SEPARATION OF VITAMIN-E ISOMERS FROM HIGH-OLEIC PALM OIL FAMES BY SUPERCRITICAL DESORPTION

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ABSTRACT

Tocotrienols present in palm oil are gaining interest for their biological activity, which might be useful for preventing and controlling pathologies such as high cholesterol and various forms of cancer. We have been studying a process for concentrating tocotrienols present in the oil obtained from a hybrid variety of palm tree (*Elaeis guineensis* x *Elaeis oleifera*, or the so-called "high-oleic" palm tree) that was developed in Colombia. Fatty acid methyl esters (FAMEs) prepared from high-oleic palm oil initially contain a tocotrienols/tocopherols (T3/T) ratio of approximately 5. In our experiments, FAMEs were initially treated with bleaching clays to remove up to 93% of carotenoids. Then, adsorption of tocopherols and tocotrienols on silica gel was carried out at 30 ºC in a batch process to remove up to 61 wt% of these compounds. The resulting solid was then desorbed with supercritical CO_2 to establish the operating conditions at which maximum separation of vitamin E isomers is obtained.

The desorption runs were arranged according to a factorial experiment. Temperatures from 40 to 70 ºC and CO_2 densities from 0.6 to 0.8 g/mL were considered as controlled variables. For each run, fractions were collected each 30 minutes during 6 h. Desorption yield and T3/T ratio were determined for each fraction. Desorption produced yields from 20 wt% for the first fraction up to 0.10 wt% for the last one. T3/T ratios from 2 up to 18 were observed for the different fractions, which indicates that the desorption process has the capability of separating tocotrienols from tocopherols.

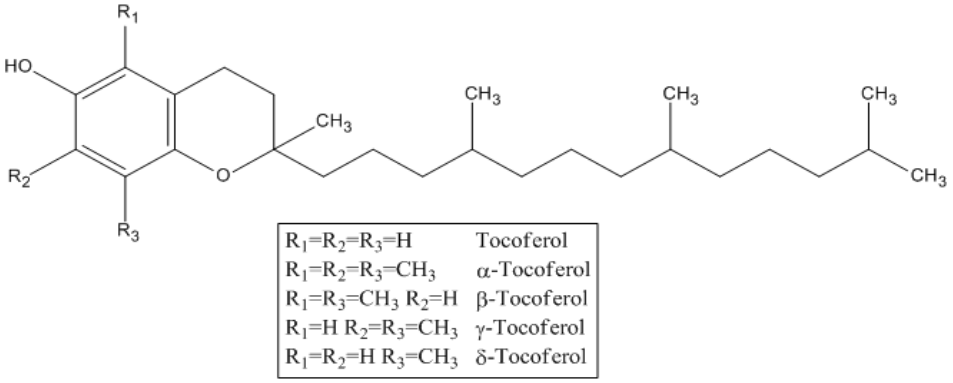
INTRODUCTION

Palm oil has become the second vegetable oil most produced in the world after soybean oil. A hybrid variety of palm tree developed in Colombia (*Elaeis guineensis* x *Elaeis oleifera*, or the so-called "high-oleic" palm tree) is the species with the greatest average annual production of oil, 6 Ton/Ha, as compared to 4 Ton/Ha for African palm tree (*Elaeis guineensis*) and 2.7 Ton/Ha for soybean. As all vegetable oils, palm oil is composed mainly by triglycerides (95%), but it also contains minor components such as carotens, sterols, and vitamin E [1]. The latter is a lipophilic vitamin composed by two homologous series of compounds (collectively called tocols): tocopherols and tocotrienols. Figures 1 and 2 show the chemical structures of these two series of compounds. Tocopherols are characterized by a saturated side chain attached to a chroman ring, while tocotrienols have an unsaturated side

chain. Four isomers for each type of compound exist [2], which are designated as α, β, δ and γ, respectively.

