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Research Article

Extraction and free radical scavenging activity of crude carotenoids from palm oil meal

Worawan Panpipat* and Manat Chaijan

Department of Food Technology, School of Agricultural Technology, Walailak University, Thasala, Nakhon Si Thammarat, 80160, Thailand.

*Email: pworawan@wu.ac.th

Abstract

The extractability of crude carotenoids from palm oil meal with different solvent mixtures and their free radical scavenging activity were investigated The recovery of crude extract was 6.89, 5.87, 5.78 and 1.70% when extracted by using acetone:hexane (2:3, v/v), ethanol:hexane (4:3,v/v), acetone:ethanol:hexane (1:1:2, v/v) and 10% alcoholic KOH, respectively. The extracts of 10% alcoholic KOH exhibited the highest carotenoid content ($p<0.05$). The lowest yield of crude extract with the highest carotenoid content was found in the extract of 10% alcoholic KOH indicating the lowest impurity. Therefore, the optimum solvent used for carotenoid extraction from palm oil meal was 10% alcoholic KOH. The major carotenoids constituted in the extract were alpha-carotene and beta-carotene. DPPH° scavenging activity of crude carotenoid extract slightly increased with increasing crude carotenoid extract concentration ranging from 50 to 200 ppm. Crude carotenoid extract at a concentration of 200 ppm was found to have the highest inhibitory activity against DPPH°. However the decrease in radical scavenging activity was observed with increasing crude carotenoid extract concentration (250-500 ppm) indicating the pro-oxidative effect of carotenoids at high concentration.

Keywords: extraction, solvent, KOH, carotenoid, palm oil meal, Thailand.

Introduction

Carotenoids are organic pigments that are naturally occurring in the chloroplasts and chromoplasts of plants and some other photosynthetic organisms like algae, some types of fungus and some bacteria [1]. The best-documented function of carotenoids is their provitamin A activity, especially that of β-carotene and to a lesser extent of β-cryptoxanthin and lutein [2]. In addition, carotenoids are efficient free-radical scavengers and they enhance the vertebrate immune system. Epidemiological studies have shown that people with high β-carotene intake and high plasma levels of β-carotene have a significantly reduced risk of lung cancer [3]. There are many origins of carotenoid in which palm fruit is one of a rich source of carotenoid [3, 4]. After the oil extracting process, palm oil meal by-product might contain some carotenoid residues. Therefore, the

utilization of this waste for carotenoid extraction was a crucial means. As a consequence, the extractability of crude carotenoids from palm oil meal with different solvent mixtures and their free radical scavenging activity were investigated.

Materials and Methods

Crude carotenoid extraction

Ground dried palm oil meals containing 4.73% moisture, 4.45% ash, 5.06% crude fat and 59.01% crude fibre were kindly obtained from Kanchanadit Palm Oil Co., Ltd. (Kanchanadit, Surat Thani, Thailand). Carotenoid extraction from palm oil meal was carried out using different solvent mixtures including 10% alcoholic KOH [5], ethanol:hexane (4:3,v/v) [6], acetone:hexane (2:3, v/v) [7], and acetone:ethanol:hexane (1:1:2, v/v) [2], with a palm oil meal/extracting solvent ratio of 1:2.5 (w/v). The percentage recovery of the crude extracts was determined gravimetrically after solvent removal by using a rotary evaporator. The extracts were dissolved in petroleum ether to obtain the final volume of 250 ml and then a spectrophotometer was used to determine the amount of total carotenoids. The suitable extracting media was selected for further study. Carotenoid constitutes were measured by using a thin layer chromatography (TLC) as described by Farombi and Britton [4]. Additionally, the effect of crude carotenoid extract concentration (50-500 ppm) on free radical scavenging activity was also analyzed by using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH°) method [8].

Total carotenoid content determination

The total content of carotenoid was spectrophotometrically determined at 450 nm according to the method of Ren and Zhang [1], with slight modification. Dried crude carotenoid extract (about 1 g) was dissolved in 250 ml of petroleum ether and subjected to absorbance measurement. The total carotenoid content was calculated by using $A^{1\%}_{1\ cm} = 2{,}500$ and expressed as mg/ g sample.

DPPH radical-scavenging activity determination

Concentration effect of crude carotenoids (50-500 ppm) extracted from plam oil meal with 10% alcoholic KOH on DPPH radical-scavenging activity was determined according to the method of Yen and Hsieh [8], with slight modification. To 0.1 ml of crude carotenoid extract solution, 3.9 ml of 0.25 mg/l of DPPH in methanol was added. The mixture was then mixed vigorously and subjected to measuring the absorbance at 515 nm using a UV-1601 spectrophotometer (A_0). After incubation at room temperature in the dark for 45 min, the absorbance of mixtures was read again at 515 nm (A_{45}). DPPH radical-scavenging activity was calculated in term of DPPH radical remaining as follows:

$$\text{DPPH radical remaining (\%)} = (A_{45}/A_0) \times 100$$

Statistical analysis

Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple-range test [9]. Statistical analysis was performed using the Statistical Package for Social Science (SPSS 8.0 for windows, SPSS Inc., Chicago, IL).

