PRODUCTION OF CAROTENOIDS AND TOCOLS CONCENTRATES FROM PALM OIL USING SUPERCRITICAL CARBON DIOXIDE

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ABSTRACT

A method using supercritical carbon dioxide to produce concentrated carotenoids and tocols from palm oil is described. The free fatty acids of crude palm oil were esterified by acid-catalysis followed by transesterification of the triglycerides into methyl esters. Methyl esters are considerably more soluble in supercritical carbon dioxide than the triglycerides and free fatty acids, and are preferentially dissolved leaving a solution of highly concentrated carotenoids (20.11 wt%) and tocols (10.66 wt%).

Keywords: carbon dioxide, carotenoids, methyl esters, palm oil, tocols.

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INTRODUCTION

Palm oil (derived from oil palm species *Elaeis guineensis*) is one of the major oils consumed worldwide. It is >90% triglycerides, 2%-7% diglycerides, <0.1% monoglycerides, 3%-5% free fatty acids and 1% minor components. The minor components include phytonutrients of nutritional attributes such as carotenoids (500-700 ppm) and tocols (600-1000 ppm) (Goh *et al.*, 1985). Carotenoids are important as the precursors of vitamin A and act as antioxidants by quenching singlet oxygen and scavenging free radicals. Tocols (tocopherols and tocotrienols) are also potent antioxidants that neutralize excess free radicals, exhibit vitamin E activity and cholesterol-lowering properties.

The commercially available carotenoids, mainly β-carotene supplements are produced by synthetic means. The synthetic carotenoids especially β-carotene taken as a supplementation is unlike natural carotenoids which is a mixture of carotenoids compounds, many of which may not be readily metabolized rapidly. Commercially available tocols are mainly obtained from soyabean deodorizer distillates.

Supercritical fluid extraction is a more environmental-friendly process as it uses a milder temperature, preserving the valuable but thermally labile components such as the carotenoids. Carbon dioxide can be used as the solvent – it is inert, non-toxic, non-inflammable and recyclable. Also, as a gas at room temperature, it leaves no residue in the products.

In view of the importance of carotenoids and tocols, effort is made in the present research to produce carotenoids and tocols directly from crude palm oil (CPO) using supercritical carbon dioxide and without any usage of organics chemicals and solvents.

EXPERIMENTAL

Materials

CPO was obtained from the MPOB Palm Oil Mill Technology Centre in Labu, Negeri Sembilan, Malaysia. All the solvents and chemicals used were of analytical or chromatographic grade, purchased from Merck (Darmstadt, Germany). Carbon dioxide of 99.995% purity was purchased from Malaysian Oxygen Berhad (Selangor, Malaysia).

Esterification/Transesterification

The free fatty acids and triglycerides in CPO were esterified and transesterified, respectively, to methyl

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esters using the method of Choo *et al.* (1988). The CPO (1 kg) was weighed into a 2-litre three-necked round bottom flask, and methanol (500 ml) added. Sodium hydroxide (5.5 g) was dissolved in the methanol. The mixture was refluxed at 60°C –70°C for 1 hr, then the glycerol layer separated and the methyl esters washed with hot water until neutral. The methyl esters were dried with anhydrous sodium sulphate and subsequently pumped dry, then subjected to supercritical carbon dioxide extraction.

Supercritical Carbon Dioxide Extraction

Figure 1 is the schematic diagram of the extraction system used. The extraction was carried out in a dynamic (flow through) system. It is assumed that a steady-state solute-solvent equilibrium is achieved as the supercritical fluid passes over the solute. The CPO methyl esters (20 g) was weighed accurately and loaded into a 50 ml high pressure extraction vessel placed in an oven (Model CO-960, Jasco, Tokyo, Japan) to control the operating temperature to 40.0 ± 0.1°C. A back pressure regulator (Model 880-81, Jasco, Tokyo, Japan) was used to control the operating pressure to 10.0 ± 0.1 MPa. Liquid carbon dioxide was pumped continuously at a constant flow of 5.0 ml min^{-1}, measured at a HPLC pump (Model PU-986, Jasco, Tokyo, Japan), into the vessel under the specific extraction conditions. A frit was installed at the outlet of the extraction vessel to prevent any physical carry over of the methyl esters. The extracts were collected at the outlet of the back pressure regulator every hour. The volumes were weighed, blanketed with nitrogen and kept in the dark at –10°C prior to analysis to prevent any degradation by heat, air and light.