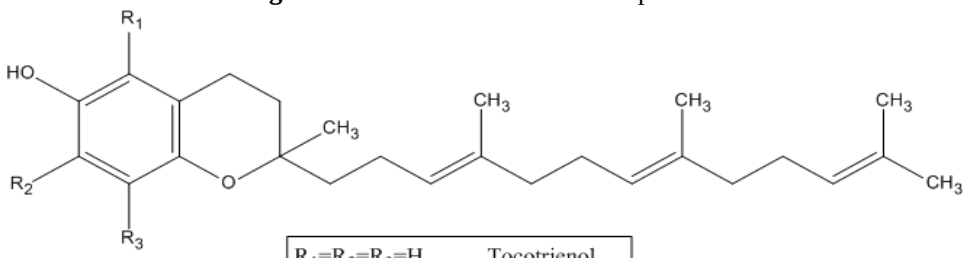
High-oleic palm is among the species with larger contents of tocols and particularly of tocotrienols. The contents of tocols in the crude oil is higher than in other sources such as rice and barley oils, and is approximately 1000 ppm, from which 20 wt% corresponds to tocopherols and 80 wt% to tocotrienols [1,3].

Due to the economic importance of palm oil and thus to the wide availability of byproducts of the oil processing, there have been efforts for developing technologies for industrial extraction of minor components. Separation of the vitamin-E isomers is particularly important because there is increasing scientific evidence on the applications of γ- and δ- tocotrienols in medicine [4,5], mainly for preventing and controlling pathologies such as high-cholesterol and several forms of cancer. As a result, there is an important opportunity for the development of high-added value products from this readily available and low-cost raw material.



$R_1=R_2=R_3=H$	Tocoferol
$R_1=R_2=R_3=CH_3$	α-Tocoferol
$R_1=R_3=CH_3$ $R_2=H$	β-Tocoferol
$R_1=H$ $R_2=R_3=CH_3$	γ-Tocoferol
$R_1=R_2=H$ $R_3=CH_3$	δ-Tocoferol

Figure 1. Chemical structure of tocopherols.



$R_1=R_2=R_3=H$	Tocotrienol
$R_1=R_2=R_3=CH_3$	α-Tocotrienol
$R_1=R_3=CH_3$ $R_2=H$	β-Tocotrienol
$R_1=H$ $R_2=R_3=CH_3$	γ-Tocotrienol
$R_1=R_2=H$ $R_3=CH_3$	δ-Tocotrienol

Figure 2. Chemical structure of tocotrienols.

Recently, some techniques for extracting vitamin-E isomers from vegetable oils have been proposed. For example, Mendes et al. [6], produced a concentrate of vitamin E from deodorizer distillate of soybean oil using supercritical CO_2. This process was achieved with a previous methylation of the fatty acids to facilitate the extraction. In other studies [7], carotens and vitamin E enriched oils have been obtained from fresh palm-pressed mesocarp fiber using supercritical CO_2. Several researchers [8-10] have been studying supercritical countercurrent extraction to obtain a product with a vitamin E concentration higher than 50%.

Further supercritical fractionation of the extracts produce pure isomers of tocopherols and tocotrienols.

Other authors have been studying the use of adsorption on silica gel for separation of vitamin E from palm fatty-acid distillate [12,13]. Efforts to use supercritical fluid chromatography to isolate carotens and vitamin E isomers have also been presented [11]. Briefly, supercritical CO_2 with a cosolvent such as ethanol has being used to fractionate a mixture of carotens and tocols by passing a stream of these components through a column loaded with a suitable adsorbent. The results have indicated that this process is technically feasible. Unlike other kinds of palm oil, the high contents of carotens present in high-oleic palm oil might interfere with adsorption of tocols.

In this paper we describe our efforts for developing a process to separate vitamin E isomers from high-oleic palm oil, by adsorbing these minor oil components on silica gel, followed by desorption with supercritical CO_2. Our aim is to develop a process that can be easily integrated into existing biodiesel production processes.

MATERIALS

Crude palm oil was obtained from a local producer (Hacienda la Cabaña, Colombia). CO_2 (99.9% purity) was obtained from Cryogas (Cali, Colombia). Neutral bleaching clays were obtained from Oil-Dri (Chicago, USA). Column chromatography-grade silica gel 60 (particle size 0.063 to 0.200 mm; specific pore volume 0.74 to 0.84 mL/g; specific area 480 to 540 m^2/g) was purchased from Merck, Darmstadt, Denmark. All chemicals used were either of analytical or high performance liquid chromatography (HPLC) grades.

