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Simultaneous Recovery of Carotenes and Tocols from Crude Palm Olein Using Ethyl Lactate and Ethanol

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Abstract. This paper demonstrates the use of ethyl lactate and ethanol as green and safe solvents to extract phytonutrients such as carotenes and tocopherols from crude palm olein (CPO) before they are lost during oil refining process. The effects of mixing time (10-40 min), temperature (10-30°C) and proportion of CPO (20-60%) were studied in terms of the extraction of individual carotenes (α - and β -carotene) and tocopherols (α -tocopherol/T, α -, γ - and δ -tocotrienol/T3) in a temperature-controlled mixer-settler system. The optimal extraction conditions were found at 20°C, 10 min of mixing, 50% of CPO using 3:2 v/v ethyl lactate/ethanol as the solvents. After four stages of extraction, 42.2% of carotenes, 86.7% of tocopherols and 44.4% of oil were recovered into an oil concentrate of 717.5 mg/L of carotenes and 1496.2 mg/L of tocopherols.

1. Introduction

Crude palm oil contains 500-700 ppm of carotenoids with approximately 35% α -carotene and 56% β -carotene [1]. They are important as a supplement of vitamin A as well as a natural antioxidant to fight against several degenerative diseases including cardiovascular diseases, cancers and macular degeneration [2]. In crude palm oil, there is also 600-1000 ppm of tocopherols (commonly known as vitamin E), with 21.3% of tocopherol (T) and 78.7% of tocotrienol (T3) [3]. As compared to T, T3 was reported to be more effective due to its unsaturated chain, which facilitates cell penetration and is highly antioxidative. T3 was reported to regulate cholesterol level, to prevent cancers, stroke and fats accumulation in liver [4]. T is commonly found in many other vegetable oils while palm and rice bran oil are among the richest natural sources of T3.

During physical oil refining process, carotenes are first partially removed by adsorption on activated bleaching earth, followed by high temperature steam deodorization which destroys the chromogenic properties of the remaining carotenes to produce a light yellow palm oil. Even though tocopherols are more thermally stable than carotenes, near to 50% of the tocopherols will be stripped off along with free fatty acids (FFA), sterols and squalene into palm fatty acid distillate (PFAD) during deodorization step. Thus, there is a need to recover these phytonutrients from crude palm oil before further refining process.

As both the extracted carotenes and tocopherols will be used as food fortifiers, the use of non-toxic, non-corrosive and non-carcinogenic solvents which are safe for human consumption is crucial. When most of the commonly used petrochemical solvents are known to pose certain degree of toxicity, ethyl



lactate is a suitable candidate solvent because it is novel, green and safe. It is produced from the carbohydrate feedstocks from the corn and soybean industries and it presents naturally in foods such as wine, beer, chicken and fruits. It is also non-ozone depleting, non-hazardous air polluting and it is biodegradable into harmless compounds such as CO₂ and water. The US Environmental Protection Agency (USEPA) approved the solvent as a Significant New Alternatives Policy Program (SNAP) solvent while US Food and Drug Administration (USFDA) has approved its direct use in food and pharmaceutical products [5]. Ethyl lactate exerts polarity in the range of acetonitrile and n-hexane. It is capable to form intra- and inter-molecular hydrogen bonding. Also, it has the ability to form Van der Waals interactions in oils [6]. As a result, ethyl lactate can dissolve in both aqueous and hydrocarbon environments and it is capable to extract compounds of a wide range of polarity. Ethyl lactate has been reported to extract various nutraceutical compounds mostly from solid matrix [7]. Limited papers reported its potential to recover compounds directly from oil sample [8], [9].

The aim of this study is to investigate the potential of ethyl lactate and ethanol as safe and green solvents to extract carotenes and tocopherols from CPO via simple multistage mixing-settling process. Commercially, there is no technology available to date to recover these phytonutrients before palm oil refining process. Hence, this study provides the experimental results which can be used for future pilot scale recovery tests.

2. Material and methods

2.1. Samples and reagents

(S)-(-)-ethyl lactate (99% purity) and ethanol (99.5% purity) were obtained from Merck. Standards such as α -carotene (98% purity) was purchased from Fluka, β -carotene (99.4% purity) and α -T (100.9% purity) came from Calbiochem. α -, γ - and δ -T3 (97% purity) were bought from Davos Life Science (Singapore). Acetonitrile (HPLC grade) and dichloromethane (99.8% purity) were obtained from Labscan. 2-propanol (HPLC grade) came from Fisher while n-hexane (HPLC grade) from Merck. Crude palm oil (*Elaeis guineensis/tenera*) was centrifuged at 7500 rpm for 15 min in order to collect the upper olein phase for use in the extraction experiment.