Results and Discussion

Recovery of crude carotenoid extract, total carotenoid content and carotenoid components in crude extract

The highest recovery of crude carotenoid extract was found in the extract of acetone:hexane (2:3, v/v), whereas the extract of 10% alcoholic KOH was found to be the lowest ($p<0.05$; Fig. 1). The

highest recovery did not represent the highest carotenoid content as indicated in Figure 2. The 10% alcoholic KOH exhibited the highest carotenoid content ($p<0.05$; Fig. 2). The lowest recovery of crude extract with the highest carotenoid content was found in the extract of 10% alcoholic KOH indicating the lowest impurity. From the results, it was noted that the highest purity of crude carotenoid extract might be obtained when the extraction was done with 10% alcoholic KOH. Therefore, the optimum solvent used for carotenoid extraction from palm oil meal was 10% alcoholic KOH.

The major carotenoid found in the extract of 10% alcoholic KOH from palm oil meal was alpha-carotene and beta-carotene (Fig. 3). The result was in agreement with Kiokias and Gordon [3] and Forombi and Britton [4], who reported that beta-carotene and alpha-carotene were the major components found in palm oil which constituted about 80-90% of total carotenoids.

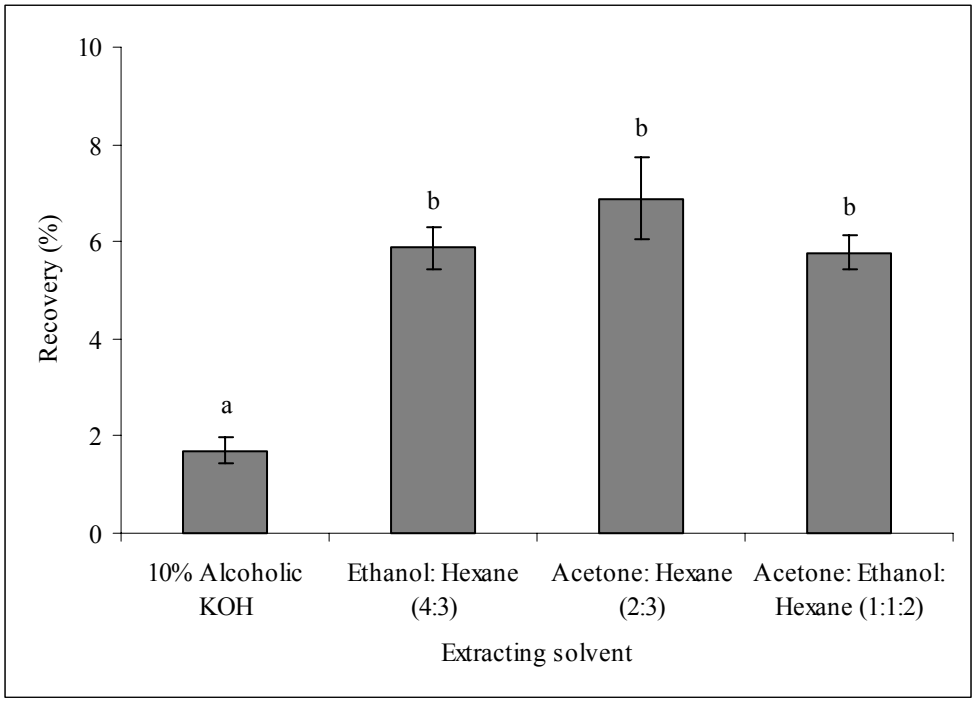

Figure 1. Effect of extracting solvent on recovery (%) of crude extracts from palm oil meal.
Values are given as mean ± SD from triplicate determinations. Different letters indicate significant differences ($p<0.05$).