EXPERIMENTAL EQUIPMENT

Adsorption of tocols on silica gel was performed in a three-necked 250 mL glass vessel, which was magnetically stirred and was located in an isothermal water bath. The vessel was covered with an opaque adhesive metallic tape to prevent the adsorbing mixture from degradation due to light exposure. An inert gas (CO_2) was preheated in a coil immersed in the isothermal bath and was supplied to the vessel using one of its necks to prevent degradation of tocos due to the presence of oxygen. A piece of tubing was introduced to the vessel through the third neck to collect samples of the liquid by using vacuum. The water bath and thus the adsorption temperature were controlled to ± 0.3 ºC.

Figure 3 shows a schematic representation of the supercritical fluid desorption apparatus that was previously built in our laboratory and was modified in this work for using a larger extractor. This desorption (extraction) vessel was fabricated in stainless steel 316, has a useful volume of 100 cm^3 and is rated for pressures up to 10000 psi at 200 °C. For operation of this apparatus, CO_2 from a cylinder is maintained at -10 °C by passing it through a heat exchanger which works with a stream of ethylene glycol as refrigerating fluid. Then, the CO_2 is brought to a desired pressure by using a Williams- Milton Roy pneumatic pump (model CP250/V225, rated for pressures up to 7000 psi), and it is passed through the desorption (extractor) vessel, which has been previously loaded with the solid obtained from the adsorption process. Both pressure and flow rate are regulated by using a micrometering valve (HiP, model 1511AF1-REG) located after the extractor, and the samples are collected in amber test tubes at the exit of this valve. Pressure is determined with a Bourdon pressure gauge (Ashcroft, model 3005HL, 0 to 5000 psi, with marks every 100 psi) placed in the extractor feed line. Pressure can thus be determined with a precision of ± 50 psi.

EXPERIMENTAL PROCEDURE

Crude palm oil was obtained from a local company (Hacienda La Cabaña, Colombia) and was filtrated in a vacuum system to eliminate the stearine fraction that is usually present in crude palm oil. Then, palm oil FAMEs were produced. 1 L of the filtered oil was mixed with 200 mL of NaOH 0.4 M in methanol. This mixture was maintained at 60 ºC, with continuous stirring under total reflux for 1.5 hours. The product was then washed with deionized water several times, until the pH of the residual water was neutral. This procedure guarantees the elimination of glycerin, methanol and NaOH from the produced FAMEs.

FAMEs were then treated to remove carotens by adsorption on neutral bleaching clays (provided by Oil-Dri, Chicago) in a 2 wt% concentration. This process was conducted in the three-necked glass vessel described above, at 90 ºC, under CO_2 atmosphere and continuous stirring during 1 h. Removal of carotens was quantified by spectrophotometry. A Thermo Scientific Evolution 60 spectrophotometer was used to measure the absorbance of FAMEs at 440 nm during the bleaching process. Using this information the bleaching efficiency was determined.

