THE EFFECT OF EXTRACTION METHODS OF WHITE SAFFRON (*Curcuma mangga* Val.) ON THE ANTIOXIDANT ACTIVITY

*Pengaruh Metode Ekstraksi terhadap Aktivitas Antioksidan Ekstrak Kunir Putih (*Curcuma mangga *Val.)*

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ABSTRACT

A study on the effect of white saffron to distilled water ratio on the antioxidant activity of white saffron extract has been conducted. White saffron rhizome was blanched in the 0.5% citric acid solution at 100C for 5 min, and grated. The grated rhizome was extracted with distilled water at the ratio of 1:1, 1:2, 1:3 and 1:4, respectively. The antioxidant activity of white saffron extract was assayed with Ferry Thyo Cyanate (FTC) and Thio Barbituric Acid (TBA) methods and compared with standard of BHA. The antioxidant activity of white saffron extract expressed as % .inhibition, and the control (without added white saffron extract) was considered as having 0 % activity. Higher decrease of antioxidant activity was found with higher ratio of white saffron to distilled water. The highest antioxidant activity of white saffron extract (FTC method 20.17% and TBA method 32.15%) was found in 1:1 ratio.

Keywords: *white saffron, antioxidant activity, extracttion method, %inhibition*

INTRODUCTION

Antioxidant is a chemical compound which is naturally existing in most of food stuff, but such compound can be decomposed and subsequently its function decreases, when materials are processed. Halliwel and Gutteridge (1985) reported that antioxidant is the substance that in low concentration is able to prevent or inhibit free radical oxidation.

Formerly, antioxidant was primarily used to preserve food stuff qualities. Since the acidity of oxidative reaction of fatty product in the food occured, antioxidant activity significantly increased. A lot of pathological conditions were caused by the presence of active oxygen consisting of superoxide, hydroxy radical, and hydrogen peroxide which function as diet antioxidant, and may be effective for preventing peroxide destruction in the biological system (Nardini *et al.*, 1995)

Antioxidant can be devided based on the solubility to the solvent. There are antioxidants that water soluble (hidrophilic), and some other antioxidants oil soluble (lipophilic). This grouping was conducted because natural antioxidant activity is different in the oil and in emulsion as the consequence of complex interfacial fenomena, effecting on the antioxidant partition in the multiphase food system. Huang *et al* (1996) and Frankel (1984) reported that the relative effectivity of lipophilic and hidrophilic antioxidant depends on lipid substances, physical condition, antioxidant concentration, oxidation time and methods used to determine lipid oxidation. They also observed, that antioxidants based on extraction method and solubility on the media either oil or water was grouped according to resource, they are syntetics antioxidant and natural antioxidant.

Commercial antioxidants are widely available in the market and are important materials, such as BHA, BHT, TBHQ, and PG which have a good stability on the various of food processing condition and gave a good stability both on the frying and roasting product. Carry through property is very important considered in the characteristic selection as an antioxidant. One of the disadvantages of sintetics antioxidant is its carsinogenic character, in the long periode and highly dosage can cause pathological effect, promote arrising malignant, and impact to reproduction (Furia and Bellanca, 1976; Chen and Tang Ho, 1992). Therefore, it is necessary to utilize the natural antioxidant materials.

Many researches on natural resources and their utilization as a natural antioxidant, such as turmeric, galingale, tomato, carrot, betel leaf rosemary, and ginger that can really promote

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oxidative stability have been published (Umi Suryanti, 1998). Study on turmeric oleoresin added to peanut oil at a temperature of 60°C showed the capability to prevent oxidation (Sukardi, 2001).

The aroma and flavour of white saffron rhizome are like ripped manggoes therefore people name it temu mangga (Fauziah, 1999). White saffron processed as a syrup has an antioxidative activity (Dwiyati, 2003) and may contain antioxidant compound, and according to Dwiyati and Sutardi (2003), it is recognized as curcuminoid compounds.

The research is conducted to obtain the appropriate distilled water to grated white saffron ratio to produce an extract with a high yield and high antioxidant activity. This research has never been done before.

The aim of the present research is to study the antioxidant activity of white saffron extract in the emulsion system by ratio variation of grated blanched white saffron rhizome to distilled water.

MATERIALS AND METHODS

White saffron rhizomes (*Curcuma mangga* Val.) were obtained from local markets in their fully mature and they have yellow colour and manggo like aroma. BHA (Butylated Hydroxy Anisole) as antioxidant standard was purchased from Sigma Chemicals (St. Louis, MO, USA). Chemical agents used were acetic acid, linoleic acid 60%, tween 20, ethanol, ammonium thyosianate, ferro chlorid, and TCA. All chemicals were AR grade and water was glass distilled water. The equipments used were centrifuge, vortex, incubator and spectrophotometer (Shimadzu UV-Vis 1601).

