Journal of Food Processing & Technology
ISSN: 2157-7110

Han et al., J Food Process Technol 2018, 9:2
DOI: 10.4172/2157-7110.1000718

Research Article Open Access

Tocotrienols Concentration Using Packed Column Supercritical Fluid

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Abstract

Palm oil is the richest source of natural tocotrienols. Tocols in palm consists of both tocopherols and tocotrienol in the amount between 600 ppm-1,000 ppm in crude palm oil. Both tocopherols and tocotrienols have been found to be beneficial to health with the tocotrienols exhibit more powerful antioxidant and anti-cancer power than the tocopherols. Effort has been made to extract and recover the valuable tocols from palm oil, where they are being made into food fortifier, nutritional supplements or as ingredients in cosmetics formulations. This paper reports on the application of supercritical fluid chromatography (SFC) for such purpose. The process and feasibility of extraction and recovery of tocols from palm using SFC are reported whereby it was found that the SFC is able to purify the palm tocols in consistent purity and production rate with no effect on the processing time. Tocols with 20% purity were obtained in one chromatographic step with SFC, using carbon dioxide as the mobile phase and ethanol as modifier at 600°C and 190 bars with specific production rate between 16-31 $g.kg^{-1}$ min. 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 three folds with the introduction of the pre-treatment step. The purity and specific production rate of the tocols obtained from pre-treated palm oil were 70% and 446.8-844.6 $g.kg^{-1}$ min respectively.

Keywords: Chromatography; Palm; Supercritical; Tocols

Introduction

Natural tocols comprises of eight isomers which are classified into two homologues series, the tocopherols and tocotrienols [1-7]. Tocopherols and tocotrienols in particular, have received vast amount of attention in recent years due to their health benefits. Studies have indicated that the tocotrienols exhibit superior antioxidative and anticancer properties [6-19]. Due to the many health benefits of the tocols, the food industry is now heading towards manufacturing tocotrienols fortified food.

Besides synthetically produced, tocopherols are found in many natural sources. The same cannot be said for the tocotrienols. There is yet any successfully proven method to synthesize the tocotrienols, while its natural sources are limited to a few types of oils and marine lives. Palm oil is the richest source of natural tocotrienols [1-6]. Their amount in crude palm oil (CPO), the oil obtained upon pressing of the palm fruits, ranging from 700-1000 ppm [1-4,6]. Earlier studies documented that palm tocols consist of α-tocopherol (α-T), α-tocotrienol (α-T3), γ- tocopherol (γ-T), γ-tocotrienol (γ-T3) and δ-tocotrienol (δ-T3) [1-4,6]. The compositions of tocopherols and tocotrienols in various vegetable oils are depicted in Table 1 [6,7]. Studies in later years reported that α-Tocomonoenol (α-T1) to be present as well in palm oil [3]. The palm tocotrienols have also been found to provide protection against neuro degenerative diseases and cardiovascular diseases [17,20-24]. The palm tocotrienols have been product of interest in recent years for applications in various industries such as nutraceuticals, pharmaceuticals, cosmeceuticals, etc. As the tocotrienols cannot be synthesized, the only way to cultivate them is through extraction from natural sources.

The major concern in any food manufacturing processes is the safety of the process and the end product. In other words, the process needs to be chemical free, environmental friendly and the end product to be non-toxic. Conventionally, extractions of lipid compounds were carried out using solvent extraction. Solvent extraction is an efficient and effective way to extract the tocols from its sources, be it oil or other solid sources. However, solvent extraction calls for the use of organic solvents. Although measures can be taken to keep the solvent residue in the product to minimal or below acceptance level, a non-toxic method that does not use any chemical solvents is preferred. This paper reports on a method for extracting and purifying tocols from palm oil by using supercritical fluid chromatography (SFC) for food fortification and/or as nutraceuticals applications.

Oil	Tocopherol (ppm)				Tocotrienol (ppm)				Total (ppm)
	α-	β-	γ-	δ-	α-	β-	γ-	δ-	
Coconut	5	-	-	6	5	1	19	-	36
Corn	112	50	602	18	-	-	-	-	782
Cottonseed	389	-	387	-	-	-	-	-	776
Olive	51	-	-	-	-	-	-	-	51
Palm	152	-	-	-	205	-	439	94	890
Peanut	130	-	216	21	-	-	-	-	367
Rice bran	324	18	53	-	236	-	349	-	980
Safflower	387	-	174	240	-	-	-	-	801
Sesame	12	6	244	32	-	-	-	-	294
Soybean	101	-	593	264	-	-	-	-	958
Sunflower	784	-	51	8	-	-	-	-	546

Table 1: Different sources of tocopherols and tocotrienols.

