Antioxidative Properties of White Saffron Extract (*Curcuma mangga* Val) in The β-Carotene Bleaching and DPPH-Radical Scavenging Methods

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ABSTRACT

Study on antioxidative properties of white saffron extract in the emulsion system of b-carotene linoleic acid (b-carotene bleaching method) and DPPH-radical scavenging method was undertaken. The objective of this study was to examine the antioxidative activity of white saffron extract in the emulsion system of b-carotene linoleic acid and for radical scavenging activity by DPPH method. The extraction was carried out as follows: fresh white saffron was peeled and blanched in the 0.5% boiling citric acid solution for 5 minutes, the blanched white saffron was grated, and added distilled water. The ratio between grated white saffron and distilled water were 1 : 1 ; 1 : 2 ; 1 : 3, and 1 : 4. The mixture was then mannually filtered through cloth to obtain white saffron extract. The antioxidant activity of white saffron extract was evaluated in the emulsion system of b-carotene linoleic acid and DPPH- radical scavenging method with reference standard of Butylated Hydroxy Anisole (BHA) and linoleic acid with no white saffron extract as a control. The results of this study showed that the oxidative inhibition of white saffron extract in the emulsion system of â-carotene linoleic acid was not significantly different from to BHA 200 ppm. The lower ratio of grated white saffron and distilled water, the lower percent free radical scavenging capacity. The higher white saffron extract concentration (white saffron : distilled water = 1: 2) the higher percent free radical scavenging capacity.

Key words : white saffron, antioxidant activity, b-carotene bleaching method, DPPH-Radical scavenging method

INTRODUCTION

White saffron is spices commonly used as a raw material of traditional medicine. White saffron (*temu mangga*) is a bush perennial and has stalk tubers. When the tuber is cutted the yellow flesh will be seen on the outside layer and slightly light yellow in the center layer. The white saffron aroma and taste are similar to a ripe mangoes. The syrup of white saffron showed antioxidative activity (Dwiyati, 2003). This is hipotyzed due to curcuminoid contain.

Antioxidant can be obtained both from natural resources such as curcuminoid from turmeric (*Curcuma domestica* Val) (Kikuzaki and Nakatani, 1993) and from synthetic material such as Butylated Hydroxi Anisole (BHA), Butylated Hydroxy Toluene (BHT), Tert Butyl Hydroxy Quinone (TBHQ) and Propyl Gallate (PG) (Sherwin, 1990 in Wanasundara

et al, 1994). Commonly, syntetic antioxidants had a great effectiveness, but the safety is still questionable. Therefore their use was tightly regulated in the most countries. The current trend of increasing consumer awareness and concern about the safety of synthetic additives in food products emphasizes the importance of continuing reseach in the application of natural antioxidants. Kim et al (1999) reported that chloroform fraction of *Rhus verniciflua* extract showed higher antioxidative activity than commercial antioxidants, such as BHA and BHT. Therefore, the development of potential natural antioxidant, especially from white saffron is needed.

The antioxidative activity of curcuminoid from *Curcuma domestica* Val. evaluated with the ratio curcumin, demetoxy curcumine and bisdemetoxy curcumin 140 ìg, 130 ìg and 190 ìg respectively, gave higher antioxidative activity than that of á-tocopherol (4 mg). The antioxidative activity of *Curcuma domestica* aseton extract was lower than that of α-tocopherol in the same quantity (Jitoe *et al.,* 1992). The individually curcuminoid had an antioxidative activity, moreover the naturally occuring curcuminoid complex containing all of three curcuminoids have the highest antioxidative activity compared to individual curcumin, and BHT, when determined by Rancimat method (Majeed et al., 1995).

The free radical scavenging activity of various curcuminoids was evaluated by using DPPH (1.1 diphenyl-2-picrylhydrazyl)-radical scavenging method. It showed that addition of curcuminoids resulted in the significant neutralization of free radicals. Tetrahydrocurcumin was being the most effective, followed by curcumin, and bisdemethoxy curcumin. Tetrahydrocurcumin is a hydrogenated product of curcumin reducing curcumin in an organic solvent using a metal catalyst. The ingredients of curcuminoid mixtures may not effect their free radical quenching ability. Five curcumin formula containing 67.2 % - 86.6 % curcumin, 8.3% – 16.7 % demethoxy curcumin, and 1.9%-4.5% bisdemethoxy curcumin suggested that the formation of percent free radical were not different (Majeed *et al.,* 1995).