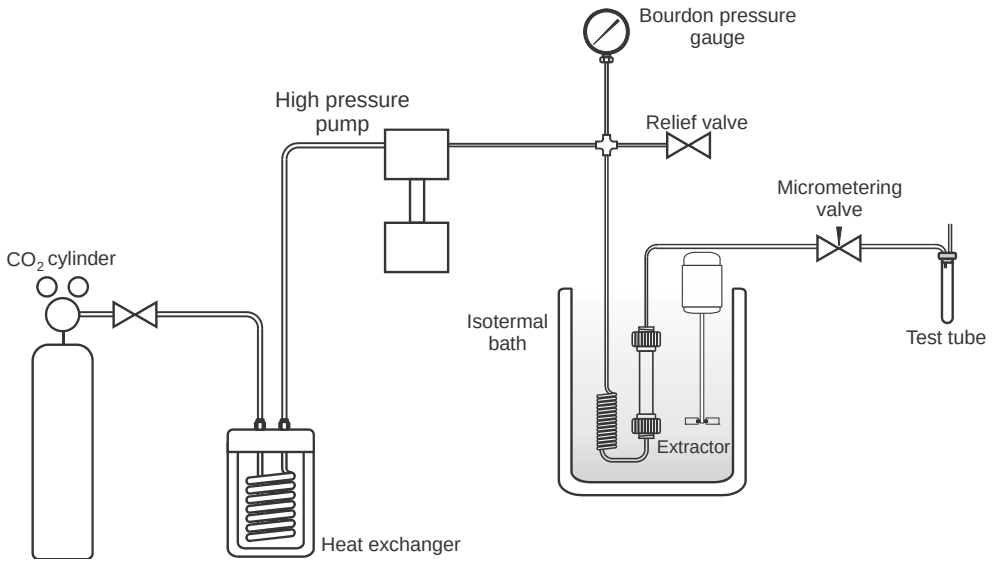


Figure 3. Experimental apparatus that was used for desorption.

The bleached FAMEs were then subjected to adsorption on silica gel. A sample of FAMEs and silica gel in a specific mass ratio were loaded into the glass vessel, which then was immersed into the isothermal bath. After temperature reached a steady value, the inert gas flow rate was set to 60 mL/min and the stirring was turned on. The system was left at these conditions for 2 h until the adsorption reached equilibrium. Samples of the liquid were obtained at 5, 15, 30, 60 and 120 min, and were analyzed by HPLC for their concentration of tocols. After 2 h the mixture was vacuum filtered and the obtained solid was saved for the supercritical desorption step.

In a desorption experiment, the product of the adsorption (silica + adsorbed compounds) was loaded into the extractor, which then was immersed into the isothermal bath. After few minutes in which the temperature reached a steady value, carbon dioxide was pumped maintaining the micrometering valve fully closed until the desired pressure was reached. At this moment, the micrometering valve was slowly opened, until steady values of both pressure and CO_2 flow rate were obtained. The desorption time considered in each run varied from 4 to 7 hours, depending on the amount of the liquid product that was obtained.

Desorption fractions were obtained each 30 min, and the samples were analyzed by HPLC [13] for determining their profile of vitamin E isomers.

EXPERIMENTAL DESIGNS

An augmented 2^2 factorial experiment was run to explore the effect of adsorption temperature and FAME`s/silica ratio on tocols adsorption. This response variable was defined as the percentage of tocols that are removed from the initial FAMEs. Temperatures from 30 to 60 °C, and FAMEs/silica ratio from 8.33 to 25 mL/g, were used as conditions for the factorial experiment. At each one of the 4 possible combinations of these conditions two experimental runs were made. Two more runs were planned at 45 °C and 12.5 mL/g (the so called "augmentation") to determine the experimental error.

Another augmented 2^2 factorial experiment was run to explore the effect of temperature and CO_2 density on the desorption yield and the tocotrienols/tocopherols ratio of the product. Desorption yield was defined as the percentage of the adsorbed material that is desorbed by supercritical carbon dioxide. Temperatures between 40 and 70 °C, and CO_2 densities between 0.6 and 0.8 g/cm^3 were considered. At each one of the 4 possible combinations of these variables one experimental run was made. Two more runs were planned at 55 °C and 0.7 g/cm^3 to obtain an indication of the reproducibility of the results. For each combination of temperature and CO_2 density, we used the Bender equation of state [14] to calculate the pressure at which the corresponding run was to be made.

RESULTS AND DISCUSSION

Figure 5 shows photographs of the raw material in different treatment steps before adsorption. Pretreatment of the crude high-oleic palm oil consisted in filtration, methylation and bleaching. As mentioned, filtration removed the stearine fraction present in crude palm oil. Methylation produced FAME´s that have viscosities lower than that of crude oil (3 vs. 35 cP at 25 ºC). Lower viscosities increase the mass transfer rate during further steps of bleaching and adsorption. The bleaching process was conducted to separate carotenes and other pigments that interfere with adsorption of tocols on silica gel. The bleaching efficiency that was determined was 93%.

Figure 6 shows the tocols adsorption from bleached FAME´s at 30 ºC and a FAME´s/silica ratio of 8.33 mL/g. Note that the adsorption took place rapidly at the initial stages and then proceeded gradually to reach equilibrium after about 40 min. For all the experiments that were run in this work, equilibrium was reached at about this time. These results are similar to those obtained by other researchers [12,15], but because in those works the FAMEs were dissolved in hexane, which decreases the viscosity of the liquid, a shorter time for reaching equilibrium was reported.

