CONCENTRATION OF MINOR COMPONENTS IN CRUDE PALM OIL

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This study deals with the enrichment of minor constituents from crude palm oil with supercritical CO_2. Direct extraction of carotene using CO_2 is not very practical, so the main component of crude palm oil, triglyceride, has to be esterified. Fatty acid ester has a solubility magnitudes higher than triglyceride in CO_2. The beginning material is crude palm oil, which has a concentration of free fatty acid up to 4%, 600 ppm tocochromanols and 500 ppm carotene. The free fatty acid was first separated, and the crude palm oil was then esterified to fatty ester methyl esters(FAME) with methanol using base catalyst. Afterwards, the glycerol was separated and the product is washed with water to remove catalyst and methanol. In a pilot countercurrent extraction apparatus, which was built according to the mixer settler principle, fatty acid methyl esters were extracted at 60 °C and 140 bar. At these operating conditions, the CO_2 has a loading around 3 wt%. The apparatus is operated with a CO_2 flow rate of 20 kg/h. With two steps of extraction, an enrichment of up to 100 times was found.

INTRODUCTION

The purpose of this study is the enrichment of minor constituents such as tocochromanols and carotenoids from crude palm oil with supercritical CO_2. Currently, interest is focusing on the nutritional aspects of the minor constituents. Tocopherol and tocotrienol are well recognized for their antioxidative effect. In general, antioxidants are suggested to reduce cardiovascular disease (CVD) and cancer. In the field of cancer chemotherapy, tocotrienols have been shown to display better anti-tumor activity than α-tocopherol, the compound usually found in vitamin E supplements. Hence, the role of tocotrienol in the prevention of cardiovascular disease and cancer may have significant implications[5]. Carotenes, in particular β-carotene, have long been known for their provitamin A activity, as they can be transformed into vitamin A in vivo. The cholesterol-lowering effect of sterols, which were isolated from soybeans, was first shown in the 1950s. Later numerous studies reported the hypocholesteremic potency of plant sterols. Since these minor constituents are growing in importance and value, their recovery from palm oil is becoming more important.

The beginning materials for the enrichment from earlier studies are always the co-products from the oil refining process, such as deodorizer distillates. This can lead to difficulties in further enrichment since there are many unknown components.

Crude palm oil is mainly composed of triglyceride, which is only little soluble in CO_2. In order to achieve an enrichment of the minor constituents, glyceride has to be removed.

Since the solubility of fatty acid methyl ester in CO_2 is magnitudes higher, triglyceride could be esterified into methyl ester to be removed. An overview of the esterification process is given in Gutsche[2]. Freedman[1] also studied variables affecting the yields of transesterification reaction. In comparison to industrial practice, where methyl esters are removed by using vacuum distillation or molecular distillation, supercritical CO_2 is used to extract the methyl ester.

MATERIALS AND METHODS

Materials:

Crude palm oil was obtained from Cargill GmbH (Germany). It contains 600 ppm tocochromanols ppm and 500 ppm carotenoids and 4% free fatty acid. Methanol (99.8%) was purchased from Riedel-deHaën.(Germany). KWD (Germany) delivered carbon dioxide 99.95 % pure.

Apparatus:

