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Extraction and characterization of antioxidant from cocoa by-products

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Abstract

Antioxidative properties of extracts from six types of cocoa by-products have been studied. Seven different solvents differing in polarity were used to extract the antioxidants from cocoa by-products. Out of 42 different extracts tested, twelve extracts from cocoa powder, natural cocoa powder, cocoa nib and cocoa shell exhibited strong antioxidant activities using a diene conjugation formation method. The characterization studies done indicated that increasing extract concentration resulted in increased antioxidant activities at all the levels studied, namely 1000, 2000 and 3000 ppm. Methanol proved to be the best solvent in extracting antioxidants from cocoa by-products, followed by mixtures of chloroform, ether and dichloroethane and mixtures of chloroform, methanol and dichloroethane. The antioxidative activities of the extracts were found to be stable at temperatures below 50°C and exhibited highest activity at neutral and alkaline pH. It appears that flavonoid compounds may be responsible for the antioxidative activities in cocoa by-products and complete elucidation of the active compounds is in progress.

Keywords: Cocoa by-products; Antioxidant; Natural antioxidant; Cocoa powder

1. Introduction

Consumers all over the world are becoming more conscious of the nutritional value and safety of their food and its ingredients. At the same time, there is a preference for natural foods and food ingredients that are believed to be safer, healthier and less subject to hazards than their artificial counterparts (Farag, Badei, Heweij, & El-Baroty, 1986). In addition, it has been reported that dietary administration of synthetic antioxidants like BHT (butylated hydroxytoluene) to rats can result in fatal hemorrhages. Evaluation of antioxidative activity of naturally occurring substances has been of interest in recent years. However, the use of natural antioxidants is limited by a lack of knowledge about their molecular composition, amount of active ingredients in the source material and the availability of relevant toxicity data (Shahidi, Wanasundara, & Amarowicj, 1994). Therefore, the aim of this project is to screen for antioxidative activities of cocoa by-products and examine factors involved in modulating these activities.

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2. Materials and methods

Three types of cocoa by-products were used in this study, namely cocoa nib, cocoa powder and cocoa shell. Three types of cocoa powder (natural cocoa powder, alkalized cocoa powder and alkalized red cocoa powder) were obtained from Kokomal, Teluk Intan, Perak, Malaysia. Other samples were obtained from Guan Chong Cocoa Manufacturer in Pasir Gudang, Johor, Malaysia.

2.1. Extraction of antioxidants

Antioxidants were extracted using a Soxhlet extractor utilizing seven different types of solvents. (Mehta, Zayas, & Yang, 1994). The ground samples were weighed into thimbles (25×100 mm) and extracted with 250-ml solvents for 16 h. After air drying for 3 h, the residues of petroleum ether extract were reextracted with the solvent combinations as shown in Fig. 1. The solvent was then removed using a rotary evaporator and kept in air-tight amber bottles until analyzed. The bottles were flushed with nitrogen for 30 s prior to storage in a freezer at −18°C.

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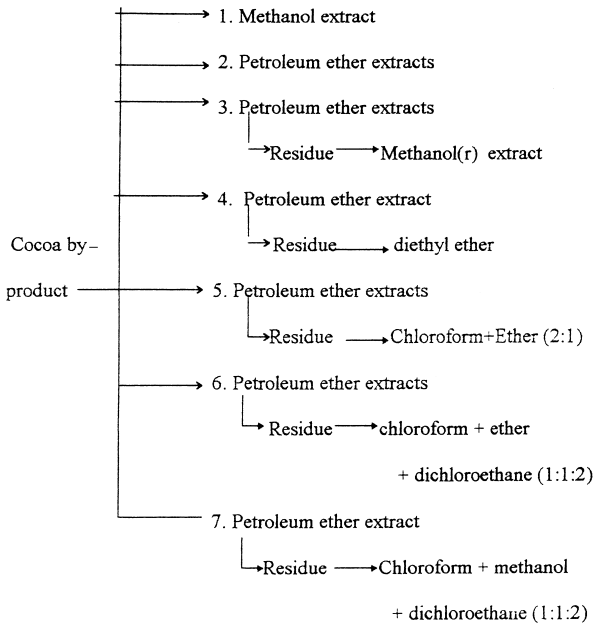

Fig. 1. Scheme for extraction of antioxidants from cocoa by-product.

