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The effects of blanching treatment on the radical scavenging activity of white saffron (*Curcuma mangga* Val.)

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Abstract: The purpose of this research was to study the effect of blanching methods on antioxidant activity of parts and whole white saffron rhizomes. White saffron rhizomes were peeled, washed, and blanched in boiling water at 100°C, or steamed for 5, 7.5, and 10 minutes in the media of distilled water, 0.05%, and 0.1% citric acid solution. The antioxidative activity of blanched white saffron was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity (RSA) method. The total phenolic content (TPC) was determined using a Folin-Ciocalteu assay using gallic acid (GA) as a standard. White saffron rhizomes blanched at 100°C for 5 minutes in 0.05% citric acid solution showed the highest antioxidant activity with RSA of 86.57%. Antioxidant activity of the first branch of white saffron rhizome (RSA of 84.89%) showed no significant difference from the main rhizome. These findings confirmed that blanching in media containing citric acid (0.05%) resulted in increased RSA and TPC in white saffron rhizome as compared to fresh or water blanched white saffron.

Keywords: Saffron, blanching, radical scavenging activity (RSA)

Introduction

Antioxidant can be obtained from both natural resources such as curcuminoid from turmeric (*Curcuma mangga* Val.) and from synthetic materials. Synthetic antioxidants such as Butylated hydroxy anisole (BHA), Butylated Hydroxy Toluene (BHT), Tertiary butyl hydroxy quinone (TBHQ) and Propyl Gallate (PG) are highly effective (Wanasundara *et al*., 1994). However, their safety is still questionable. Therefore, their use is tightly regulated in most countries. The current trend of increasing consumers' awareness and concern about the safety of synthetic additives in food products emphasizes the importance of continuing research in the application of natural antioxidants. Several studies have also reported that the natural antioxidants showed higher antioxidantive activity than synthetic commercial antioxidants such as BHA and BHT (Kim *et al*., 1999).

White saffron which is locally known as *temu mangga* is a bush perennial and has stalk rhizomes. When the rhizome is cut, the yellow flesh is clearly visible on the outside layer and slightly light yellow flesh in the center layer. The white saffron aroma and taste are similar to those of ripe mangoes. The white saffron extract showed antioxidative activity and this is due to curcuminoid and tannin content (Pujimulyani and Sutardi, 2003).

The antioxidative activity of curcumin, demethoxy curcumin, and bisdemethoxy curcumin were 20, 9 and 8 times higher than that of α–tocopherol, respectively (Toda *et al*., 1985). Jitoe *et al*. (1992) examined the antioxidative activity of curcuminoids in alcohol-water system and reported that each compound gave an antioxidative activity of approximately 2.5 times higher than α–tocopherol.

White saffron in the form of syrup, instant powder, effervescent tablet (Pujimulyani and Wazyka, 2005), and dried sweets (Pujimulyani and Wazyka, 2009) still showed antioxidant activity. These products were prepared by heating (boiling) but they keep showing antioxidant activity this suggested that some of antioxidant components within the white saffron are heat stable, and some other compounds may change into different compounds having higher antioxidative activity.

Blanching is one of the most important preparation step in processing of various frozen vegetables. The primary objective of blanching is to inactivate enzymes that cause unfavorable effects on the quality of frozen vegetables (Barrett and Theerakulkait, 1995). However, the severity of the process should be limited in order to maintain chemical and physical quality (Barrett *et al*., 2000). Food processing not only improved flavor of foods but also increased the bioavailability of nutrients (Chau *et al*., 1997).

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Blanching can be done in water at 85-100°C or by steaming. Short time blanching is effective to reduce the incidence of degradation reactions during the storage. However, blanching also produces modifications in cellular structure and composition (Viña *et al*., 2007). The purpose of this research was to study the effect of blanching methods on antioxidant activity of whole and parts of white saffron rhizome.