Extraction

White saffron rhizomes were peeled, washed and blanched in the 0.5% citric acid solution at 100°C for 5 min and grated. White saffron extract was prepared by extracting grated tubers with a 1:1, 1:2, 1:3 and 1:4 ratio of grated rhizomes to water, and finally filtered with cheese cloth. White saffron extract was ready to be used as the antioxidant source in most of the assays.

The Assay of Antioxidant Activity

The antioxidant activity of white saffron extract was measured by the modified method of Huang *et. al.* (1996b). The linoleic acid (1.0 ml) was added with 0.1 ml tween 20 and mixtured for 1 min. The white saffron extract (0.8 ml) was macerated to the mixture and stirred for 1 min. To obtain emulsion system, the mixture was added with 2.1 ml distilled water and stirred for 2 min and again 2.0 ml of distilled water was added for every 2 min while stirring until the total volume of added distilled water was 8.1 ml. The obtained emulsion system was incubated at 37°C for 10 days. The peroxide value (meq/ kg oil) was assayed by ferry thyocyanate (Mitsuda *et. al.*, 1967, Osawa and Namiki 1981), and the malonaldehyde (mg/ kg oil) was assayed by the method of thiobarbituric acid (Ottolenghi, 1959). The assays were done 0, 2, 4, 6, 8 and 10 days incubation, respectively. A 200 ppm of BHA in the absolute ethanol was used as standard.

The antioxidant activities were also calculated as % inhibition and the control (without added white saffron extract) was considered as having 0 % activity.

The antioxidant activity (% inhibition) was calculated using the following equation:

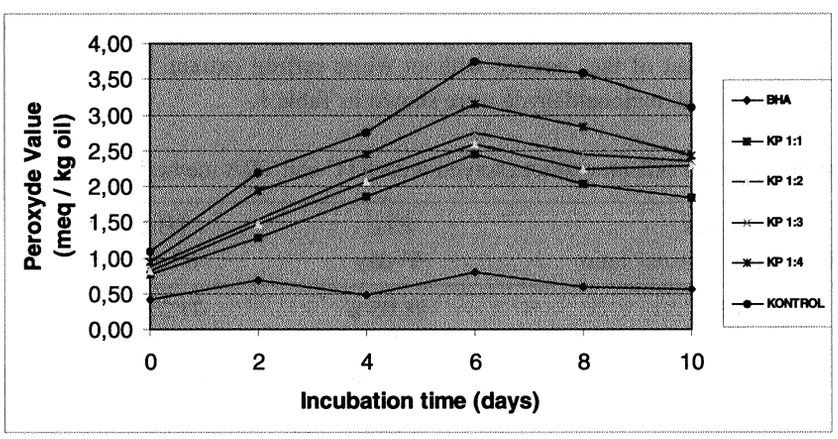

Figure 1.

Antioxidant activity of white saffron extract assayed with Ferry Thiocyanate (FTC) method
BHA : Buthylated Hidroxy Anisole (an antioxidant synthetic)
KP 1:1, KP 1:2, KP 1:3, KP 1:4 (white saffron to distilled water ratio)
Kontrol : control of linoleic acid whithout white saffron extract

$$\% \text{ inhibition} = \left(1 - \frac{(A_6 \text{ sample} - A_0 \text{ sample})}{(A_6 \text{ control} - A_0 \text{ control})}\right) \times 100\%$$

A_6 = absorbance at 6 days
A_0 = absorbance at 0 days (start)

RESULTS AND DISCUSSION

The Antioxidant Activity

Antioxidant activity assesment using ferry thyocyanate method was conducted to determine the capability of white saffron extract to inhibite formation of peroxides as a product of primarilly oxidation of linoleic acid that is present in emulsion system. The principle of analysis using ferry thyocyanate method is peroxide as oxidation product of linoleic acid reacted with ferro chloride. Ferro ion will be oxydated by peroxide to be ferry ion. Next, those ferry ions will react, forming ferry thyocyanate which have red colour. The higher red colour intensity showed the more peroxides.

Antioxidant activity determination of white saffron extract using Thiobarbituric Acid (TBA) method was based on the antioxidant activity of white saffron extract to inhibit malonaldehyde formation.

The assayed results of antioxidant activity using ferry thyocyanate (FTC) and using TBA method were presented in Figure 1 and Figure 2.

