ENHANCING THE SEPARATION AND PURIFICATION EFFICIENCY OF PALM OIL CAROTENES USING SUPERCRITICAL FLUID CHROMATOGRAPHY

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ABSTRACT

Palm oil is the richest source of natural plant carotenoids in terms of retinol or pro vitamin A equivalent. The carotenoids found in palm oil consist of carotenes and xanthophylls, with about 500 to 700 ppm carotenes in crude palm oil. Effort has been made to extract and recover the valuable carotenes from palm oil, where they are being made into nutritional supplements or as ingredients in cosmetics formulations. This article reports on the application of supercritical fluid chromatography (SFC) for such purpose. While SFC has been conventionally used as an analytical tool, its application in preparative or even pilot scale has been gaining popularity of late. The process and feasibility of extraction and recovery of carotenes from palm using SFC are reported whereby it was found that the SFC is able to purify the palm carotenes in consistent purity and production rate with no effect on the processing time. Carotenes were obtained in one chromatographic step with SFC, using carbon dioxide as the mobile phase and ethanol as modifier at 60°C and 190 bar. The purity of the end product and production rate however, can be greatly enhanced with the introduction of a pre-treatment step, prior to the purification by SFC. The purity of the end product increased by more than four-fold with the introduction of the pre-treatment step. The technical and economical feasibility of the purification of carotenes from palm, including the pre-treatment process, were studied and discussed in this article.

Keywords: carotenes, palm, supercritical fluid chromatography.

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INTRODUCTION

The oil palm is the richest source of natural plant carotenoids, in terms of retinol (pro vitamin A) equivalent (Yap *et al.*, 1991; Ooi *et al.*, 1994; Goh *et al.*, 1985). The presence of carotenes in other vegetable oils, such as corn oil, peanut oil, soyabean oil, rapeseed oil, linseed oil, olive oil, sunflower oil and cottonseed oil are relatively lower than the amount of carotenes found in palm oil. In terms of retinol equivalent (pro vitamin A), palm oil contains about 15 to 300 times more retinol equivalents than carrots, green leafy vegetables and tomatoes. Due to the presence of carotenoids, palm oil is orange in colour, mainly due to the large amount of β-carotene.

The carotenoids found in palm oil consist of carotenes and xanthophylls. There are about 500 to 700 ppm carotenes in crude palm oil (Yap *et al.*, 1991; Ooi *et al.*, 1994; Goh *et al.*, 1985). While α- and β-carotene constitutes the major carotenes,

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there are a total of 13 types of carotenes present in palm oil. Much less is known about the chemistry of xanthophylls in palm oil although a few compounds, namely, dehydroretinal, ξ-caroten-dione and β-carotene-5,6-epoxide isomers have been reported (Tay and Gwendoline, 2006).

It has been reported in many studies that palm carotenes exhibit beneficial properties such as being anti-cancer, as well as antioxidative. It was reported that α- and β-carotene in palm oil protect the cells against free radical damage as well as preventing xerophtalmia (Choo *et al.,* 1992; Canfield, 1995; Ziegler, 1989; 1996; Sommerburg *et al.,* 2015).

In view of the beneficial properties of carotenes, many studies have been carried out to extract and recover them from palm. Carotene in general, is sensitive to light; heat, as well as acidic conditions. Although present in considerable amount in crude palm oil, unfortunately, the amount of carotenes is greatly reduced during the deodorisation stage in palm oil processing. Methods such as preparative liquid chromatography (LC), formation of micelles, supercritical fluid extraction, as well as adsorption and desorption have been reported for the separation and recovery of carotenes from various sources (Ooi *et al.,*1994; Choo *et al.,* 2005; Lesellier *et al.*, 1999; Latip *et al.,* 2000; 2001; Liew *et al.,*1995; Maoka *et al.,* 2002; Larsen and Christensen, 2005; Damayanti *et al.*, 2014).

This article reports on a method for producing carotenes from palm oil by using supercritical fluid chromatography (SFC). The use of SFC has been primarily for analyses purpose as the supercritical fluid offers both chromatographic behaviour of LC and gas chromatography (GC) (King and List, 1996a; Smith, 1998; King, 1993; Lee and Markides, 1990; Mendes *et al.,* 1999; Miriam *et al.,* 1990; Shen *et al.,*2002; King *et al.,* 1996b). It has been used for the analyses of many lipid compounds such as tocols, carotenes and squalene (Latip *et al.,* 2001; Mendes *et al.,* 1999; Ng *et al.,* 2006; 2004a, b; Laakso, 1992). The use of SFC in preparative scale for the concentration and separation of high value compounds have been gaining popularity of late (Taylor and King, 2002; Searle *et al.*, 2004).

The starting material for this process is the palm phytonutrients concentrate, which is obtained through vacuum distillation of palm oil methyl ester (POME). The mild conditions employed for the esterification/transesterification of crude palm oil into POME enabled the carotenes to remain intact after the reaction. After reaction, the POME was subjected to vacuum distillation whereby a raffinate that is rich in phytonutrients, including carotenes, is produced. The distilled POME is then used as biodiesel or feedstock for oleochemicals whereas the phytonutrients concentrate is used as feedstock for further recovery of the carotenes, tocols and coenzyme Q that are contained within.