Materials and Methods

Fresh white saffron rhizomes (*Curcuma mangga* Val.) were harvested from local farm in Yogyakarta. Chemicals used were 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), Folin-ciocalteu reagent, gallic acid (GA), sodium carbonate, ethanol from Sigma Chemical Co., and distilled water. The equipments used were spectrophotometer (Shimadzu UV-Vis 1601), vortex, centrifuge (BUCHI Rotavapor R-114) and an incubator.

Preparation of extract

The white saffron rhizomes were peeled, washed, and blanched using water blanching at 100°C or steam blanching for 5, 7.5, and 10 minutes using blanching media of distilled water, 0.05%, or 0.1% citric acid solution. In addition, white saffron rhizomes which were consisted of main rhizome, first branch, second branch, and third branch of the rhizomes were peeled, washed, and blanched at 100°C for 5 minutes in 0.05% citric acid solution. The whole, sliced (6 mm, 4 mm, and 2 mm thickness), and grated white saffron rhizome were blanched at 100°C for 5 minutes in 0.05% citric acid solution. Blanched white saffron and the remaining blanching media were evaluated for its antioxidant activity. In another set of experiment whole or grated white saffron rhizomes were peeled, washed then blanched at 100°C for 0, 5, 10, 15, and 20 minutes in distilled water or 0.05% citric acid solution. Antioxidant activity and TPC were evaluated at predetermined time interval.

DPPH free radical scavenging activity

DPPH free radical scavenging capacity of white saffron extracts was determined according to Chen and Ho, 1995; Amarowicz *et al*., 2000; Kikuzaki *et al*., 2002; Xu and Chang, 2008. Sample preparation of ethanol extracts was randomly chosen and peeled by a stainless steel knife, then washed. The white saffron (1 g) was added with 10 volumes of ethanol (10 mL) and then was mixed with vortex (1 minute), incubated for 1 h at room temperature, and filtered. The supernatant obtained was determined using DPPH as follows: a 4 ml of 0.05 mM DPPH was added with 1 ml diluted extract of white saffron, incubation at room temperature for 30 minutes, 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:

$$\%\ RSA = 1 - \frac{sample\ \ absorbance}{control\ \ absorbance}\ x\ 100\ \%$$

Determination of total phenolic content

The total phenolic content (TPC) was determined by a Folin Ciocalteu assay (Singleton and Lamuela-Raventos, 1999) using gallic acid (GA) as the standard. The sample (1 mL), 0.4 mL of Folin-Ciocalteu's reagents solution were mixed in a tube and incubated for 8 minutes at room temperature. Then 7% $NaCO_3$ (4 mL) was vortexed and then a dose of distilled water was added. The mixture was allowed to stand for 2 h at room temperature. The absorbance was measured at 765 nm against distilled water as a blank. The total phenolic content was expressed as gallic acid equivalent (mg of GAE/100 g sample) through the calibration curve of gallic acid. Linearity range of the calibration curve was 10 to 70 µg/ mL (r=0.9998).

Result and Discussion

Radical DPPH scavenging activity

DPPH assay was conducted in order to evaluate the radical scavenging activity of blanched white saffron, as shown in Figures 1 and 2. In general, white saffron prepared by water blanching showed higher RSA than that of steam blanching. Ordinary water blanching is likely to have higher thermal efficiency than the ordinary steam blanching (Hui, 1992). The use of citric acid in the blanching medium resulted in higher antioxidant activity than the use of water only or as compared to raw white saffron. It was suspected that during the blanching process, the antioxidants in the form of glycoside were hydrolized into aglikon and sugar (Yue and Xu, 2008). Bilberry extract heated at 80°C for 30 min, 100°C for 20 min, and 125°C for 10 min had higher free radical scavenging capability than the unheated extract. In addition, some degradation products of anthocyanins were also reported to have antioxidant capability (Seeram *et al*., 2001).

