b	0.464±0.051	0.124±0.016 ^a	0.029±0.001 ^a	0.068±0.008 ^a	0.100±0.006 ^a	0.91 ± 0.08 ^a
Blanching aquadest 10 min	0.091±0.016 ^a	0.416±0.072	0.113±0.020 ^a	0.203±0.038 ^a	0.092±0.023 ^{ab}	0.104±0.022 ^a	1.02 ± 0.17 ^a

The means with the same letter in the same row are not significantly different at 5% levels with Duncan's multiple range tests. (Data are expressed as mean ± standard deviation)

The content of gallic acid of blanched white saffron in 0.05% citric acid solution and aquadest for 10 minutes decreased significantly compared to that of non blanched white saffron. It was suspected that blanching white saffron for 10 minutes damaged gallic acid more than blanching it for 5 minutes. There was no significant decrease of gallic acid when blanching was done for that duration. It was found that gallic acid dissolved in hot water [17]. The C and EGCG content of blanched white saffron in 0.05% citric acid media increased significantly compared to that of the non blanched. The increase of C and EGCG in blanched white saffron may be due to the hydrolysis of phenolic complex during the blanching process. This is similar to the study of [7] that there was a formation of a hydroxyl group, such as phenolic acid and flavonoid, during heating. Reference [18] reported that the increase of antioxidant activity in tannic acid during heating was due to the thermal hydrolysis of hydroxyl group into new galloyls.

B. Effect of Blanching on DPPH Free Radical Scavenging Capacity of White Saffron

DPPH free radical scavenging capacities of blanched white saffron is shown in Fig. 2. The DPPH value of blanched white saffron in 0.05% citric acid media, 100°C, for 5 minutes showed significant increase compared to that of fresh white saffron, but if the blanching time was extended up to 20 minutes, the different of the antioxidant activity was not significant compared to that of 5 minutes [19].

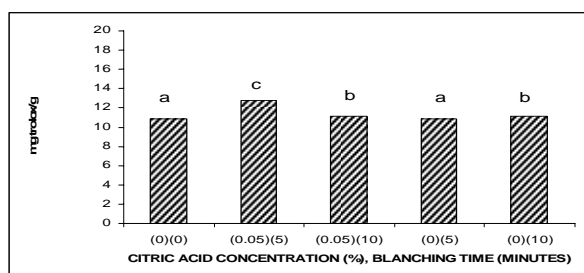


Fig. 2 DPPH free radical scavenging capacities of blanched white saffron

This was probably due to the hydrolysis of glycoside to aglycone and sugar. The aglycone quercetin in this research showed higher antioxidant activity than the glycoside. Similar study was reported by [12], that glycoside anthocyanin was

hydrolysed into aglycone anthocyanidin. Reference [20] reported that aglycone had higher antioxidant activity compared to glycoside. Similar study was reported that blanching beans [7] and broccoli [16] increased the antioxidant activity which was determined using DPPH method.

IV. CONCLUSIONS

The phenolic substances identified in blanched white saffron are GA, C, EC, EGC, EGCG, and GCG. The content of C and EGCG in the blanched white saffron showed a significant increase compared to that of the non-blanched. Blanching white saffron in 0.05% citric acid media at boiling temperature for 5 minutes increased the antioxidant activity and TPC significantly compared to that of the non-blanched.

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