2.2. Extraction process

In the temperature controlled mixer-settler system, a total volume of 250 mL solution composed of CPO, ethyl lactate and ethanol was prepared into the vessel. The proportion of CPO, ethyl lactate and ethanol vary for each run. The temperature of the solution was controlled at 10-30°C using an oil jacket controlled by the circulating bath (Lab Companion, Model RW-0525G). The solution was mixed at 360 rpm using a four-bladed stirrer for 10-40 min. After that, the mixture was left to settle for an hour with the temperature being controlled throughout. Lastly, the volume of the upper (oil) and lower (solvent) phases were measured and collected for analysis. Reversed- and normal-phase high performance liquid chromatography (HPLC) was employed to determine the concentration of carotenes and tocopherols, respectively, in both the oil and solvent phase. All the experiments were repeated and the average values were reported.

The process performance was evaluated mainly based on the enrichment factor, percentage recovery and percentage of oil recovery as defined by equation (1)-(4).

$$\text{Enrichment factor} = \frac{\text{mass of carotenes or tocopherols extracted (mg)/volume of oil extracted (mL) into solvent phase}}{\text{mass of carotenes or tocopherols (mg)/volume of oil (mL) present in feed}} \quad (1)$$

$$\text{Percentage recovery (\%)} = \frac{\text{mass of carotenes or tocopherols extracted (mg)}}{\text{mass of carotenes or tocopherols present in feed (mg)}} \times 100\% \quad (2)$$

$$\text{Total recovery (\%)} = \frac{\text{total mass of carotenes and tocopherols extracted (mg)}}{\text{total mass of carotenes and tocopherols present in feed (mg)}} \times 100\% \quad (3)$$

$$\text{Percentage oil recovery (\%)} = \frac{\text{volume of oil extracted (mL)}}{\text{volume of oil present in feed (mL)}} \times 100\% \quad (4)$$

2.3. High performance liquid chromatography (HPLC)

An Agilent 1260 Infinity Series HPLC system was used along with a quaternary pump, an autosampler, a variable wavelength detector (VWD) and ChemStation software for system control and data collection. Carotenes (α - and β -carotene) separation was carried out in a Purospher STAR RP-18 encapped column (5 μ m; 4.6 x 250 mm) maintained at 30°C. The mobile phase consists of 85% acetonitrile and 15% dichloromethane at 1.5 mL/min. The detector was set at 450 nm and the total run time was 25 min. Tocols (α -T, α -, γ - and δ -T3) separation was achieved in a Zorbax Rx-SIL column (5 μ m; 4.6 x 250 mm) maintained at 30°C. The mobile phase consists of 99% of n-hexane and 1% of 2-propanol at 0.8 mL/min. The detector was set at 292 nm and the total run time was 15 min.

3. Results and discussion

3.1. The use of ethanol as co-solvent

Table 1, 2 and 3 present the results of extraction when operated at 20°C, 25 min of mixing and 50% of CPO at a total capacity of 250 mL. When ethyl lactate was used as the sole solvent, the total carotenes were concentrated by 0.92 while the total tocots were concentrated by 2.25 (table 1). Tocots exert greater affinity towards ethyl lactate because they are more polar than carotenes due to the presence of hydroxyl group and unsaturated side chain. Even though ethyl lactate exerts polarity in the range of acetonitrile and hexane, it tends towards polar solvent. Therefore, compounds of higher polarity are more readily extracted. Similarly, the enrichment factor improved from 1.69 to 3.02 in the order of α -T, α -T3, γ -T3 and δ -T3 with increasing polarity. Since β -carotene was more concentrated than α -carotene, β -carotene is more polar than α -carotene. A total recovery of 13.5% was achieved including both carotenes (7.6%) and tocots (18.6%) in a single stage system. The oil recovery was 8.2%.

Table 1. The concentration of each carotenes and tocots in feed, solvent and oil phase (after solvent removal) when 100% of ethyl lactate was used as the solvent.