The blanching of white saffron increased antioxidant activity as compared to its raw material. This was probably due to polyphenol compounds degradation to simple phenolics during blanching. Similar study on the blanching of corn (Randhir *et al*., 2008) and wheat (Cheng *et al*., 2006) increased

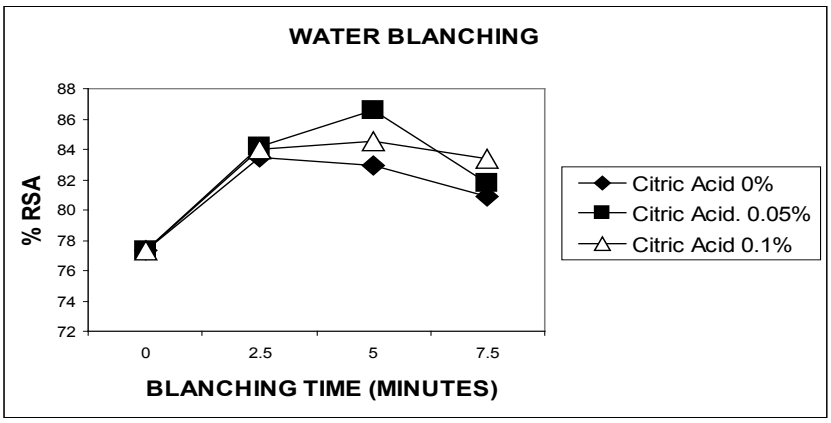

Figure 1. The effect of water blanching condition on antioxidant activity of white saffron (*Curcuma mangga* Val)

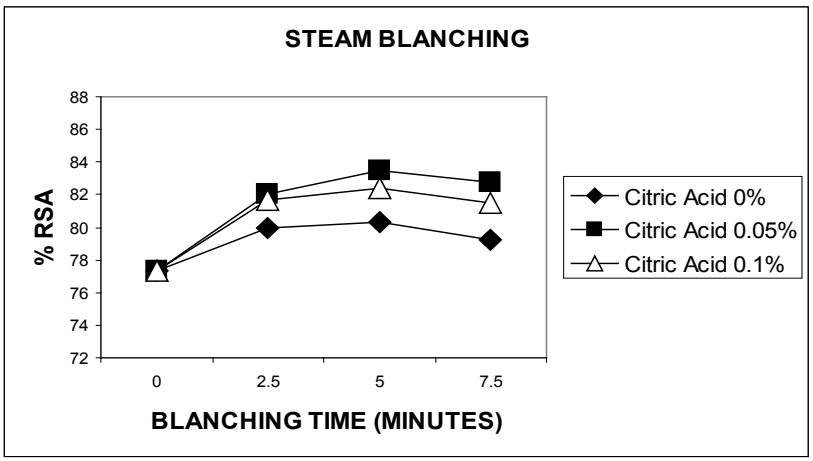

Figure 2. The effect of steam blanching condition on antioxidant activity of white saffron (*Curcuma mangga* Val)

Figure 3. The antioxidant activity of parts of white saffron (*Curcuma mangga* Val). Different letter in the chart mean significant difference.

their total phenolic contents. The antioxidant activity of different parts of white saffron is shown in Figure 3.

Branch no. 3 of white saffron rhizomes showed the smallest antioxidant activity and the main rhizomes had the highest antioxidant activity. It was found that the older the rhizomes, the higher the antioxidant activity. This was in part due to it contained higher of phenolic antioxidant compounds. The antioxidant activity of grated white saffron, and the blanching media is shown in Figure 4.

Figure 4 showed that blanched sliced 2 mm and grated white saffron have the lowest antioxidant activity. This was, in part, due to leaching of antioxidant compound in to the blanching media. The antioxidant activity of the whole white saffron was the highest, compared to grated ones. Leaching of antioxidant components were observed. This could be seen in the fact that the higher the disruption of white saffron rhizomes, the higher the antioxidant activity of the blanching media. With the grated white saffron, the higher the damage of cell matrix of white saffron, the higher the antioxidant activity of the blanching media. Similar study was reported by Puuponen-Pimia *et al*. (2003) that the heating treatment facilitated the release of bioactive compounds in vegetables from the cellular matrix. The antioxidant activity of white saffron extracts are shown in Figure 5.