Specific production rate of the carotenes, in terms of weight per weight of stationary phase is also described.

MATERIALS AND METHODS

Preparative supercritical fluid chromatography flow rate of CO_2 was 5.0 ml min^{-1} with 0.2 ml min^{-1} absolute ethanol as modifier. Temperature of the column was set at 60°C and pressure at 19 MPa.

The starting material (phytonutrients concentrate or saponified phytonutrients concentrate) was dissolved in dichloromethane and injected into the SFC. Carotenes were identified by UV spectrophotometry based on their λ_{max} and collected at the outlet of the SFC system. The fraction collected was then dried with nitrogen and analysed for its carotenes content using the MPOB Test Method.

Carotenes Analyses

Analyses of total carotenes were carried out in a method as described in the MPOB Test Method where the sample was dissolved in hexane and the absorbance at 446 nm was obtained using a UV spectrophotometer. The concentration of the carotenes was calculated with the formula:

$$\text{Carotene concentration (ppm)} = \frac{383 \text{ x abs x vol}}{\text{Sample weight}}$$

where,
383 = extinction coefficient for carotenes;
Abs = absorbance at 446 nm;
Vol = volume of the sample (ml); and
Sample weight = weight of sample used for analyses

Saponification

Saponification of the phytonutrients concentrate was carried out by subjecting 0.2 g of the phytonutrients concentrate to reflux with 30 ml absolute ethanol, 1 g pyrogallol and 3 ml KOH (50%) for 1 hr. Thereafter the mixture was cooled to room temperature and extracted using hexane. The oil layer was collected and the aqueous layer was re-extracted with hexane until the oil layer turned into pale yellow colour. The oil layer was then pooled and solvent removed via rotary evaporation. The composition of carotenes before and after saponification is depicted in *Table 1*.

Materials

Palm phytonutrients concentrate was obtained from the short path distillation of palm oil methyl ester in a method as described by Ooi *et al*. (1994). Carbon dioxide was of chromatographic grade (99.995%) obtained from the Malaysian Oxygen

TABLE 1. COMPOSITION (%) OF SELECTED PHYTONUTRIENTS IN PYTONUTRIENTS CONCENTRATE BEFORE AND AFTER SAPONIFICATION

Saponification	Squalene (%)	Carotenes (%)	Tocols (%)
Before (labelled as PC)	1.5	2.6	1.9
After (labelled as PPC)	9.8	17.5	10.2

(Malaysia). Absolute ethanol was from Merck (Darmstadt, Germany). Preparative SFC was carried out using JASCO SFC system. Column used was Silica, 5 μm, 20 x 250 mm.

RESULTS AND DISCUSSION

Figure 1 shows the specific production rate of carotenes obtained from various loading capacities of phytonutrients concentrate, whereby the concentration of the carotenes in the starting material was 2.6%. The concentration of the carotenes in the fraction collected was 30%, maximum. To achieve the 30% concentration of carotenes, it was observed that the yield of the fractions decreased from 41% for loading at 12 g kg^{-1} to 25% for loading at 30 g kg^{-1}. The highest specific production rate was 101.8 g kg^{-1} min, achieved at the loading capacity 27 g kg^{-1}.

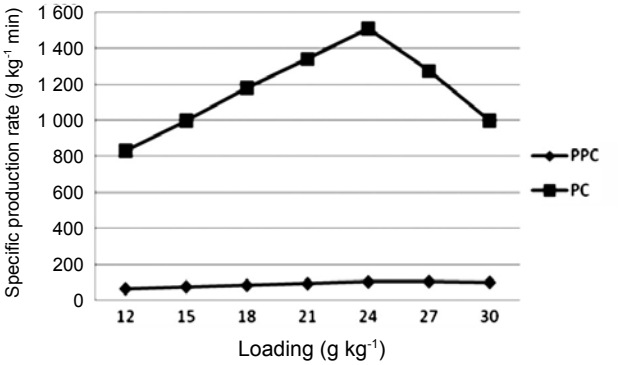

Figure 1. Specific production rate of carotene.

The concentration and yield of the carotenes obtained from PC and SPC after purification by SFC is as depicted in *Table 2*. The carotenes content was much higher in saponified phytonutrients concentrate than phytonutrients concentrate. Upon saponification, the SPC was injected into the SFC. The specific production rate and yield of the carotenes from phytonutrients concentrate and saponified phytonutrients concentrate are depicted in *Table 3*.