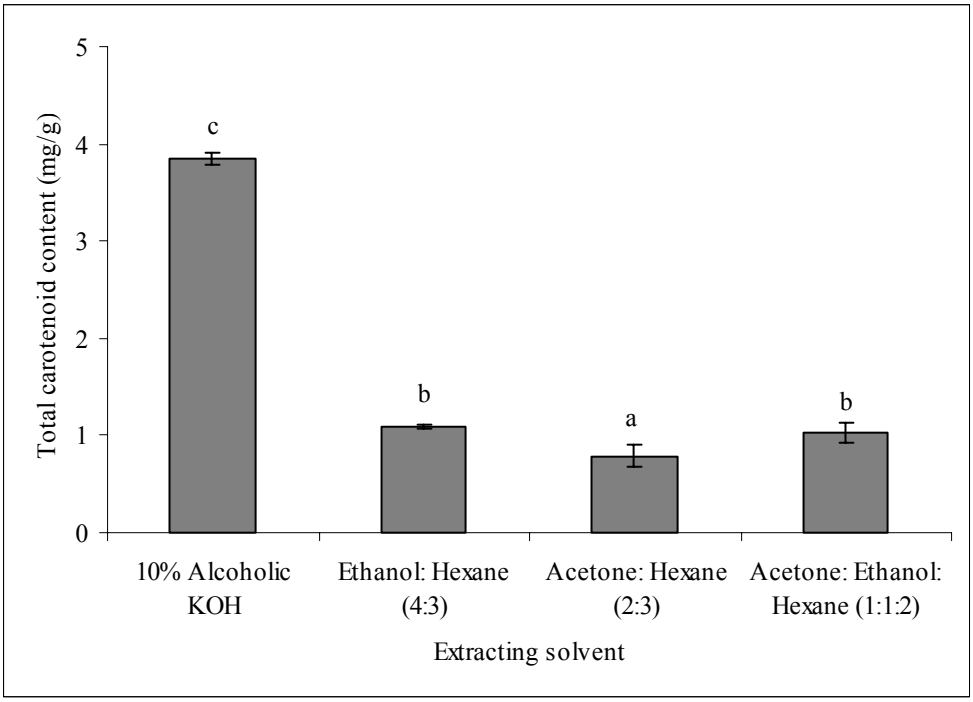

Figure 2. Effect of extracting solvent on total carotenoid content of crude carotenoid extracts recovered from palm oil meal.

Values are given as mean ± SD from triplicate determinations. Different letters indicate significant differences ($p<0.05$).

Figure 3. Thin layer chromatogram of components separated from crude carotenoid extract recovered from palm oil meal with 10% alcoholic KOH.

A =alpha-carotene (Rf =0.96), B = beta-carotene (Rf =0.94).

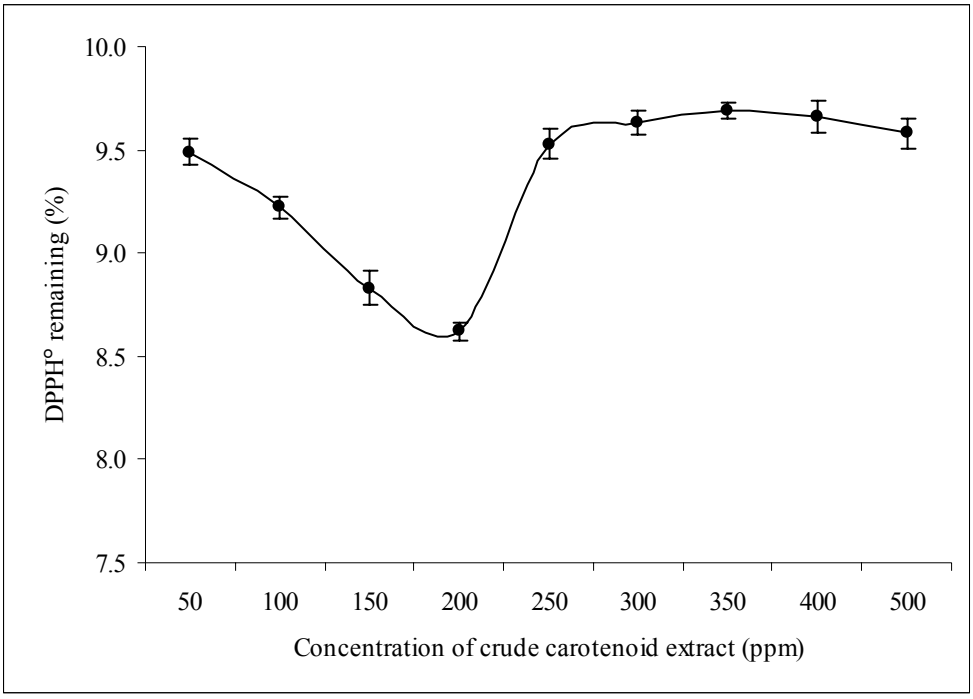

Figure 4. DPPH radical-scavenging activity of different concentrations of crude carotenoid extract recovered from palm oil meal with 10% alcoholic KOH.
Values are given as mean ± SD from triplicate determinations.

DPPH radical-scavenging activity of crude carotenoid extracts recovered from palm oil meal with 10% alcoholic KOH

DPPH° scavenging activity of palm oil meal crude carotenoid extract was concentration dependent (Fig. 4). From the result, DPPH° scavenging activity of crude carotenoid extract slightly increased with increasing crude carotenoid extract concentration ranging from 0 to 200 ppm as indicated by a gradual decrease in remaining DPPH° (Fig. 4). The result suggested that crude carotenoid extract at a concentration of 200 ppm was found to have the highest inhibitory activity against DPPH°. However, the decrease in radical scavenging activity was observed with increasing crude carotenoid extract concentration (250-500 ppm) indicating the pro-oxidative effect of carotenoids at high concentration. This result was in accordance with Kiokias and Gordon [3], who found the autooxidaiton of carotenoids at a high concentration *in vitro*.

Conclusion

The 10% alcoholic KOH was a suitable extracting media for carotenoid recovery from palm oil meal. The extract was composed of two major components including alpha-carotene and beta-carotene. Crude carotenoid extract exhibited free radical scavenging activity, depending on its concentration.

Acknowledgments

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