The antioxidant activity of blanched white saffron extract in water was significantly lower than that of 0.05% citric acid. Figure 5 shows that blanched white saffron extract for 5 minutes have higher antioxidant activity than fresh extract. Blanching of white saffron extract for 5 minutes was not significantly different from antioxidant activity resulted from blanching for 10, 15 and 20 minutes. The antioxidant activities of whole and grated white saffron (*Curcuma mangga* Val.) are shown in Figure 6.

Blanching treatment in 0.05% citric acid, 100°C and 0% (distilled water) for 5, 10, 15, and 20 minutes increased antioxidant activity of all samples compared with fresh material. This might be due to the degradation of anthocyanin to anthocyanidin and sugar. The increased of antioxidant activity because of the blanching treatment was suspected due to the process of heating which caused antioxidant compound to be extracted more easily.

Some studies reported that anthocyanidin was produced from anthocyanins using acid hydrolysis method (Zhang *et al*., 2004; Sadilova *et al*., 2006). Yue and Xu (2008) reported that about 30% of degrated anthocyanins were thermally converted to anthocyanidin when the extract was heated at 100°C for 30 minutes. It indicates that anthocyanin could be either broken down to small molecules or it lose its conjugated sugar to become its coresponding authocyanidin during high heating treatment.

Total phenolic content (TPC)

TPC of whole and grated white saffron extracts are presented in Figure 7. Significant differences ($P<0.05$) in TPC were found 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 similar study on the blanching of corn (Randhir *et al*., 2008) and the blanching of wheat (Cheng *et al*., 2006) could increase their total phenol.

Boiled frozen broccoli increased the amount of phenolic compound from 0.964 to 2.50 mg/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 released of phenolics from insoluble complexes due to the blanching has made them more accessible for extraction (Gawlik-Dziki, 2008).

Conclusion

Blanching of white saffron rhizomes in 0.05% citric acid solution at 100°C for 5 minutes resulted in the highest RSA and TPC. Different parts of white saffron rhizomes had different antioxidant activity, but the antioxidant activity of white saffron rhizome branch no. 1 (RSA of 84.89%) was not significantly different from its main rhizome. The smaller the pieces of blanched whole, sliced or grated white saffron, the lower its antioxidant activity. Blanching of whole white saffron rhizomes for 5 minutes resulted in antioxidant activity and TPC which were not significantly different from white saffron blanched for 10, 15, and 20 minutes. Blanching in citric acid (0.05%) resulted in a higher RSA and TPC, in all tested white saffron compared to water as the media or compared to raw white saffron.

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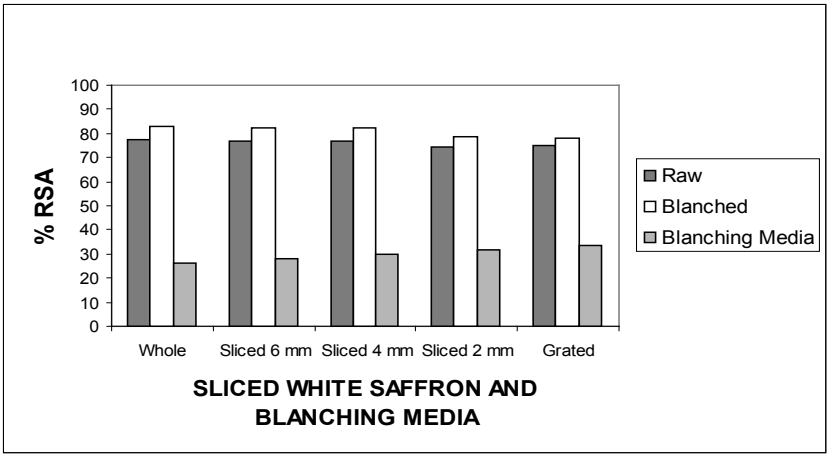

Figure 4. The antioxidant activity of white saffron (*Curcuma mangga* Val.)