Concentration (mg/L)	Feed	Solvent	Oil	Enrichment factor
α -carotene	311.4	286.2	313.4	0.91
β -carotene	461.2	426.9	463.9	0.92
Total carotenes	772.6	713.1	777.3	0.92
α -T	242.9	409.5	227.8	1.69
α -T3	222.4	465.5	200.3	2.06
γ -T3	326.2	859.7	277.9	2.61
δ -T3	85.4	261.3	69.5	3.02
Total tocots	877.0	1996.0	775.4	2.25

After mixing and settling of a solution made up of 125 mL of CPO and 125 mL of ethyl lactate, 78 mL of the solvent phase was collected. It was only 62.5% of the initial volume of ethyl lactate introduced into the vessel. Ethyl lactate diffused and retained in the oil phase due to the formation of Van der Waals interactions. In order to create a better two-phase system with stronger immiscibility, ethanol, which is strongly polar, green and safe, was introduced as a co-solvent. The addition of ethanol aims to refrain ethyl lactate from diffusing into the oil phase. [10] reported that more β -carotene was extracted from carrots powder with increasing amount of ethanol as a co-solvent in ethyl lactate. Therefore, ethanol was chosen to improve the overall performance. 40% of ethanol was used with 60% of ethyl lactate as the mixed solvents because ethanol was found to form another individual top phase as the volume goes beyond 50%. This was due to the density differences of ethanol (789 kg/m³), CPO (888 kg/m³) and ethyl lactate (1034 kg/m³). In the presence of 40% ethanol, the volume of solvent phase improved to 89 mL, which was 71.2% from the initial volume (125 mL) of solvents added. After ethanol addition, the enrichment factor of carotenes and tocots improved to 0.96 and 2.68, respectively (table 2). Again, the extraction of tocots was much better due to their polarity difference. Similar results were reported by [11] as more T dissolved in ethanol than carotenoids. Even though the

oil recovery increased to 11.0%, the total recovery improved to 21.4% with carotenes and tococls recovery increased to 10.5% and 29.3%, respectively

Table 2. The concentration of each carotenes and tococls in feed, solvent and oil phase (after solvent removal) when 40% ethanol + 60% ethyl lactate (3:2 v/v ethyl lactate/ethanol) was used as the premixed solvents.

Concentration (mg/L)	Feed	Solvent	Oil	Enrichment factor
α -carotene	243.8	199.9	249.3	0.81
β -carotene	402.1	419.3	400.0	1.04
Total carotenes	645.9	619.2	649.2	0.96
α -T	239.5	531.2	203.5	2.21
α -T3	271.8	657.0	224.3	2.42
γ -T3	363.6	1087.0	274.4	2.99
δ -T3	89.8	306.2	63.1	3.41
Total tococls	964.6	2581.5	765.5	2.68

In table 3, 100% ethanol was used as the solvent for extraction. The enrichment factor of tococls was further improved to 2.76 but it dropped drastically to 0.56 for carotenes. Since ethanol is a strongly polar solvent, it is not suitable to be used alone to recover carotenes, which are non-polar in nature. 6.1% of carotenes and 30.2% of tococls were recovered with a total recovery of 20.1%. The oil recovery was 10.7%. Apart from carotenes, the extraction performance of 100% ethanol was comparable to the results as obtained by using 40% of ethanol and 60% of ethyl lactate in table 2. Therefore, 40% ethanol and 60% ethyl lactate (equivalent to 3:2 v/v ethyl lactate/ethanol) were used in the subsequent extraction experiments to improve the yield.

Table 3. The concentration of each carotenes and tococls in feed, solvent and oil phase (after solvent removal) when 100% of ethanol was used as the solvent.

Concentration (mg/L)	Feed	Solvent	Oil	Enrichment factor
α -carotene	275.4	146.5	291.0	0.53
β -carotene	342.4	199.4	359.0	0.59
Total carotenes	617.7	345.9	650.0	0.56
α -T	212.0	474.5	178.5	2.21
α -T3	200.3	497.2	162.1	2.53
γ -T3	330.6	1017.1	245.3	3.05
δ -T3	87.7	320.6	59.7	3.66
Total tococls	830.5	2309.3	645.6	2.76

3.2. The effect of mixing time

Table 4 shows the enrichment factors and recoveries as obtained after 10, 25 and 40 min of mixing at 20°C and 50% of CPO using 3:2 v/v ethyl lactate/ethanol as the solvents. As the mixing time increased from 10 to 25 min, the enrichment factor and recovery for both carotenes and tococls were improved. A longer period of agitation induced higher chances of bringing the solvent and CPO into direct contact and hence, there was improved mass transfer into the solvent phase. Further increase in the mixing time to 40 min reduced both the enrichment factors and recoveries. The formation of stable emulsion after prolonged mixing might affect the extractability negatively [12]. Also, competitive extraction might occur when other compounds such as FFA, glycolipids, sterols and squalene [13] in CPO were co-extracted. As a result, carotenes and tococls back-diffused into the oil phase after prolonged period of mixing.