Table 3 shows the result from the repeatability study of the process whereby the standard deviation of the process ranged from 1.90 - 2.63 with confidence level ranged from 0.41 – 0.56. Preparative chromatography sees the injection of large amount of starting material or solutes per weight of the stationary phase as compared to analytical work in order to achieve the feasible production rates. However, this is at the expense of column efficiency, resolution, purity and yield. The loading capacity, recovery and purity of the carotenes can best be related or represented in terms of specific production rate (PR). Specific production rate is a function of product produced over weight of starting material at a period of time; expressed in the unit of g kg^{-1} min. Calculation of PR was carried out using the formula:

$$PR = LR \,.\, Cd \,.\, \frac{Y}{t}$$

where,

PR = specific production rate (g of product/kg stationary phase. Min);

LR = load ratio (g loaded/kg stationary phase);

TABLE 2. DETERMINATION OF SPECIFIC PRODUCTION RATE FOR CAROTENES OBTAINED FROM VARIOUS LOADING CAPACITIES OF PHYTONUTRIENTS CONCENTRATE BY NORMAL PHASE SUPERCRITICAL FLUID CHROMATOGRAPHY

		PC			SPC		
Loading (g kg^{-1})	Injection interval (min)	Concentration of carotenes in feed (%)	Yield (%)	Specific production rate (g kg^{-1} min)	Concentration of carotenes in feed (%)	Yield (%)	Specific production rate (g kg^{-1} min)
12	20	2.6	41	64.0	17.5	79	829.5
15	20	2.6	38	74.1	17.5	76	997.5
18	20	2.6	35	81.9	17.5	75	1 181.3
21	20	2.6	33	90.1	17.5	73	1 341.4
24	20	2.6	32	99.8	17.5	72	1 512.0
27	20	2.6	29	101.8	17.5	54	1 275.8
30	20	2.6	25	97.5	17.5	38	997.5

Note: PC – phytonutrients concentrate. SPC – saponified phytonutrients concentrate.

TABLE 3. REPEATABILITY DATA ON THE PRODUCTION OF CAROTENES FROM DIFFERENT STARTING MATERIALS

Run	Phytonutrients concentrate		Saponified phytonutrients concentrate	
	Concentration (%)	Yield (%)	Concentration (%)	Yield (%)
1	31	32	77	72
2	35	31	74	78
3	37	32	72	75
4	29	37	73	74
5	33	34	72	71
6	31	32	72	72
7	32	33	71	74
8	29	30	72	73
9	30	31	70	71
10	30	30	72	71
Average	31.7	32.2	72.5	73.1
Min	29	30	70	71
Standard deviation	2.63	2.10	1.90	2.23
Confidence level	0.56	0.45	0.41	0.48

Cd = percentage of desired component in starting material;
Y = yield of desired compound of the loaded amount (%); and
t = injection interval (min).

In order to achieve higher concentration of carotenes in the fraction recovered from SFC, the phytonutrients concentrate was subjected to saponification process. This is to remove the excess glycerides or methyl esters that remained in the starting material. The glycerides and methyl esters elute at the same retention time as the carotenes, thus, lowering the concentration of carotenes in the fraction collected. Upon saponification, the concentration of the carotenes increased from 2.6% to 17.5% (*Table 1*). The saponified phytonutrients concentrate was then used as starting material in purification by SFC.

The concentration and yield of the carotenes obtained from phytonutrients concentrate and saponified phytonutrients concentrate after purification by SFC which is depicted in *Table 2* showed that the concentration of carotenes increased from 29% min to 70% min when saponified phytonutrients concentrate is use as the starting material. This shows that a semi purified starting material is able to yield higher concentrations of the desired compound in the final product.

For phytonutrients concentrate and saponified phytonutrients concentrate, it was found that the optimum loading was 24 g of starting material per kg of stationary phase where the yield and specific production rate of the carotenes were at the highest. In order to maintain the concentration of the carotenes in the product to remain at 30% and 70% respectively for both phytonutrients concentrate and saponified phytonutrients concentrate, the fraction need to be limited at narrower range when the loading of the starting material is high due to peak expansion. As such, loading higher than 24 g kg^{-1} gave poorer yield and specific production rate.

Reserved stationary phase is conventionally used for the separation of carotenes. Under supercritical condition however, the carotenes took a long time to be eluted when reversed stationary phase is used without increasing the percentage of the modifier in the mobile phase. This is not in favour in this process as the idea is to reduce the usage of organic solvent as much as possible to avoid the whole process to be liquid chromatography like. Moreover, as the carotenes are collected as total carotenes and not as their individual components, a normal stationary phase was chosen for this purpose. However, the performance of normal stationary phase has been associated with inconsistent separation, retention time and peak distortion.

Repeated injections and separations by the SFC revealed that there are not much changes in the concentration and yield of the carotenes produced (*Table 3*). The retention time of the carotenes as well as the duration of the process remained constant. This shows that the results obtained are highly repeatable, giving confidence in the process.

CONCLUSION

Separation and purification of carotenes using SFC showed to that the carotenes were produced in consistent purity, yield and specific production rate. A material per stationery phase of 24 g kg^{-1} was deemed the optimum loading capacity. Starting material that has undergone pre-treatment yield carotenes of higher purity, yielded and specific production rate. The SFC is repeatedable with consistent performance.

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