

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.)

Dwiyati Pujimulyani¹⁾, Agung Wazyka¹⁾, Sri Anggrahini²⁾, and Umar Santoso²⁾

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, extraction 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. Halliwell 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 occurred, 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 divided based on the solubility to the solvent. There are antioxidants that water soluble (hydrophilic), 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 phenomena, effecting on the antioxidant partition in the multiphase food system. Huang *et*

al (1996) and Frankel (1984) reported that the relative effectiveness of lipophilic and hydrophilic 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 synthetic 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 synthetic antioxidant is its carcinogenic character, in the long period and highly dosage can cause pathological effect, promote arising 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, galangale, tomato, carrot, betel leaf rosemary, and ginger that can really promote

¹⁾ Fakultas Teknologi Pertanian, Universitas Wangsa Manggala, Yogyakarta, Indonesia, Jl Wates km 10 Argomulyo, Yogyakarta 55753

²⁾ Fakultas Teknologi Pertanian, Universitas Gadjah Mada, Jl. Sosio Yustisia, Bulaksumur, Yogyakarta 55281.

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 mangoes 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 mango 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 thiosyanate, 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 mixed 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 ferric thiocyanate (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:

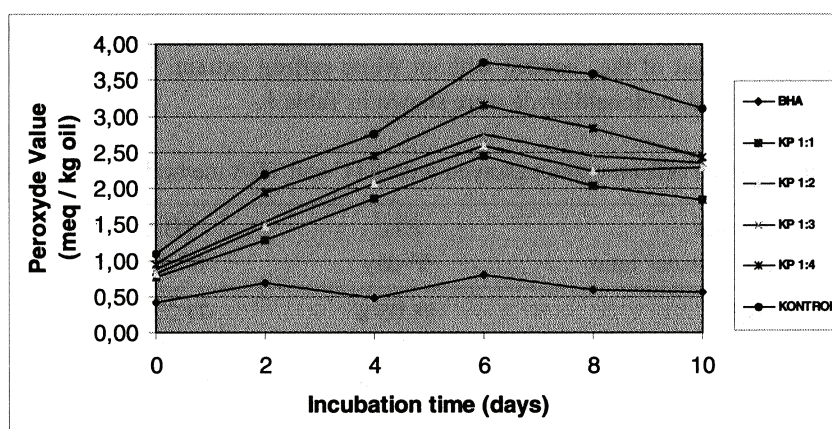


Figure 1.

Antioxidant activity of white saffron extract assayed with Ferric Thiocyanate (FTC) method

BHA : Butylated Hydroxy 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 without white saffron extract

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

A₆ = absorbance at 6 days

A₀ = absorbance at 0 days (start)

RESULTS AND DISCUSSION

The Antioxidant Activity

Antioxidant activity assesment using ferry thiocyanate 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 thiocyanate 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 thiocyanate 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 thiocyanate (FTC) and using TBA method were presented in Figure 1 and Figure 2.

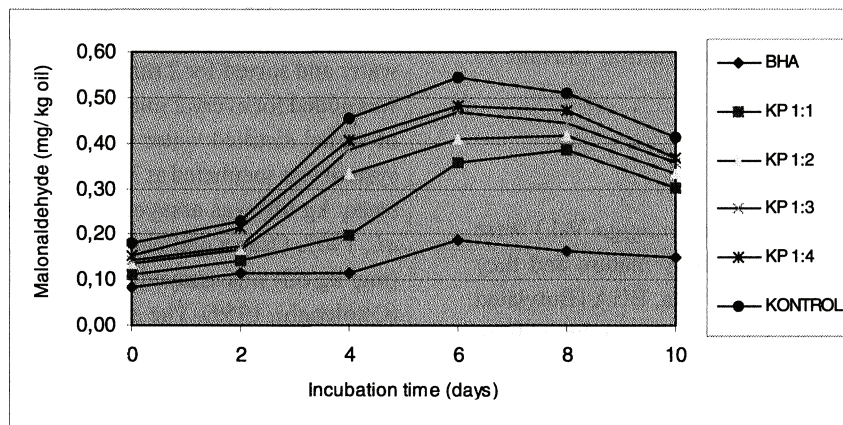


Figure 2.

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	FTC	TBA
White saffron to water ratio	6 th day	6 th 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 colourum 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 ($P \leq 0.05$) compared to BHA. Turmeric rhizome extract contained several compounds such as curcumin 54.5%, demetoxo curcumin 13%, and bisdemetoxo 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 ($P \leq 0.05$) 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 ($P \leq 0.05$) 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.