The improvement of enrichment factors and recoveries as mixing time increased by 15 min from 10 min were not pronounced. Mixing time had little effect on the process performance. Therefore, further increase of the mixing time by 1.5 times was not necessary. In the industry, multistage extraction is

preferred to improve the overall extraction yield. A short mixing or operating time is desirable to reduce the overall operational takt time. Thus, the subsequent experiments were carried out at 10 min of mixing.

Table 4. The enrichment factor and recovery of carotenes, tocols and oil when operated at mixing time of 10, 25 and 40 min.

Mixing time (min)		10	25	40
Enrichment factor	Carotenes	0.88	0.96	0.77
	Tocols	2.56	2.68	2.26
Recovery (%)	Carotenes	10.1	10.5	9.0
	Tocols	28.0	29.3	26.1
	Total	20.3	21.8	18.6
Oil recovery (%)		11.2	11.2	11.7

3.3. The effect of temperature

Figure 1 and 2 illustrate the results of the enrichment factors and recoveries when operated at 10, 20 and 30°C after 10 min mixing of 50% of CPO with 3:2 v/v ethyl lactate/ethanol. Temperature as low as 15.2°C was reported to be superior to extract α -T from olive oil [9]. Lower operating temperature is preferred to avoid thermal decomposition of heat-sensitive compounds such as carotenes and tocols at high temperature. However, higher temperature system provides more energy, the kinetics will be higher to induce mass transport. In addition, solvents become less viscous for diffusion at higher temperature and hence, the diffusivity or rate of diffusion is expected to be enhanced.

As the operating temperature of current system reduced from 20 to 10°C, there were minimal effects on the enrichment factors and recoveries. There was slight reduction for carotenes as the kinetic of mass transport was slower at 10°C. Longer mixing time might be required to improve the yield. In contrast, the performance of tocols extraction remained almost unchanged. Thus, additional cooling below normal atmospheric temperature was not necessary.

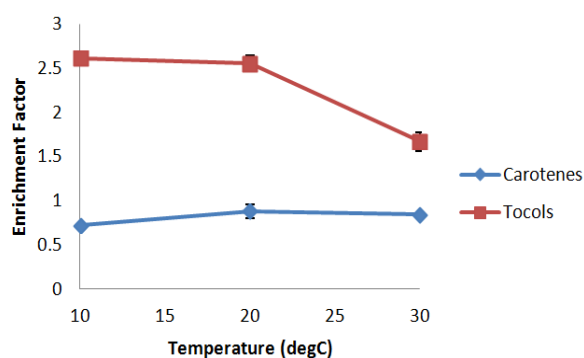


Figure 1. The enrichment factor of carotenes and tocols at 10, 20 and 30°C.

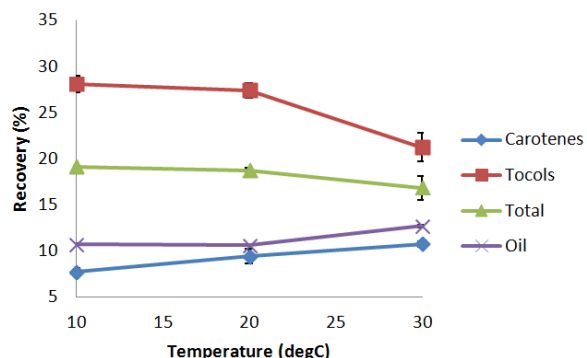


Figure 2. The percentage recovery of carotenes, tocols and oil at 10, 20 and 30°C.

As the temperature increased further to 30°C, the enrichment factor and recovery of carotenes remained almost unchanged while they reduced drastically for tocols. Under such conditions from 10 to 30°C, temperature was not a determining factor to affect the extraction of carotenes. The solvents content in the oil phase ranged from 31.2, 32.2 and 42.0% as temperature increased from 10 to 30°C. The oil recovery was also getting higher at 30°C. With increasing temperature, more solvent diffused into the oil phase and vice versa. As a result, the selectivity reduced as more oil but less tocols were extracted at higher temperature. Similar results were reported by [14] whereby selectivity reduced at higher temperature. As there was more oil extracted at higher temperature with no additional carotenes and tocols being recovered, 20°C was chosen as the optimal temperature with best results.