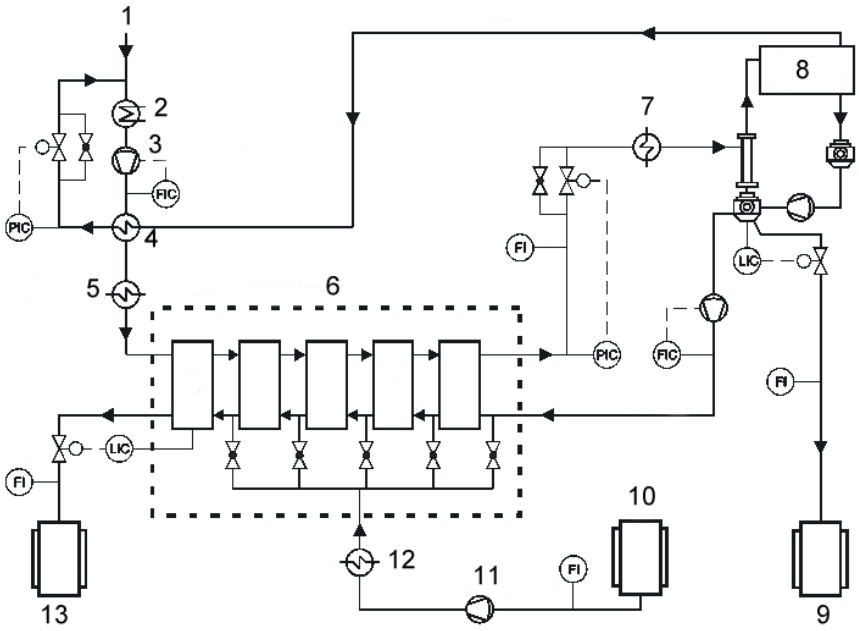

Figure. 1 Schematic diagram of the mixer settler apparatus (1)CO_2 supply (2)condenser (3)diaphragm pump (4)heat exchanger (5)gas preheater (6)Mixer settler stages in an air-circulation oven (7)gas warmer (8)droplets separator (9)extract container (10)feed container (11)syringe pump (12)feed preheater (13)raffinate container

With supercritical CO_2, fatty acid methyl ester made from crude palm oil is extracted using a five stage counter-current extraction apparatus constructed according to the principle of mixer settler. In Figure. 1, a schematic diagram of the pilot scale extraction apparatus used in this work is shown. It is designed to operate up to 300 bar and 120 °C [4].

The used solvent can be recycled. The prewarmed feed is supplied to the first stage of the mixer-settler apparatus by a syringe pump (11). Firstly, CO_2 is liquidized with a condenser

(2). Then it is brought up to the operating pressure with a triplex diaphragm-pump (Lewa, G3H) (3). This follows with the heat exchange (4) between the pressurized CO_2 and unloaded CO_2 which is flowing back to the condenser. The CO_2 is then brought up to the operating temperature (5). The five-stage mixer-settler apparatus is placed in an air-circulation oven (Heraeus)(6) that delivers 53 m^3 /min air to keep the temperature constant. Each stage of mixer-settler is made of a side-channel pump, a diffuser and a cyclone. In the side-channel pump of a mixer-settler stage, the feed and the CO_2 are well mixed, since the pump provides good contact between the two phases. Afterwards, the mixture enters a diffuser to extend the retention time for equilibrium. It enters then a cyclone, where phase separation takes place. The separated raffinate is collected in a reservoir and transported to the next stage. While CO_2 flows against the feed in a counter-current manner, it is enriched with the light phase (FAME). After CO_2 passes through the five stages of extraction, the pressure is decreased and the gas is heated to counteract Joule Thompson effect. Through the decreased pressure the solubility of CO_2 is also decreased. The extract can then be obtained with cyclone while the CO_2 is recycled. The raffinate from the last stage is obtained through the expansion of the raffinate flow.

Enrichment procedure:

The enrichment procedure of minor constituents in crude palm oil performed in this work is shown in Figure. 2 Free fatty acid was removed firstly. This is followed by the transesterification of palm oil to fatty acid methyl ester. The glycerol produced was removed while the methyl ester obtained washed with water. Using mixer settler apparatus with supercritical fluid, the methyl ester extracted. The raffinate was then subjected to trans-esterification to esterify glycerides in the raffinate. A second step of extraction was done to further increase the contents of minor constituent. After the two-step extraction, a concentrate of minor constituent was obtained.