2.2. Determination of antioxidant activity of the extracts

Antioxidant activities were determined using a diene conjugation formation method (Lingnert, Vallentin, & Eriksson, 1979). The substrate consisted of 10 mM of linoleic acid emulsified with an equal amount of Tween 20 in buffer, pH 7.0. The mixture was then homogenized at high speed for 1 min. 10 μl of extracts were mixed with 5 ml emulsion and incubated at 50°C for 20 h. Absorbance was then measured at 234 nm. Samples were measured in triplicate. Four extracts with the highest antioxidative activity were selected for characterization study at various pH values and temperatures.

2.3. Effect of pH and temperature

The effects of pH and temperature on the antioxidative activities of the extracts were also determined. Phosphate–HCl buffer was used for pH 3 and 5, while phosphate–NaOH buffer was used for pH 7, 9 and 11. The range of temperature used was 30 to 90°C. All experiments were carried out in triplicate. Statistical analyses were accomplished using Statistical Analysis System software utilizing Duncan's Multiple comparison test. Level of significance used was at $p = 0.05$.

3. Results and discussion

Out of 42 extracts from six cocoa by-products that were tested, 12 extracts from three by-products exhibited high antioxidative activities. Methanol and methanol-residue extracts (residue of petroleum ether extract) showed the highest activity. This is in agreement with Yen, Wing, and Duh (1996) who said that methanol is a widely used and effective solvent for extraction of antioxidant. Four samples exhibited strong antioxidative activities, namely cocoa powder, cocoa nib, cocoa shell and cocoa powder. On the other hand, alkalized cocoa powder and alkalized red cocoa powder showed very little antioxidative activities. The same findings was reported by Ziegleder and Sandmeir (1982) who noticed that the antioxidant activity of cocoa products was reduced by 30 to 50% with alkali treatment.

3.1. Effect of increasing concentration of extract

Methanol, methanol-residue (residue of petroleum ether reextracted with methanol), chloroform–methanol–dichloroethane (CMD) and chloroform–ether–dichloroethane (CED) extract were used in the characterization study. The effects of concentration of these extracts on the oxidation of linoleic acid are shown in Fig. 2. Increasing the concentration of the extracts up to 2000 ppm resulted in an increase in antioxidative activities and increasing concentration of extract thereafter had no effect on antioxidative activities. The pattern was observed for all the extracts except for that of CMD where the activities increased significantly for all concentrations studied. This result is similar to that of Lee et al. (1986) who demonstrated that antioxidative activities increased when concentration of ginger extract was increased. In all the extracts investigated, the methanol (residue) extract appeared to have the highest and most stable antioxidative activity.

3.2. Effect of temperatures and pH

The antioxidative activities of extracts incubated at 30, 40, 50, 70 and 90°C (at concentration 3000 ppm) are shown in Fig. 3. The extracts were stable up to 50°C, after which the antioxidant activities started to decrease significantly for all samples tested. Extracts from methanol (residue) proved to be most stable while that from a mixture of chloroform, methanol and dichloroethane exhibited the least antioxidative activities. The result suggests that when foods are heated at temperatures normally encountered during boiling or other cooking methods ($t > 50$°C), antioxidative activities would be expected to be destroyed.

The influence of pH on the stability of the extracts can be seen in Fig. 4. The antioxidative activity of the different extracts increases with increasing pH from 3 to 11, indicating strong dependence on the pH of the system. This is contrary to the study done by Yen (1993) who reported that a methanol extract from peanut hulls showed high antioxidative activities at neutral and acidic pH. This may be due to the different samples used and different compounds being extracted in the two cases.