3.4. The effect of the volume percentage of CPO

Figure 3 and 4 show the enrichment factor and recovery of carotenes, tocopherols and oil at 20, 30, 40, 50 and 60% of CPO when operated at 20°C and 10 min of mixing time using 3:2 v/v ethyl lactate/ethanol as the solvents. Referring to figure 3, 50% of CPO gave the highest enrichment factors for carotenes and tocopherols at 0.88 and 2.56, respectively. As the volume of CPO was less than 50%, there was lesser amount of carotenes and tocopherols available for extraction and hence, the driving force (concentration difference) for mass transfer would be lower. As opposed to tocopherols, the enrichment factor of carotenes remained almost constant near at 0.9. This implied that it was the maximum enrichment factor which could be achieved using 3:2 v/v of ethyl lactate/ethanol as the solvents. Any further increase in the amount of CPO will not improve the enrichment factor of carotenes. As the volume of CPO increased to 60%, the amount of carotenes and tocopherols increased along with other minor compounds such as FFA, glycolipids, squalene and sterols. Thus, the enrichment factor became lower largely due to competitive co-extraction.

The maximum recovery occurred at the lowest volume percentage of CPO at 20% as shown in figure 4. From 60% of CPO, the recovery of carotenes and tocopherols increased by 33.2 and 62.4%, respectively. The total recovery at 20% of CPO reached 55.1% while the recoveries of 36.5% carotenes and 71.6% tocopherols were achieved. The oil recovery increased along with phytonutrients recovery from 4.1 to 38.8% as the volume of CPO reduced. Since the volume of solvents increased when CPO reduced, more compounds including carotenes, tocopherols and oil were extracted leading to high recovery. Carotenes and tocopherols were minor components while oil (triglycerides) present in bulk. Therefore, the concentration difference was maintained for oil even at the lowest volume of CPO. As a result, more oil was extracted causing low enrichment factor with reducing volume of CPO as can be seen in figure 3.

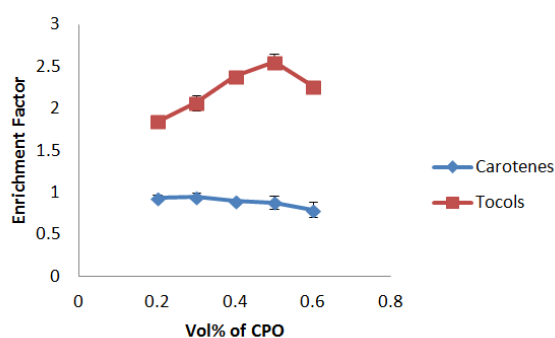


Figure 3. The enrichment factor of carotenes and tocopherols at 20, 30, 40, 50 and 60% of CPO.

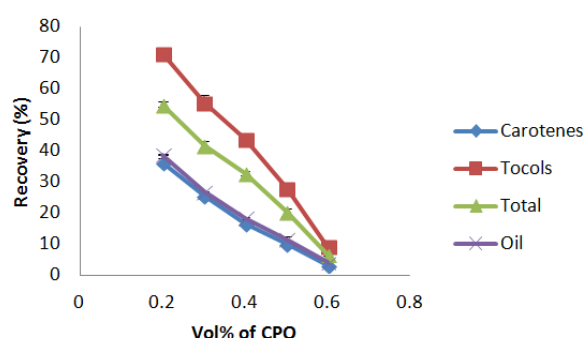


Figure 4. The percentage recovery of carotenes, tocopherols and oil at 20, 30, 40, 50 and 60% of CPO.

As the remaining treated oil phase is returned to the oil refineries, the increase of the subsequent oil throughput due to solvent addition and the recovery of oil into solvent phase are not wanted. As the volume of CPO increased, both oil throughput and solvent content in the oil phase increased as illustrated in figure 5. As the volume of CPO increased, less oil diffused into the solvent phase, but more solvents diffused into the oil phase. The oil throughput should be near to unity while the oil recovery should be minimized. Hence, the optimal point was controlled at 50% of CPO when the oil throughput is 1.25 with 32.5% of solvents in the oil phase while 11.6% of oil was recovered into the solvent phase. Subsequent solvent removal from the oil phase could be carried out before conventional oil refining process. However, the presence of these solvents in the oil does not affect the safety of the palm oil because they are both food grade and safe for human consumption. At 50% of CPO, the highest enrichment factors were achieved at a total recovery of 20.3%. Test at 10% of CPO was also investigated, but was not reported here because the oil phase became too little (~11.5 mL) to be measured accurately. In addition, the overall operation takt time would increase as only 10% of CPO could be treated in a single run with lower enrichment factor for tocopherols as more oil was recovered. The

optimum with the best extraction performance was found at 50% of CPO, 10 min of mixing at 20°C using 3:2 v/v ethyl lactate/ethanol as the solvents.