Quantification of Carotenoids

The carotenoids content was determined using MPOB Test Method p2.6 (2005). The off-line approach was used to prevent overloading of the carotenoids from excessive concentrations. Each fraction of the sample extracted (0.1 g) was homogenized and weighed to the nearest ± 0.0001 g into a 25 ml volumetric flask. The sample was dissolved in *n*-hexane and diluted to the mark. The solution was transferred into a 1 cm quartz cuvette and the absorbance measured at 446 nm against *n*-hexane using a spectrophotometer (Hitachi U-2000, Japan). The total carotenoids content was expressed as ppm β-carotene.

Quantification of Tocols

Quantification of the individual tocols by HPLC was done as described by Puah *et al.* (2007a). Each fraction of the sample extracted (0.1 g) was homogenized and weighed accurately into a 25 ml volumetric flask, then dissolved in *n*-hexane and diluted to the mark. The HPLC system was an Agilent 1100 Series model (Agilent Technologies, USA) complete with quaternary pump, fluorescent detector and standard auto-sampler. A Zorbax SIL normal phase silica column, 150 mm x 4.6 mm i.d. (5 μm) (Agilent Technologies, USA), with mobile phase of *n*-hexane/tetrahydrofuran/2-propanol (1000:60:4, v/v/v) and flow rate of 1.0 ml min^{-1} were used. The compounds were detected at an emission wavelength of 326 nm and excitation wavelength of 292 nm. Quantification was by a five-point external standard calibration assay, with R^2 values > 0.99.

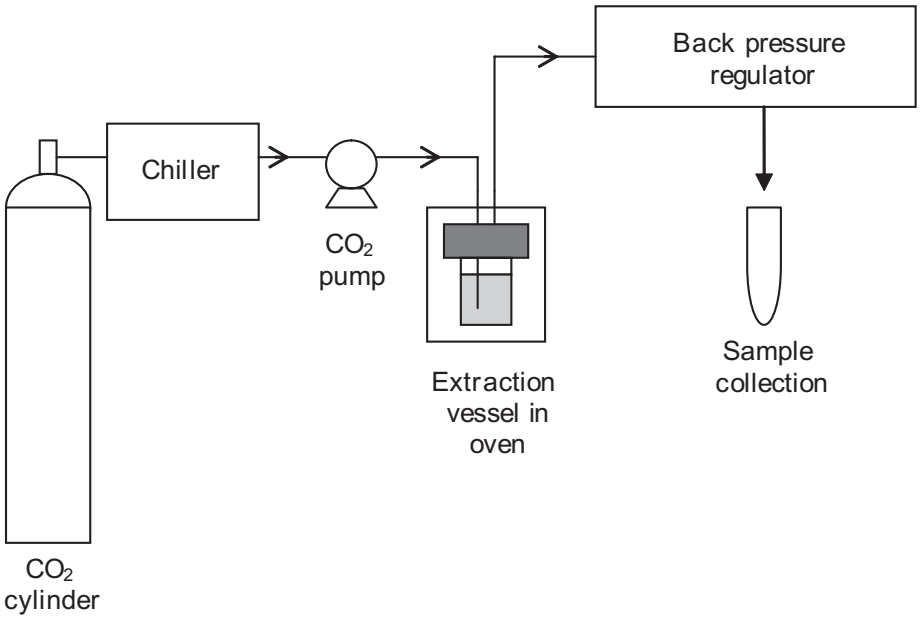

Figure 1. Schematic diagram of supercritical fluid extraction system.

RESULTS AND DISCUSSION

The CPO methyl esters contained 550 ppm (0.055 wt%) carotenoids and 900 ppm (0.090 wt%) tocols. *Table 1* shows the carotenoids and tocols extracted at 40°C and 10 MPa. *Table 1* showed that the concentration of carotenoids and tocols increase with time. The seven fractions were collected at constant temperature of 40°C and constant pressure of 10 MPa. The concentration of carotenoids and tocols in Fractions 1 – 5 are relatively low. Fraction 6 is the concentrated fraction of tocols (10.66 wt%) while Fraction 7 is the concentrated fraction of carotenoids (20.11 wt%).

yield was low with only 19.27% of the carotenoids recovered. Low operating temperature of 40°C was used and thus the quality of carotenoids was not significantly affected.

Tocols in CPO have also been shown to have low solubility in supercritical carbon dioxide under 30 MPa pressure (Puah *et al.*, 2007b). Thus, esterification enhanced the solubility of fatty acids in supercritical carbon dioxide by converting the polar acid groups to less polar esters. *Table 1* shows the tocols concentrated from 0.090 wt.% in the feed material to 10.66 wt.%, or >100-fold increase. However, again, the yield was low with only 16.16% recovery.