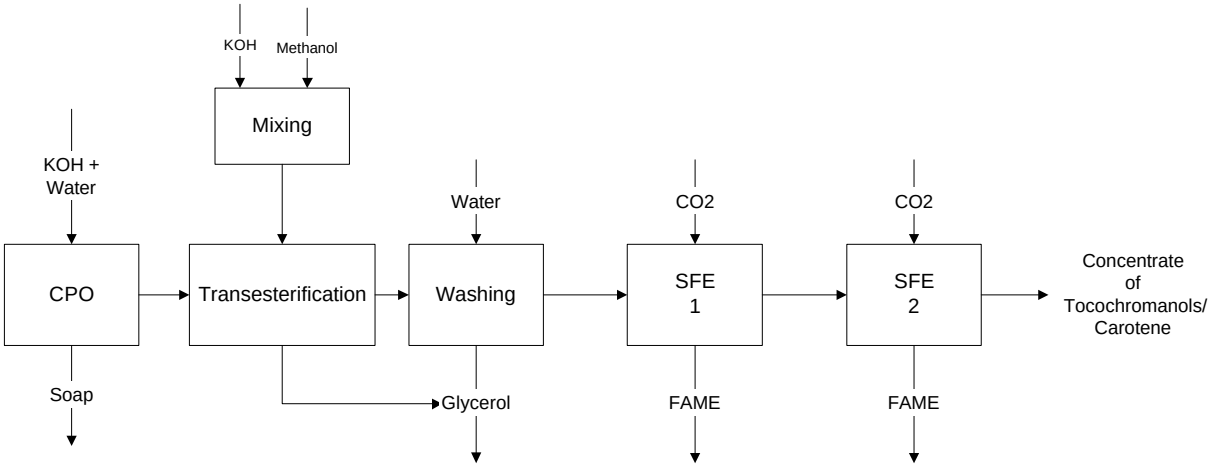

Figure. 2 Enrichment procedure

Analysis:

GC analysis:

The analysis of the sample drawn from counter-current extraction experiments were partly done with gas chromatography. (Hewlett Packard HP 5890 A capillary gas chromatograph) . The column used was a J & W Scientific fused silica(DB-5ht) column (30 m x 0.25 mm ID with 0.1 μm coating). The oven temperature was maintained at 150 °C for 2 minutes and raised to 230 °C at a gradient of 5 °C/min and to 270°C at a gradient of 2 °C/min. The internal standard is squalane. Preliminary to the calculation of sample composition, determination of response factor was done using reference standard. From the area of the peaks in the chromatograph, the contents of each component are calculated.

In order to obtain reliable quantitation of mono- and diglycerides, derivatization of these glyceride species must be done. By silylation of the free hydroxyl groups of mono-and diglycerides, excellent peak shapes of mono-and diglycerides can be achieved. The silylating agent was N-methyl-N-trimethylsilyl-trifluoracetamide (MSTFA). Tetradecane was used as internal standard. The oven temperature program for glyceride analysis is as followed: it was maintained at 80 °C for 2 minutes and raised to 360°C at 10 °C/min and kept at 360°C for 20 minutes.

Tocochromanols analysis:

Tocochomanols contents in the samples were analysed with HPLC equipped with a detector Gynkotek RF 1002 fluorescence and a pump (Shimadzu LC-6A). The column used was LiChrosorb Diol 5μm (250 x 4.6 mm). The mobile phase used was hexane and butyl-methyl-ether with 96 [wt%] and 4 [wt%] at a rate of 1.3 ml/min. Approximately 20 mg sample was weighted and dissolved with 1 ml Hexane. 20 μl was then injected. The calibration was done with four isomer of tocopherol, which was also analyzed as standard solution.

Carotenoids analysis:

The samples were dissolved and diluted in a solvent mixture of 30% acetone and 70% hexane. (Volume percentage) and analyzed by UV spectroscopy at a wavelength of 450 nm. The carotenoids content was then calculated using the calibration curve, which was obtained using known concentration of β-carotene.

RESULTS:

1. First step extraction:

After being deacidified, the crude palm oil was esterified and used as feed for the extraction experiments. The experiments were conducted at 140 bar, 60 °C using mixer settler apparatus. The CO_2 flow rate was 20 kg/h. The feed flow rate was 640 g/h. The raffinate flow rate was 40 g/h and the extract flow rate was 600 g/h. At 60 °C and 140 bar, the gas load of methyl palmitate is 0.041 g/g_{co2} while the gas load of methyl oleate is 0.02 g/g_{co2}[3]. These two methyl esters are the main components in palm fatty acid methyl ester.

The composition of feed, raffinate, and extract is shown in Table 1 and Table 2. In general, a ten times enrichment of the minor constituent was obtained. For β-carotene, it is even thirteen times. The contents of fatty acid methyl ester was decreased from 96 [wt%] to 56 [wt%], while glycerides were also enriched in the raffinate.