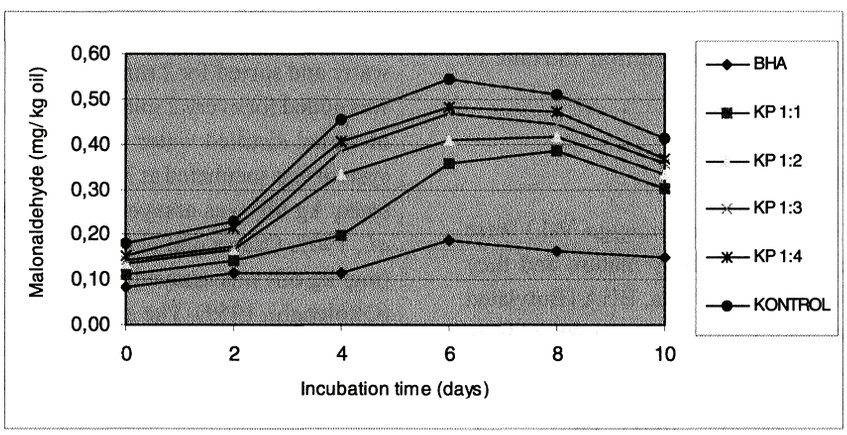

Figure 2.
Antioxidant activity of white saffron extract assayed with Thio Barbituric Acid (TBA) method
BHA : Buthylated Hidroxy Anisole (an antioxidant synthetic)
KP 1:1, KP 1:2, KP 1:3, KP 1:4 (white saffron to distilled water ratio)
Kontrol : control of linoleic acid whithout white saffron extract

White saffron extract has evidently an antioxidant activity in the emulsion system. It can be seen from the amount peroxides or malonaldehyde which is lower than that of the control without white saffron extract. The antioxidant activity presented % inhibition of formation of peroxides and malonaldehyde were shown in Table 1.

Table 1. Antioxidant activity of white saffron extract assayed with FTC and TBA method expressed as % inhibition

Samples White saffron to water ratio	FTC 6th day	TBA 6th day
BHA	88.05 a	71.22 a
1:1	20.17 ab	32.15 ab
1:2	11.16 ab	24.31 ab
1:3	5.29 b	9.92 b
1:4	3.83 b	12.46 b

Average of 2 batch, 3 replicates analysis
Different letter behind number at the same coloum showed significantly different (P£ 0.05)
Control: 0% of inhibition

The highest antioxidant activity, either assayed by FTC or TBA, occurred on white saffron extract prepared with white saffron to distilled water ratio of 1:1.

Antioxidant activity of white saffron extract with the ratio of white saffron rhizome to distilled water (1:1 and 1:2) was not significantly different (P≤ 0.05) compared to BHA. Turmeric rhizome extract contained several compounds such as curcumin 54.5%, demetoxy curcumin 13%, and bisdemetoxy curcumin 13% and other compounds 16.9% (Khurana and Ho, 1980). According to Majeed *et al* (1995), curcuminoid as individual had antioxidant activity, but natural complex curcuminoid containing all of three types of curcumin had the highest antioxidant activity compared to both each curcumin and BHT, when it was determined by Rancimat method. Whereas, white saffron extract : with ratio of grated rhizome to distilled water (1:3 and 1:4) was significantly different compared with BHA.

Evidence showed that white saffron extract has an antioxidant activity in the emulsion system when assayed with TBA method. This result was seen from malonaldehyde formation that lower than control (Figure 2). Antioxidant activities of white saffron extract (white saffron to distilled water ratio of 1 : 1 and 1 : 2) were not significantly different (P£0.05) compared to BHA. This might be due to the presence of curcuminoid 176.07 ppm in the white saffron (Dwiyati and Sutardi, 2003). Whereas, white saffron extract (white saffron to distilled water ratio of 1 : 3 and 1 : 4) was significantly different (P£0.05) compared with BHA, that was lower than BHA. It might be caused by the lower concentration of white saffron extract, and subsequently has lower antioxidant activity. It may be due to the lower curcuminoid content.

CONCLUSIONS

Higher decrease of antioxidant activity was found with higher ratio of white saffron to distilled water. The antioxidant activity of white saffron extract assayed with FTC method was 3.83% to 20.17%, while assayed TBA method was 9.92% to 32.15 %. The highest antioxidant activity of white saffron extract (FTC method 20.17% and TBA method 32.15%) was found in 1:1 ratio of grated white saffron rhizome to distilled water.

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