The SFC has been primarily used for analyses purpose as the supercritical fluid offers both chromatographic behaviour of LC and GC [25-31]. It has been used for the analyses of many lipid compounds

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Received January 22, 2018; **Accepted** February 09, 2018; **Published** February 16, 2018

Citation: Ng MH, Choo YM, Chuah CH, Hashim MA (2018) Tocotrienols Concentration Using Packed Column Supercritical Fluid. J Food Process Technol 9: 718. doi: 10.4172/2157-7110.1000718

such as tocols, carotenes and squalene [2,3,29,32-36]. In large scale chromatography, larger amount of solutes must be injected per weight of stationary phase than in analytical scale is needed to achieve feasible production [37,38]. However, increasing the injected amount into the column will lead to decreasing column efficiency [37,38]. The use of SFC in preparative scale for the concentration and separation of high value compounds have been gaining popularity since the early 2000s [39-41]. It has been reported in the past that with some modifications in the process variables optimized for maximum productivity, SFC separation of compounds is technically possible.

The SFC is an excellent choice for the production of tocols from palm as it is environmental friendly, safe and the inert supercritical carbon dioxide does not change the properties of the tocols. In addition, carbon dioxide used in the SFC is non-toxic, thus making the tocols, or end products safe for consumption.

Materials and Methods

Materials

All solvents used were of chromatographic grades purchased from Merck (Darmstadt, Germany). Tocotrienols standards were purchased from Davos Life Sciences Pt. Ltd. (Singapore).

Palm oil was obtained from Carotino (Johor, Malaysia). Carbon dioxide was of chromatographic grade (99.995%) obtained from Malaysian Oxygen (Malaysia). Absolute ethanol was from Merck (Darmstadt, Germany). Preparative SFC was carried out using JASCO SFC system. Column used was Silica, 5 µm, 20 × 250 mm.

Preparative supercritical fluid chromatography

CO_2 flowrate was 5.0 ml min^{-1} with 0.2 ml min^{-1} absolute ethanol as modifier. Temperature of the column was set at 600°C and pressure at 19 MPa. The starting material, palm oil (PO) was dissolved in dichloromethane and injected into the SFC column with a manual syringe injection loop filler. Following a series of adsorption and desorption, different components of palm oil were eluted from the column at different time, based on their polarity. Products eluted from the SFC outlet were collected once the real time reading from the UV spectrophotometer. The fraction collected was then dried with nitrogen and analysed for its tocols content. Fractions were collected based on cut shaving technique.

Chromatography analyses of palm tocopherols and tocotrienols

A Waters HPLC coupled with a fluorescence detector was used for HPLC analyses of palm tocols. Column used was silica 4.6 mm I.D. × 250 mm length. Chromatography were carried out under condition: hexane: THF: IPA (1000: 60: 4 v/v), flowrate 1.0 ml/min. The excitation and emission of fluorescence detector were set at 295 and 325 nm respectively.

Saponification

Saponification of palm oil was carried out by subjecting 0.2 g palm oil to reflux with 30mL 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 color. The oil layer was then pooled, and solvent removed via rotary evaporation. The composition of tocols before and after saponification is depicted in Table 2. The preparative SFC procedure was repeated for the SPO.

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

Table 2: Composition (%) of selected phytonutrients in palm oil before and after saponification.

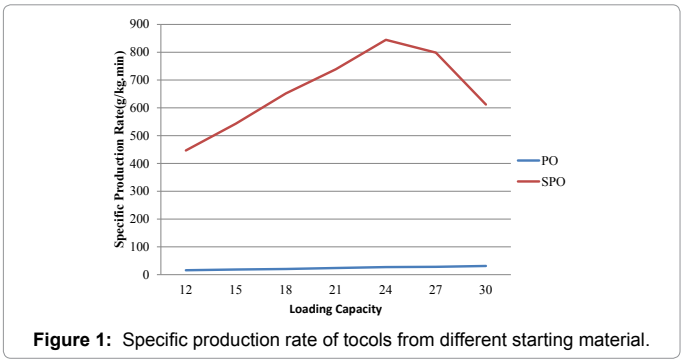

Figure 1: Specific production rate of tocols from different starting material.

Results and Discussion

Conventionally, the SFC is primarily used as an analytical tool rather than as extraction or purification medium. Preparative SFC involves 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 in the expense of column efficiency, resolution, purity and yield. More often than not, the required products' purity or concentration cannot be achieved without any sacrifices in yield.

The tocols peaks were identified based on the increased in intensity at 290 nm recorded by the UV detector. 290 nm is the wavelength where the tocols absorb maximum UV. Timing for the fraction collections were estimated visually from the SFC chromatogram. The initial concentration of tocols in the starting material prior to loading into SFC were: palm oil, 1.9% and saponified palm oil, 10.2%. Upon extraction and purification, its concentrations were increased up to 20% and 70% respectively. The loading capacity, recovery and purity of the tocols throughout the SFC process can best be related or represented in terms of specific production rate (PR).