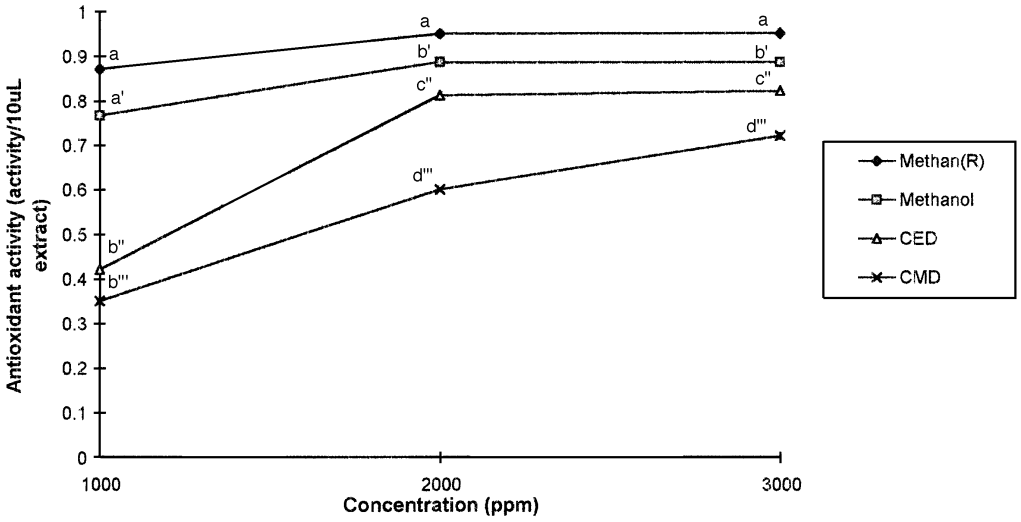

Fig. 2. Effect of concentration of cocoa by-product extract on antioxidant activity (at 40°C in buffer pH 7). CED: mixture of chloroform, ether and dichloroethane (1:1:2). CMD: mixture of chforoform, methanol and dichloroethane (1:1:2). a,b,c,d,e: values with the same letter are not significantly different ($p > 0.05$).

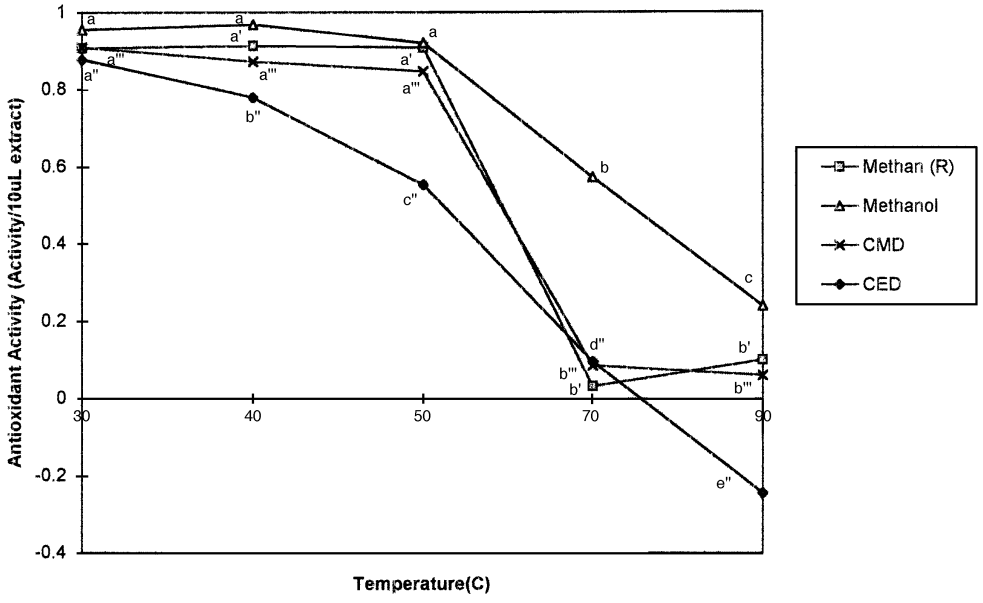

Fig. 3. Effect of incubation temperature on antioxidant activity (AOA) for different extracts. AOA values represent means of three replications at 3000 ppm at pH 7. a,b,c,d,e: values with the same letter are not significantly different ($p > 0.05$). CED: mixture of chloroform, ether and dichloroethane (1:1:2). CMD: mixture of chloroform, methanol and dichloroethane (1:1:2).

Fig. 4 shows the antioxidant activity of methanol (residue) extract at different pHs and temperatures. The study shows that the antioxidant is stable at all temperatures (except at 90°C) and is stable at all pHs tested. This indicated that the active component in the extract might be flavonoid in nature. Flavonoids have been reported to function well in th pH range of 7 to 10 (Jovanovic, Steenken, Mihajlo, Marjanovic, & Simic, 1994). In addition, other researchers have reported that flavonoids exhibited high antioxidant activity at 25°C (Nieto et al., 1993). Furthermore, Swan (1979) reported that cocoa had a high content of flavonoid compounds. Complete elucidation of the compounds is now underway.

In conclusion, methanol proved to be the best solvent for extracting antioxidant from cocoa by-products. The compounds responsible for antioxidative activities were detected in cocoa shell, cocoa nibs, natural cocoa powder and cocoa powder. Increasing the concentration of extract up to 2000 ppm increased the antioxidative activities for all the extracts studied except CMD extract, where it was found that the activities increased significantly at all concentrations tested. Characterization also revealed that the antioxidant activities