Table 2 shows the adsorption of tocols at equilibrium. The results shown correspond to duplicated runs and show a high reproducibility. Note that the percent of adsorbed tocols increase with decreasing FAME´s/silica ratio and also with decreasing temperature. On the other hand, the effect of each operating variable on the tocols adsorption is independent of the level at which the other variable is considered; for example, the adsorption increases with decreasing FAME´s/silica ratio both at low and high values of temperature. Thus, there is not an interaction between the two operating variables.

With these results, and considering that a larger percentage of tocols adsorption is needed before an industrial process can be developed, an additional adsorption experiment was performed at 30 ºC, and a FAME´s/silica ratio of 3.5, during 2 h. As a result, a tocols

adsorption of 60.58% was obtained. Silica gel loaded with this amount of tocols was then used as raw material for the supercritical desorption experiments.

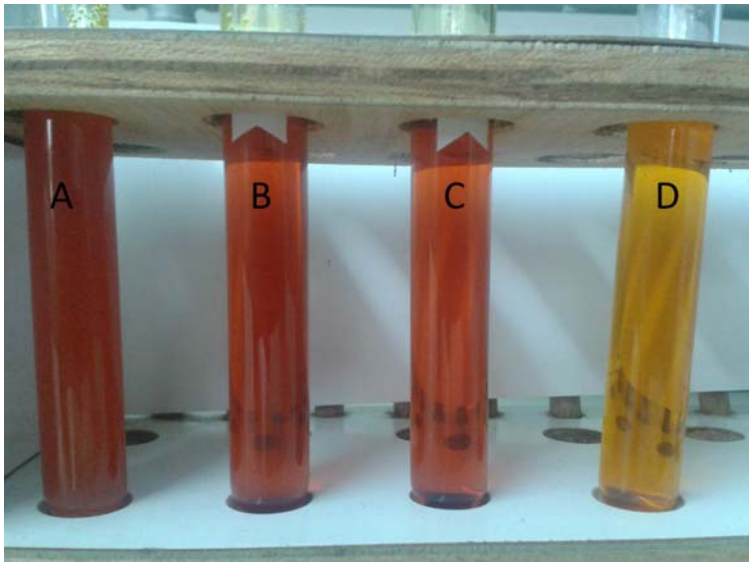


Figure 4. Photographs of the raw material in different pretreatment steps before adsorption process. (A) Crude palm oil, (B) filtered palm oil, (C) FAMEs, (D) Bleached FAMEs

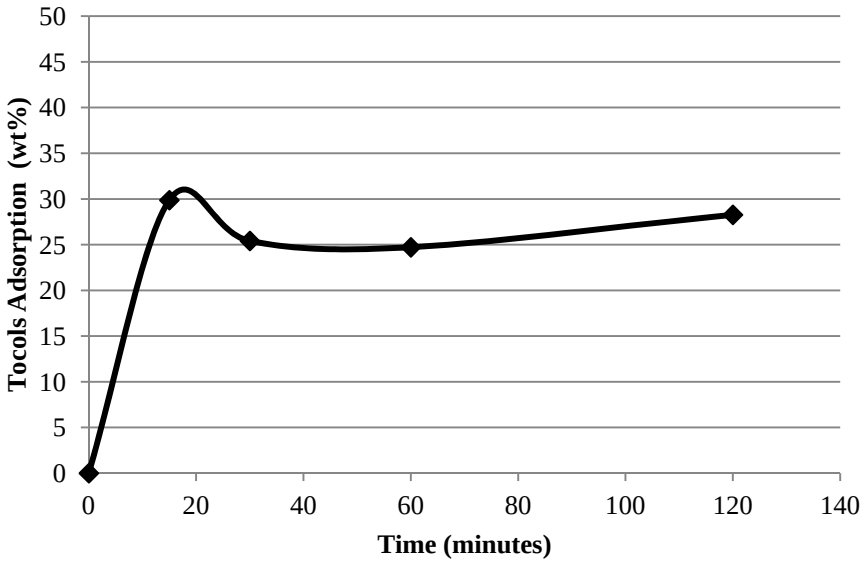


Figure 5. Tocols adsorption from bleached FAME´s at 30 ºC and a FAME´s/silica ratio of 8.33 mL/g.