The antioxidative activity of curcuminoid compounds (curcumin, demethoxy curcumin and bisdemethoxy curcumin) was 20, 9 and 8 times higher than á-tocopherol, when it was determined by modified active oxygen (Toda *et al.,* 1985). Jitoe *et al.* (1992) examined the antioxidative activity of curcuminoid in the alcohol-water system and reported that each compound gave an antioxidative activity, and calculated 2.5 times higher than á-tocopherol. Kakaiau *et. al.*, (1974) and Revankar *et. al.,* (1975) in Majeed *et. al.*, (1995) reported that antioxidative activity of curcuminoid as a food additive had a potention to prevent the oxidation of oil and fat during storage and heat processing.

The objective of this study was to examine the antioxidative properties of white saffron extract in the emulsion system of β-carotene linoleic acid, and by DPPH radical scavenging method.

MATERIALS AND METHODS

The main substance of this study was white saffron tubers (*Curcuma mangga* Val) purchased from local market. Chemicals used were linoleic acid, Tween 40, ethanol, DPPH, BHA (pa) purchased from Sigma Chemical, and chloroform.

The equipment used in this study were spectrofotometer (Shimadzu UV-1601), rotary evaporator centrifuge (BUCHI Rotavapor R-114), incubator, and aerator.

Preparation of white saffron extract

White saffron tubers were selected, washed, peeled, washed, and grated. The grated white saffron was added with distilled water in the ratio of 1 : 1, 1 : 2, 1 : 3, 1: 4. and then, mannually pressed through filter cloth to obtain white saffron extract. White saffron extract were determined their antioxidative activity in the emulsion system of β-carotene linoleic acid (β-carotene bleaching method) (Miller, 1971 in Wanasundara *et al.,* 1994) and by DPPH radical scavenging method (Hatano *et al.*, 1998 in Duh, 1998). The experimental diagram of this study is shown in Figure 1.

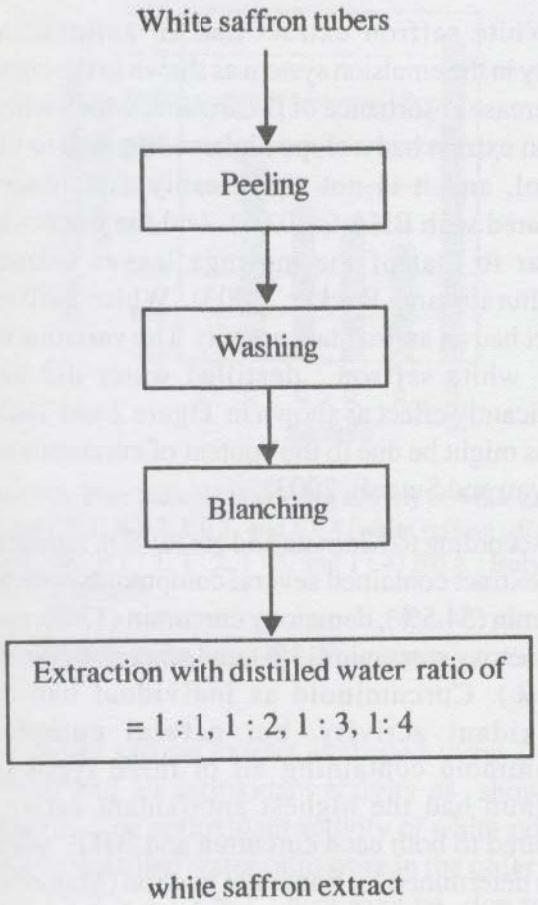

Figure 1. The experimental diagram of this study Evaluation of antioxidant activity:a).

a) β-carotene bleaching method

b). DPPH- Radical scavenging method

Antioxidant activity in the emulsion system of β-carotene linoleic acid (β-carotene bleaching method)

Antioxidant activity was determined by β-carotene bleaching method (Miller, 1971 in Wanasundara *et al.*, 1994; Sacchetti, et al, 2004) with a slightly modification.