TABLE 1. EXTRACTION AND CONCENTRATION OF CAROTENOIDS AND TOCOLS FROM PALM OIL METHYL ESTERS USING SUPERCRITICAL CARBON DIOXIDE AT 40°C AND 10 MPa

Fraction	Weight (g)	Carotenoids		Tocols	
		Concentration (wt.%)	Recovery (%)	Concentration (wt.%)	Recovery (%)
Feed	20	0.055	na	0.090	na
1	6.19	0.003	1.27	0.007	2.23
2	5.73	0.002	0.81	0.014	4.15
3	5.44	0.004	1.40	0.028	8.17
4	2.39	0.047	7.22	0.20	25.30
5	0.13	1.51	12.64	5.11	35.11
6	0.034	2.67	5.93	**10.66**	16.16
7	0.016	**20.11**	19.27	8.85	8.71

Note: na – not applicable.

The solubility of a compound in supercritical carbon dioxide depends on its molecular weight, polarity and solvent strength. The triglycerides in CPO have molecular weights of 807-885, and as larger molecules have lower solubility in the carbon dioxide solvent. Carotenoids also have very low solubility in supercritical carbon dioxide even under pressure at up to 30 MPa (Puah *et al.*, 2005).

In addition, the carotenoids are reportedly transported together with the triglycerides. Further, the solubility of fatty acid methyl esters is several magnitudes higher than that of their corresponding triglycerides (Güclü-Üstündag and Temelli, 2000). The transesterification converted the large molecules of triglycerides into smaller molecule fatty acid methyl esters, making it much easier to separate them from the carotenoids. Removal of the triglycerides concentrated the carotenoids in the final product. *Table 1* shows the carotenoids concentrated from 0.055 wt.% to 20.11 wt.%, a >350-fold increase. However, it should be noted that the

Another possible influence on the solubility of a compound in supercritical carbon dioxide is the vapour pressure of the compound. Vapour pressures of compounds with their corresponding carbon numbers are in the order: fatty acid esters > fatty acids > triglycerides. The fatty acid esters are easier to be extracted compared to its fatty acids and triglycerides with the same corresponding carbon numbers. Therefore, converting fatty acids and triglycerides into fatty acid esters enable their removal to enhance concentration of carotenoids and tocols. Therefore, the concentration of free fatty acids in the CPO will not affect the results as they are converted into methyl esters.

Figure 2 is the flow diagram for production of carotenoids and tocols from CPO. The figure shows that the concentrations of tocols and carotenoids were not affected by the esterification and transesterification underwent. This was due to the reactions being carried out at a mild temperature, thus not altering or destroying them.

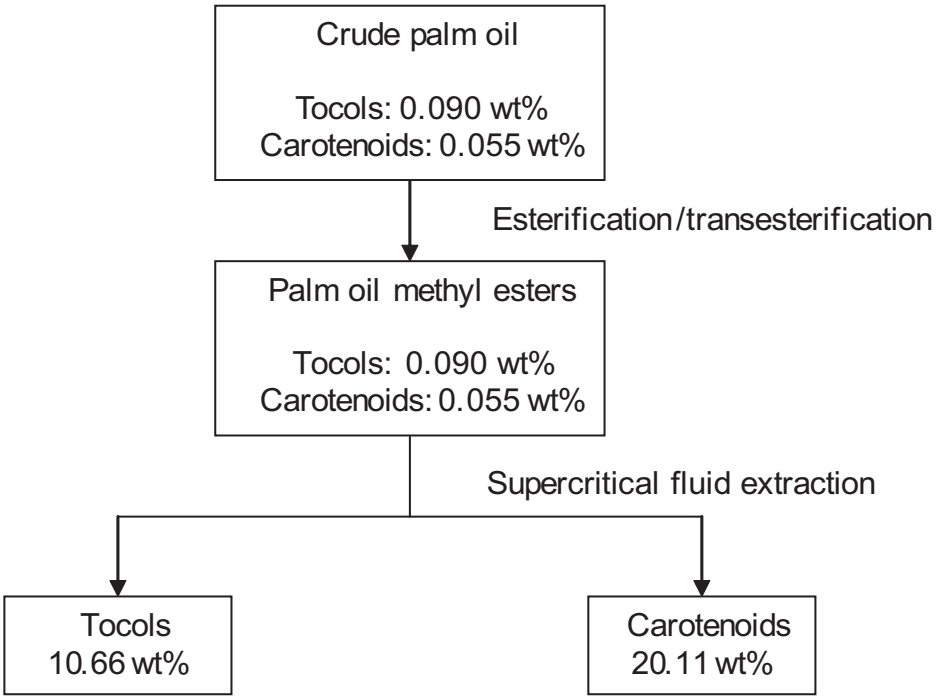

Figure 2. Flow diagram for production of carotenoids and tocols concentrates from crude palm oil.

CONCLUSION

Supercritical fluid extraction is a potential technology for extracting concentrated carotenoids (350x increased concentration) and tocols (100x increased concentration) from CPO.

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