Specific production rate is a function of load ratio and 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:

$PR = LR \times Cd \times Y/t$

Where,

PR: Specific production rate (g of product/kg stationary phase. Min)

LR: Load ratio (g loaded/kg stationary phase)

Cd: Percentage of desired component in starting material

Y: Yield of desired compound of the loaded amount (%)

T: Injection interval (min)

Figure 1 shows the specific production rate of tocols obtained from various loading capacities of palm oil and saponified palm oil. The specific production rates of SPO for each loading capacity studied were much higher compared to that of PO at similar loading capacity. Saponification is a pre-treatment process to remove the excess glycerides or saponifiable compounds in palm oil. Presence of

Loading (g kg^{-1})	Injection interval (min)	PO			SPO		
		Concentration of Tocols in Feed (%)	Yield (%)	Specific Production Rate (g kg^{-1} min)	Concentration of Tocols in Feed (%)	Yield (%)	Specific Production Rate* (g kg^{-1} min)
12	20	1.9	14	16.0	10.2	73	446.8
15	20	1.9	13	18.5	10.2	71	543.2
18	20	1.9	12	20.5	10.2	71	651.8
21	20	1.9	12	23.9	10.2	69	739.0
24	20	1.9	12	27.4	10.2	69	844.6
30	20	1.9	11	31.4	10.2	40	612.0

Table 3: Determination of specific production rate for tocols obtained from various loading capacities of palm oil and saponified palm oil by normal phase SFC.

Run	Phytonutrients Concentrate		Saponified Phytonutrients Concentrate	
	Concentration (%)	Yield (%)	Concentration (%)	Yield (%)
1	21	11	71	69
2	22	13	69	72
3	19	16	72	70
4	25	11	72	68
5	20	14	70	67
6	23	15	68	69
7	21	12	69	71
8	20	14	68	70
9	22	13	71	72
10	22	13	72	69
Average	21.5	13.2	70.2	69.7
Min	19	11	68	67
Standard Deviation	1.72	1.62	1.62	1.64
Confidence level	0.37	0.35	0.35	0.35

Table 4: Repeatability data on the production of tocols from different starting materials at 24 g/kg loading capacity.

glycerides and / or methyl esters is not favourable in the SFC process as they elute at almost the same retention time as the tocols. The co-elution resulted in lower concentration of tocols in the fraction collected. The excess glycerides and methyl esters were turned into water soluble compounds in the saponification process and removed by water washing. Upon saponification, the concentration of the tocols increased from 1.9% to 10.2% (Table 2).

The concentration and yield of the tocols obtained from PO and SPO after purification by SFC is as depicted in Table 3. The initial tocols content was much higher in SPO than PO. The specific production rate and yield of the tocols from PO and SPO are depicted in Table 4. The concentration and yield of the tocols obtained from PO and SPO after purification by SFC which is depicted in Table 2 showed that the concentration of tocols in the fraction increased from ~10% to ~70% when SPO is used as the starting material. The SFC is able to concentrate the tocols by 6-7 folds of its initial concentration in the starting material. For PO and SPO, 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 tocols were at the highest. In order to maintain the concentration of the tocols in the product to remain at 20% and 70% respectively for both PO and SPO, the fraction collection need to be carried out at narrower peak range when the loading of the starting material is high due to peak expansion and to avoid overlapping of non-tocols neighbouring peaks. As such, loading higher than 24 g kg^{-1} gave poorer yield and specific production rate.

Table 4 shows the result from the repeatability study of the process at loading capacity 24 g/kg whereby the standard deviation of the process ranged from 1.62-1.72 with confidence level ranged from 0.35-0.3756. Repeated injections and separations by the SFC revealed that there is not much changes in the concentration and yield of the tocols produced (Table 4). The retention time of the tocols 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

The tocols content in palm oil increased by at least three folds in one chromatographic step using SFC with specific production rate between 16-31g.kg^{-1} min. The purity and specific production rate are greatly enhanced when the palm oil undergo a pre-treatment step before loading into the SFC. Purity and specific production rate of the tocols obtained from pre-treated palm oil were 70% and 446.8-844.6 g.kg^{-1} min respectively. This will render the whole SFC process for the production of high concentration tocols to be more efficient and cost effective. The process for concentration of tocols by SFC also serves as a guide for the application of SFC for production rather than its usual function as analytical tool.

Funding

The work was carried with funding from Malaysian Palm Oil Board as part of their research activities.

Acknowledgement

The authors wish to thank the Director General for his permission to publish this paper. Thanks are also due to the supporting staff of Clean and Emerging Technologies, Engineering and Processing Research Division for their technical assistance.

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