In a 20 mg linoleic acid was added 200 mg of tween 40, and 1.0 ml of 0.02% β-carotene solution in chloroform, and then evaporated in a rotary evaporator at 40°C. This mixtures was added 50 ml destilled water which had been aerated for 24 hours in order to obtain emulsion system of β-carotene linoleic acid. After that, in a 0.4 ml extract of white saffron was added to 4.6 ml of the emulsion system, and incubated at temperature 50 °C. Absorbance values of emulsion system in the incubation time 0, 30, 60, 90, 120, and 150 minutes were measured at π 470 nm on Spectrophotometer Shimadzu UV-1601. The antioxidant activity was defined as percent inhibition calculated according to the following equation:

$$\% \text{ inhibition} = \left[1 - \frac{(A_0 \text{ sample} - A_{150} \text{ sample})}{(A_0 \text{ control} - A_{150} \text{ control})}\right] \times 100\%$$

Where A_0 = absorbance 470 nm at initial incubation (0 min)

A_{150} = absorbance 470 nm after 150 min incubation time

Antioxidant activity assay using a,a diphenyl picrylhydrazil (DPPH-Radical scavenging method)

White saffron extract sample was diluted with the absolute ethanol in order to obtain a given concentration. The mixture was then centrifuged at 300 rpm for 10 minutes. Supernatant obtained were determined using DPPH as followes: a 4 ml of 0.5 mM DPPH was added with 1 ml diluted extract of white saffron, and the absorbance was measured at 517 nm wave length. A 200 ppm of BHA in the ethanol was used as reference standard, control was ethanol without extract.

The capacity of free radical scavenging activity (Radical Scavenging Activity) was calculated by the following equation :

$$\text{Radical Scavenging Activity (\%)} = \left[1 - \frac{(\text{sample absorbance at 517 nm})}{(\text{control absorbance at 517 nm})}\right] \times 100\%$$

Experimental design

Data was analyzed by using completed randomized design single factor. When there was significant difference it was followed by Duncan's Multiple Range Test (DMRT) at 95% confidence interval.

RESULTS AND DISCUSSION

Antioxidant activity in the β-carotene bleaching method

Antioxidant activity in the emulsion system was determined by using β-carotene bleaching method. The principle of this method was to evaluate white saffron extract protection as an antioxidant resource which determined the oxidation of β-carotene linoleic acid by O_2 in the water (O_2 saturated water) and heating. The results of those evaluation in the emulsion system of β-carotene linoleic acid is shown in Figure 2.

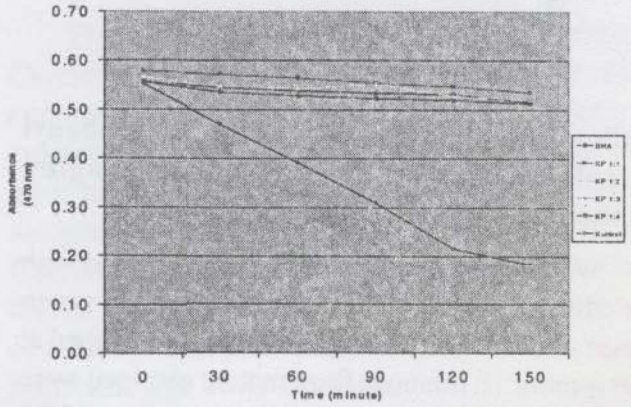

Figure 2. The antioxidant activity of white saffon extract in the emulsion system of α-carotene linoleic acid at 50°C for 150 min.
KP 1 : 1 ; KP 1 : 2 ; KP 1 : 3, and KP 1 : 4 (white saffon : distilled water ratio = 1 : 1 ; 1 : 2 ; 1 : 3, and 1 : 4 BHA : Butylated Hydroxy Anisole
Kontrol : control, ethout without white saffon extract.

The oxidation inhibition level of white saffron extract in the emulsion system of b-carotene linoleic acid is shown in Table 1.

Table 1. The inhibition of β-carotene bleaching by white saffron extract

Sample	% inhibition
BHA	89.09b
Extract of KP:Dw=1:1	88.26b
Extract of KP: Dw = 1:2	87.69b
Extract of KP: Dw = 1:3	87.19b
Extract of KP: Dw = 1:4	85.90b
Control (ethanol without extract)	0.000a

KP: white saffron
Dw: Distilled water

White saffron extract had an antioxidant activity in the emulsion system as shown in the curve of decrease absorbance of β-carotene, which white saffron extract had a slope higher compared to the control, and it is not significanly differences compared with BHA ($p<0.05$). And the trend was similar to that of the moringa leaves extract (Siddhuraju and Becker, 2003). White saffron extract had an antioxidant activity. The variation of ratio white saffron : destilled water did not significantly effect as shown in Figure 2 and Table 1. This might be due to the content of curcuminoid (Dwiyati and Sutardi, 2003).