ACKNOWLEDGMENT

The authors wish to thank to the Directorate General of Higher Education (DGHE) Ministry of National Education of Republic of Indonesia, for providing fund for this study through HIBAH PEKERTI II/I in 2004.

REFERENCES

- Chen, W., H., and Tang Ho, C., 1992. *Effect of Rosemary Extract and Major Constituents on Lipid Oxidation and Soybean Lipoxigenase Activity*, JAOCS, 69 (10) : 999-1002.
- Dwiyati P., 2003. *The Effect of Blanching on Antioxidant Properties Of White Saffron Syrup (Curcuma mangga Val)* Agritech, vol 23, No 3 D; 137-141.
- Dwiyati P. and Sutardi, 2003. *Curcuminoid Content and Antioxidative Properties on White Saffron Extract (Curcuma mangga Val)*, *Proceeding International Conference on redesigning Sustainable Development on Food and Agricultural System for Developing Countries. Fac. of Agric. Tech., Gadjah Mada University.*
- Fauziah, 1999. *Temu-temuan dan Empon-empon, Budidaya dan Manfaatnya*. Kanisius. Yogyakarta.
- Frankel, E.N., 1984. *Lipid Oxidation: Mechanism Products and Biological Significance*, J.of Am Oil Chemist. Doc. 61:12.
- Furia, T.E.. and Bellanca, N., 1976. *Development of New Non Abdorbbable Polymery Antioxidant for Use in Food*. JAOCS, 53;132-137.
- Halliwell, B and Gutteridge, J.M.C, 1985. *Free Radicals in Biology and Medicine* Clarendon Press, Oxford.
- Huang, S.W., E.N. Frankel, K. Schwarz, and J.B. German. 1996a. *Effect of pH on Antioxidant Activity of a-tocopherol and Trolox in Oil-in-water Emulsions*. J. Agric. Food Chem. 44, 2496-2502.
- Huang, S.W., E.N. Frankel, K. Schwarz, and J.B. German. 1996b. *Antioxidant Activity of Carnosic Acid and Methyl Carnosate in Bulk Oils and Oil-in-water Emulsions*. J.Agric.Food Chem. 44, 2951-2956.
- Khurana, A.L. and C.T. Ho, 1980. *High Performance Liquid Chromatography Analysis of Curcuminoid and Their Photooxidative Decomposition Compounds in Curcuma longa L.* J. Liquid Chrom., 11:229-230.
- Kikuzaki, H. and N. Nakatani, 1993. *Antioxidant Effects of Some Ginger Constituents*. J. Food Sci. 58 : 1407-1410.
- Majeed, M., Vladimir B, Uma S and R. Rajendran, 1995. *Curcuminoids Antioxidant Phytonutriens*. Nutriscience. Publ. Inc. Piscataway. New Jersey.
- Mitsuda, H., Yasumoto, K. and Iwami, K. 1967. *Antioxidative Action of Indole Compounds During the Autoxidation of Linoleic Acid* Eiyo to Shokuryou 19 (3)210.

- Osawa.T. and Namiki, M. 1981. *A Novel Type of Antioxidant Isolated from Leaf Wax of Eucalyptus Leaves*.Agric. Biol.Chem. 45 : 753.
- Nardini, M., D. Aquino, M. Tomassi, G., Gentili, Di Felice, M. and Seaccini, C., 1995. *Dietary Fish Oil Enhances Plasma and LDL Oxidative Medification in Rats*, J. Nutr. Biochem, 6, 474-478.
- Ottolenghi, A. 1959. *Interaction of Accorbic Acid and Mitochondrial Lipid* Arch. Biochem. Biophys. 79:355.
- Sukardi, 2001. *Pengaruh Waktu Pemanasan dan Kombinasi Ekstrak Jahe, Kunyit, Temulawak dan Kencur Terhadap Aktivitas Antioksidan*, (Thesis), Faculty of Agricultural Technology, Gadjah Mada University.
- Umi Suryanti, 1994. *Kajian Fraksi Oleoresin Jahe sebagai Antioksidan Alami pada Minyak Kacang*. UGM Yogyakarta.
- Umar Santosa, 1996, *Nutritional Studies on the Coconut (Cocos nucifera L.) Water*, (Disertation), Tokyo University of Agriculture, Tokyo. Japan.