Table 1: Contents of minor constituents in first step extraction experiments

Minor components	**Feed** [ppm]	**Raffinate** [ppm]	**Extract** [ppm]
α-tocopherol	135	585	40
α-tocotrienol	131	796	0
β-tocopherol	0	33	0
γ-tocopherol	0	31	0
β-tocotrienol	7	266	0
γ-tocotrienol	283	3381	20
δ-tocotrienol	54	1070	0
Tocochromanols	610	6162	60
Squalen	400	4000	0
Campesterol	100	600	0
β-Sitosterol	200	2000	0
Sterols	300	2600	0
β-carotene	550	7000	17

Table 2: Contents of major constituents in first step extraction experiments

Major components	**Feed** [%]	**Raffinate** [%]	**Extract** [%]
Methyl myristate	0,99	0,10	1,10
Methyl palmitate	42,50	13,81	44,78
Methyl linoleate+ Methyl oleate	47,50	38,74	48,67
Methyl stearate	4,43	3,50	4,46
Monoglyceride	0,60	10,00	0,30
Diglyceride	0,40	9,00	0,00
Triglyceride	0,40	20,00	0,00

2. Second step extraction:

After many extraction experiments at 140 bar, 60 °C using mixer-settler apparatus, enough raffinate was collected. It was then esterified again, and used as the feed of the second step extraction experiment. The CO_2 flow rate was 20 kg/h. The feed flow rate was 600 g/h. The raffinate flow rate was 40 g/h and the extract flow rate was 560 g/h. The composition of feed, raffinate, and extract is shown in Table 3 and Table 4.

In the second step of the extraction experiment, β-carotene and sterols also became 10 times concentrated. The enrichment ratio for tocochromanols and squalene are lower, only around 6.5 and 5 times. This is resulted from the higher solubility of squalene and tocochromanols, as can be seen from the content of extract. The contents of fatty acid methyl ester was decreased from 93 to 20 %.

Table 3: Contents of minor constituents in second step extraction experiments

Minor components	**Feed** [ppm]	**Raffinate** [ppm]	**Extract** [ppm]
α-tocopherol	623	3941	93
α-tocotrienol	836	5510	106
β-tocopherol	26	147	0
γ-tocopherol	15	136	0
β-tocotrienol	191	1273	11
γ-tocotrienol	2550	16401	264
δ-tocotrienol	764	4907	29
Tocochromanols	5004	32316	504
Squalen	4600	22000	1800
Campesterol	600	5352	0
β-Sitosterol	1800	17454	0
Sterols	2400	22806	0
β-carotene	5500	54000	270

Table 4: Contents of major constituents in second step extraction experiments

Major components	**Feed** [%]	**Raffinate** [%]	**Extract** [%]
Methyl myristate	0,51	0,00	0,59
Methyl palmitate	32,16	3,21	36,11
Methyl linoleate+ Methyl oleate	54,01	11,88	56,30
Methyl stearate	5,94	1,89	6,16
Monoglyceride	1,5	9,2	0,8
Diglyceride	0,3	7,2	0
Triglyceride	0,3	10	0

CONCLUSIONS

The concentration of minor constituents in crude palm oil using supercritical fluid was studied. After two steps of extraction, tocochromanols were concentrated from 600 ppm to 33000 ppm. Squalene was concentrated from 400 ppm to 22000 ppm. Sterols were concentrated from 300 ppm to 23000 ppm. β-carotene was concentrated from 550 ppm to 54000 ppm. An enrichment of 60 to 100 times was obtained. As the extraction experiments were only performed at 140 bar, 60°C, operating condition can be further optimised to increase the enrichment ratio. From the above discussion, it is demonstrated that SFE can be an alternative method to recover minor constituents in crude palm oil.

REFERENCES :

[1] FREEDMAN B., PRYDE E., Journal of american oil chemists' society, Vol. 61, **1984**, p. 1638

[2] GUTSCHE B., Fett/Lipid, Vol. 99, **1997,** p. 418

[3] INOMATA H.; KONDO T., Fluid Phase Equilibria, Vol. 46, **1989**, p. 41

[4] PIETZONKA W., Mehrstufige Gegenstromapparatur nach dem Mixer-Settler-Prinzip für die Extraktion mit überkritischen Fluiden., **1997**, ETH Zürich, PhD thesis

[5] THERIAULT A., CHAO J., Clinical Biochemistry, Vol. 32, **1999**, p. 309