According to Khurana and Ho (1980), turmeric tuber extract contained several compounds such as curcumin (54.5%), demetoxy curcumin (13%), and bisdemetoxy curcumin (13%), and other compounds (16.9%). Curcuminoid as individual had an antioxidant activity, but natural complex curcuminoid containing all of three types of curcumin had the highest antioxidant activity campared to both each curcumin and BHT, when it was determined by Rancimat method (Majeed *et al.*, 1995).

Antioxidant activity using DPPH method

DPPH assay was conducted in order to evaluate the radical scavenging activity of white saffron extract. Analysis of antioxidant activity using DPPH method was conducted to white saffron extract with various ratio of white saffron : destilled water and various concentration of white saffron extract (white saffron : distilled water ratio 1 : 2).

Antioxidant activity using various extraction ratio of white saffron : destilled water

White saffron extract antioxidant activity is that was defined as the percent of free radical scavenging capacity (*Radical Scavenging Activity*) was described in Figure 3.

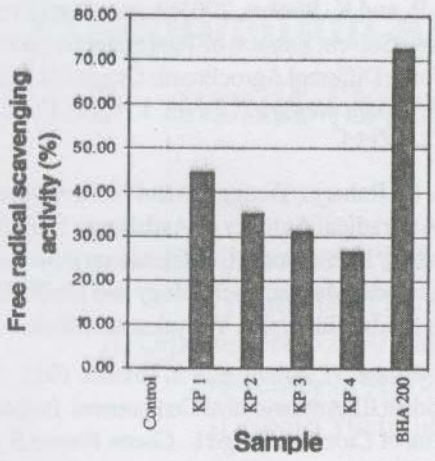

Figure 3. Free radical scavenging activity of white saffron extract KP 1, KP 2, KP 3, and KP 4 (white saffron : distilled water ratio = 1 : 1, 1 : 2, 1 : 3, and 1 : 4) BHA : Butylated Hydroxy Anisole

Control : ethanol without white saffron extract

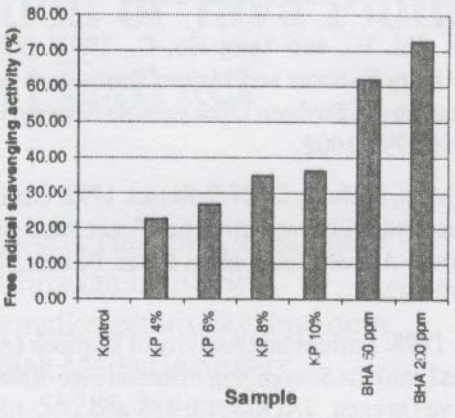

Figure 4. Percent of white saffron extract free radical scavenging activity 1 : 2 of various consentration KP 4%, KP 6%, KP 8%, KP 10% (white saffron extract of 4%, 6%, 8%, and 10% in the system) BHA : Butylated Hydroxy Anisole
Kontrol : control, ethanol without white saffron extract

White saffron extract evaluated using DPPH method had an antioxidant activity as shown in Figure 3. The antioxidant activity of white saffron extract : destilled water ratio were in the order of 1 : 1 > 1 : 2 > 1 : 3 > 1 : 4, it may be due to the curcuminoid content. It is in accordance with previous report (Majeed et al., 1995), curcuminoid had an antioxidant activity as a free radical scavenger. According to Khurana and Ho (1980), the tumeric tuber extract contains curcumin (54.6%), demetoxy curcumin (13%), and bisdemetoxy curcumin (13%) and ather compound (16.9%).

Antioxidant activity using various concentration

The sample white saffron : destilled water ratio 1 : 2 was used for further experiment were shown in Figure 4.

As shown in Figure 4, the various consentration of white saffron extract (white saffron:destilled water = 1 : 2) had significant impact on radical scavenging activity. It was caused the higher consentration of white saffron extract, the higher antioxidant activity. It may be due to a higher curcuminoid content. Culivier et al (1992) reported that the efficiency of curcumin cold be explained by increased resonance stabilization of the free radical by delocalization of the unpaired electron. It is in accordance with the previous report by Suryanto *et al* (2003) that the andaliman extract, as the concentration ranging from 200 to 900 ppm showed increasing activity on scavenging DPPH free radical. . It may be due to a higher antioxidant conteint.

CONCLUSION

In conclusion, white saffron extract exhibited antioxidant activity in the assay using the emulsion system of b-carotene linoleic acid. In the assay DPPH method white saffron extract also showed free radical scavenging activity. The higher consentration of white saffron extract, the higher antioxidant activity

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