Table 2. Adsorption of tocols at equilibrium.

Temperature (°C)	**FAMEs/silica ratio** (mL/g)	**Tocols adsorption** (%)
60	8.33	14.78 ± 0.90
30	8.33	24.25 ± 0.47
45	12.5	17.25 ± 0.49
60	25.0	4.56 ± 0.62
30	25.0	11.64 ± 2.20

Table 3 shows desorption yields and tocotrienols/tocopherols (T3/T) ratios for supercritical desorption of tocols from silica gel. The replicated results obtained at the central point (i.e., runs 3 and 4) indicate a reproducibility in the range of 2 wt%. An analysis of variance of these data indicated that the effect of temperature and CO_2 density are significant at 15 and 30% levels of significance, respectively. Note that desorption yield increases with increasing temperature, and also with increasing density at higher temperature. However, the magnitude of the experimental error is too large, as can be seen in the two runs at 55 ºC, to analyze the statistical significance of the interaction between temperature and CO_2 density.

Table 3. Desorption yields and tocotrienols/tocopherol ratios for supercritical desorption of tocols from silica gel.

Run	**Temperature** (°C)	**Pressure** (psi)	**CO_2 density** (g/cm^3)	**Desorption yield** (wt%)	**Tocotrienols/tocopherols (T3/T) ratio**
1	70	2500	0.6	56.49	5.54
2	40	2380	0.8	39.28	12.12
3	55	2450	0.7	44.42	14.21
4	55	2450	0.7	38.97	15.92
5	40	1415	0.6	38.83	22.25
6	70	4560	0.8	88.23	17.05

For each run in Table 3, fractions were collected each 30 minutes and were analyzed by HPLC to determine their contents of vitamin E isomers. To illustrate, Figure 6 shows the T3/T ratio obtained for the desorption run 6 of Table 3. The first 2 blocks are the T3/T ratios for filtered palm oil and FAMEs, respectively. The rest of the blocks are the T3/T ratios for the fractions taken from 1 to 7 h of desorption. Run 6 showed the highest desorption yield and also a T3/T ratio that increases with increasing time. We believe that the affinity of silica is higher for tocols than for FAMEs, carotenes and other compounds contained in the feed material. As a result, the first fractions are richer in these compounds. On the other hand, polarity of tocotrienols is larger than that of tocopherols, causing a weaker interaction between tocopherols and silica, and thus they elute first. Clearly, supercritical desorption generates a separation of vitamin E isomers.

CONCLUSIONS

Supercritical desorption is capable of producing a separation of the vitamin E isomers present in high-oleic palm oil. The operating conditions are moderate and there exists a potential for integrating this method with the regular processes that are being used by producers of biodiesel from this raw material.

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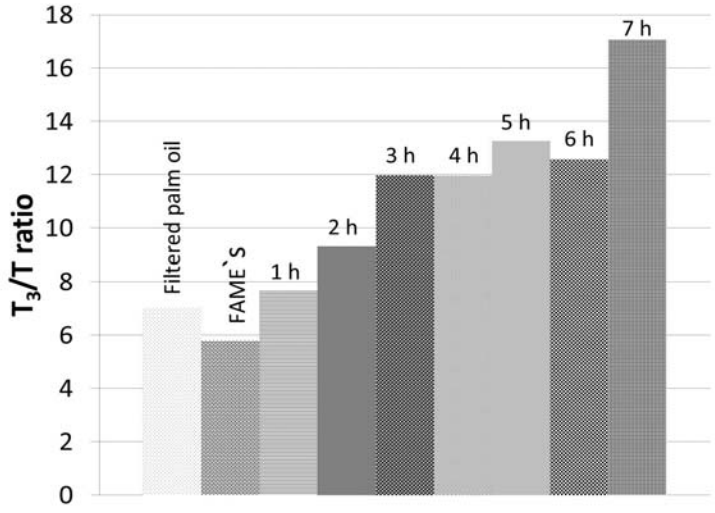

Figure 6. The tocotrienols/tocopherols ratio profile obtained for the desorption run 6 (4500 psi and 70 ºC).

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