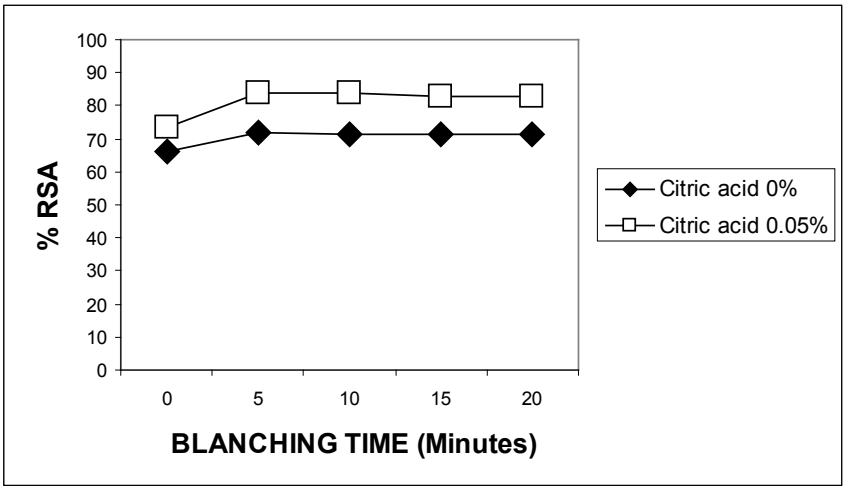

Figure 5. The antioxidant activity of white saffron (*Curcuma mangga* Val.) extract.

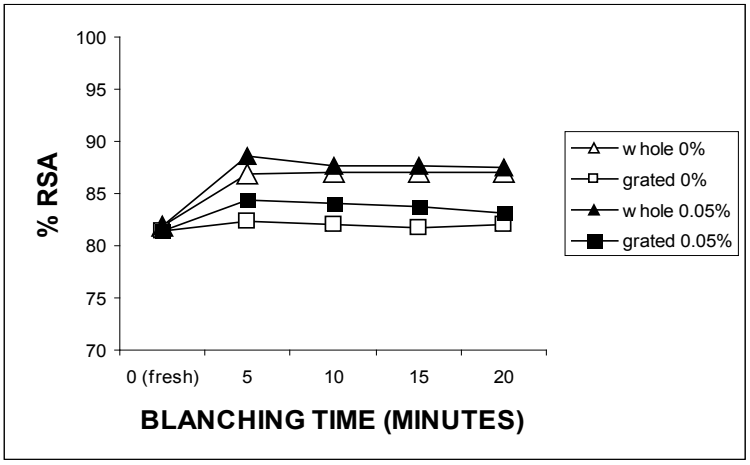

Figure 6. The antioxidant activity of whole and grated white saffron (*Curcuma mangga* Val.)

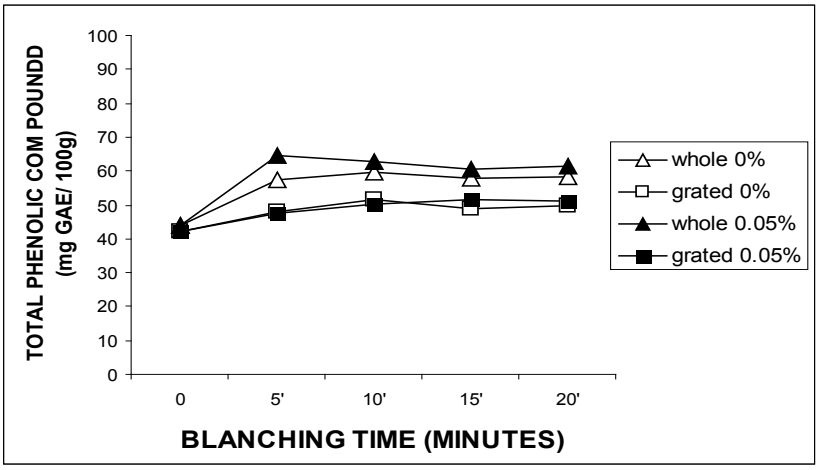

Figure 7. The total phenolic content of whole and granted white saffron (*Curcuma mangga* Val.)

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