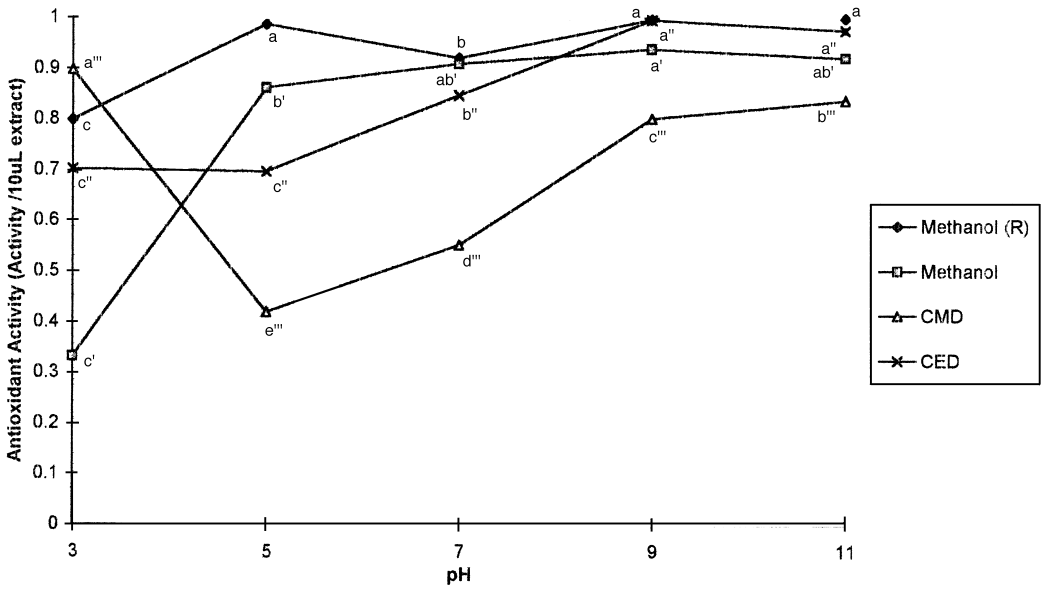

Fig. 4. Effect of pH on the andoxidant activity (AOA) of different extracts. AOA values represent means of three replications at 3000 ppm at pH 7. a,b,c,d,e: values with the same letter are not significantly different ($p > 0.05$). CED: mixture of chloroform, ether and dichloroethane (1:1:2). CMD: mixture of chloroform, methanol and dichloroethane (1:1:2).

increased with increasing pH and were stable at temperatures below 50°C.

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References

Farag, R. S., Badei, A. Z., Heweij, F. M., & El-Baroty, G. S. A. (1986). Antioxidant activity of some spices essential oils on linoleic acid oxidation in aqueous media. *Journal of the American Oil Chemists Society, 66*, 792–799.

Jovanovic, S. V., Steenken, S., Mihajlo, T., Marjanovic, B., & Simic, M. G. (1994). Flavonoids as antioxidant. *Journal of the American Chemical Society, 116*, 4846–4851.

Lee, Y. B., Kim, Y. S., & Ashmore, C. R. (1986). Antioxidant properties in ginger rhizome and its application to meat products. *Journal of Food Science, 51*, 20–23.

Lingnert, H., Vallentin, K., & Eriksson, C. E. (1979). Measurement of antioxidative in model system. *Journal of Food Processing and Preservation, 3*, 87–103.

Mehta, R. L., Zayas, J. F., & Yang, S. S. (1994). Ajowan as a source of natural lipid antioxidant. *Journal of Agricultural and Food Chemistry, 42*, 1420–1422.

Nieto, S., Garrido, A., Sanhueza, J., Loyola, L. A., Morales, Glauco, Leighton, F., & Valenzuela, A. (1993). Flavonoids as stabilizers of fish oil: an alternative to synthetic antioxidant. *Journal of the American Oil Chemists Society, 70*, 773-778.

Shahidi, F., Wanasundara, U. N., & Amarowicj, R. (1994). Natural antioxidant from low pungency mustard flour. *Food Research International, 27*, 489–493.

Swan, T. (1979) Economic importance of flavonoid compounds: foodstuff. In T. A. Geissman (Ed.), The chemistry of flavonoid compounds (pp. 513–549). New York: Macmillan.

Yen, G. C., & Duh, P. D. (1993). Antioxidant properties of methanolic extracts from peanut hulls. *Journal of the American Oil Chemists Society, 70*, 383–386.

Yen, G. C., Wing, S. C., & Duh, P. D. (1996). Extraction and identification of antioxidant components from the leaves of mulbery (*Morus alba* C). *Journal of Agricultural and Food Chemistry, 44*, 1687–1690.

Ziegleder, G., & Sandmeir, D. (1982). Antioxidant action of cocoa. *Zucerund Suesswarenwirtschchaft, 35*, 217–218, 220–222