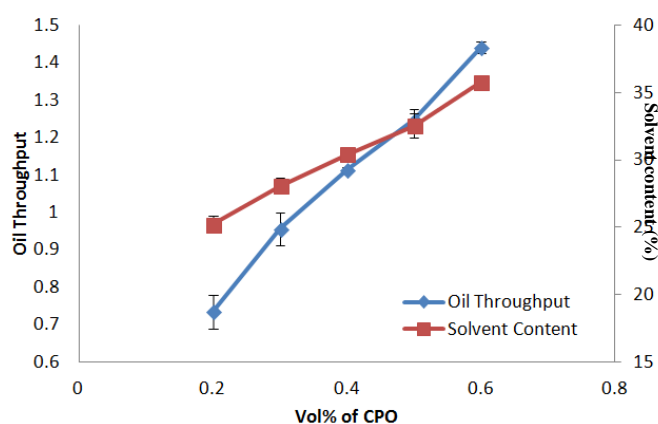


Figure 5. The oil throughput and solvent content in the oil phase from 20 to 60% of CPO.

3.5. Multistage extraction

At 20°C, 10 min of mixing and 50% of CPO using 3:2 v/v ethyl lactate/ethanol as the solvents, four stages of mixing-settling were carried out. Upon completion of a stage, the solvent phase at the bottom was withdrawn, measured and analyzed. Fresh premixed solvents were then added for another cycle of mixing-settling. Table 5 is a compilation of the enrichment factors and recoveries in each stage while table 6 compiles the cumulative results, including the previous fraction(s) collected. The cumulative enrichment factor for carotenes increased while it decreased for tocols along the stages. The improvement of the cumulative enrichment factor for carotenes was not obvious because the enrichment factor remained close to unity in each stage. Conversely, the cumulative enrichment factor for tocols reduced in subsequent stages. This was because the concentration of tocols reduced after each extraction cycle and hence, the concentration of the extracted tocols reduced from 2074.1 to 887.8 mg/L even though higher separation (enrichment factor) was achieved.

Table 5. The enrichment factor and percentage recovery of carotenes, tocols and oil in each stage.

Stage		1	2	3	4
Enrichment factor	Carotenes	0.82	0.90	0.98	1.09
	Tocols	2.73	2.68	2.94	3.37
Recovery (%)	Carotenes	10.1	15.3	13.2	12.7
	Tocols	33.5	45.6	39.4	39.3
	Total	21.8	28.3	22.1	19.4
Oil recovery (%)		12.3	17.1	13.5	11.7

Table 6. The cumulative enrichment factor and percentage recovery of carotenes, tocols and oil over stages.

Stage		1	2	3	4
Enrichment factor	Carotenes	0.82	0.88	0.92	0.96
	Tocols	2.73	2.34	2.11	1.96
Recovery (%)	Carotenes	10.1	23.8	33.9	42.2
	Tocols	33.5	63.8	78.1	86.7
	Total	21.8	44.0	56.2	64.6
Oil recovery (%)		12.3	27.3	37.0	44.4

After these four cycles, four parts of solvents were utilized for extraction per part of CPO. This was similar to the solvent consumption at 20% of CPO as presented earlier in section 3.4. It was found that

with the same amount of solvent being consumed per part of CPO, multistage extraction achieved higher enrichment factors and recoveries. When single stage was carried out, the percentage oil recovery at 38.6% was only slightly lower as compared to multistage at 44.4%. Therefore, multistage extraction performed better than single stage system when the same amount of solvent was used.

4. Conclusions

The process of carotenes and tocopherols recovery was optimized at 20°C, 50% of CPO, 10 min of mixing using 3:2 v/v ethyl lactate/ethanol as the solvents. The operation was fast, simple, green and safe which can be retrofitted the existing palm oil refining line as a cheaper alternative of red palm oil production. It was carried out in a temperature-controlled mixer-settler unit to recover 42.2% of carotenes and 86.7% of tocopherols in CPO enriched by 0.96 and 1.96 for carotenes and tocopherols, respectively, after four extraction cycles.

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