SEPARATION OF VITAMIN-E ISOMERS FROM PALM OIL FAME`S BY SUPERCRITICAL DESORPTION

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Abstract. Palm oil is among the species with larger contents of Vitamin E. This vitamin is composed of eigth isomers; namely, α, β, γ, δ tocopherols and tocotrienols, respectively. The scientific literature reports several important medical applications of γ and δ tocotrienols, and thus a separation process for concentrating these compounds is desirable.

We have been studying a process based on adsorption on silica gel of the vitamin E isomers from the fatty acids methyl esters (FAME`s) of palm oil, followed by desorption with supercritical $CO_2$. Initially, FAME`s prepared from palm oil were treated with bleaching clays to remove up to 90% of carotens. Then, the adsorption of tocopherols and tocotrienols on silica gel was carried out at 30 ℃ in a batch process with continuous stirring under inert atmosphere. Up to 30 wt% of vitamin E removal from FAME`s was obtained. The resulting solid was desorbed with supercritical carbon dioxide in a lab-scale apparatus to establish the operating conditions at which maximum separation of vitamin E isomers is obtained.

The desorption experiments were arranged according to a factorial design, in which temperatures from 40 to 70 ℃, and CO_2 densities from 0.6 to 0.8 g/cm^3 were considered as controlled variables. For each desorption experiment, fractions were collected each 30 minutes during 6 h. Desorption yield and Tocotrienols/tocopherols ratio were then determined for each fraction. Typically, yields from 20 wt% for the first fraction to 0.10 wt% for the last one were obtained. Tocotrienols/tocopherols ratios from 8.2 for the first fraction to 1.8 for the last one were observed. These results indicate that separation of vitamin E isomers by supercritical desorption is technically feasible.

Keywords: Palm oil, tocotrienol, tocopherol, adsorption, supercritical desorption

1.   Introduction

African palm or oil palm (*Elaies guineensis*) is the plant with the greatest average annual production of oil, close to 4000 Kg/ha, and is widely cultivated in tropical countries. Palm oil has become the second vegetable oil most produced in the world after soybean oil. As all vegetable oils, palm oil is composed mainly by triglycerides (95%) but 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 croman 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. Palm is among the species with larger contents of tocols and particularly of tocotrienols. The contents of tocols in crude palm oil is higher than in other sources such as rice and barley oils, and is between 600 and 1000 ppm, from which 18 to 20 wt% correspond to tocopherols and 78 to 82 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], and thus there is an important opportunity for the development of high-added value products from readily available and low-cost raw materials.
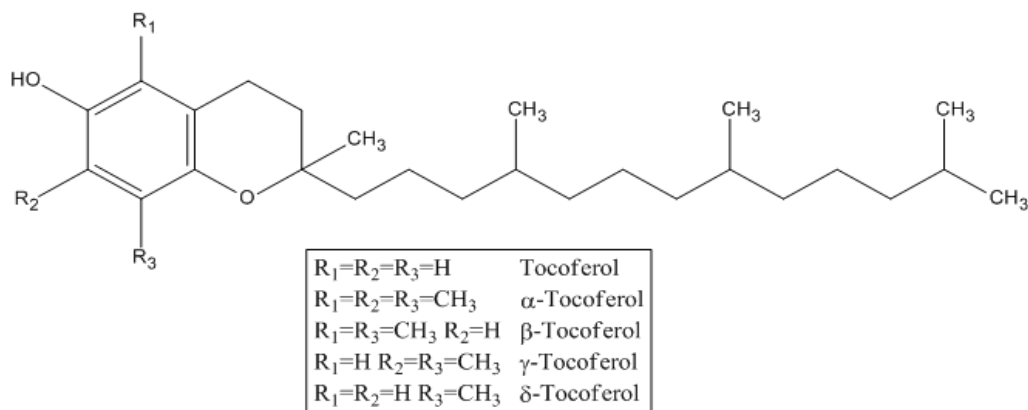

$R_1=R_2=R_3=H$	Tocoferol
$R_1=R_2=R_3=CH_3$	α-Tocoferol
$R_1=R_3=CH_3$ $R_2=H$	β-Tocoferol
$R_1=H$ $R_2=R_3=CH_3$	γ-Tocoferol
$R_1=R_2=H$ $R_3=CH_3$	δ-Tocoferol

Figure 1. Tocopherol´s chemical structure.

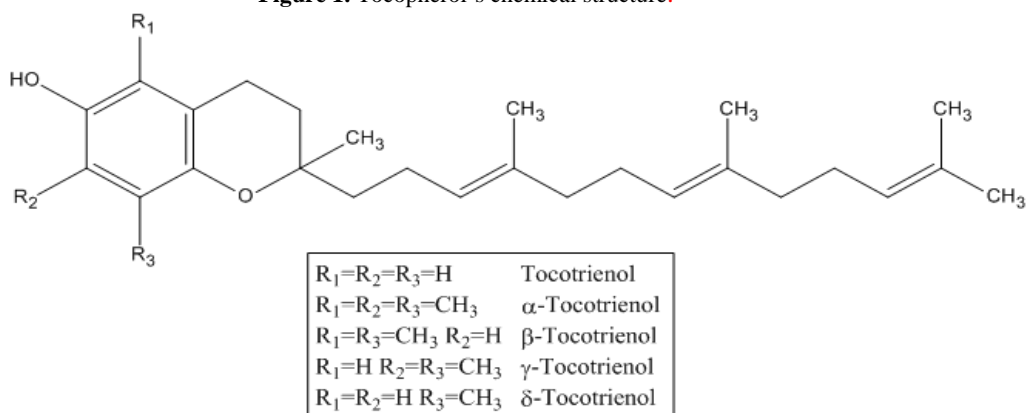|	
$R_1=R_2=R_3=H$	Tocotrienol
$R_1=R_2=R_3=CH_3$	α-Tocotrienol
$R_1=R_3=CH_3$ $R_2=H$	β-Tocotrienol
$R_1=H$ $R_2=R_3=CH_3$	γ-Tocotrienol
$R_1=R_2=H$ $R_3=CH_3$	δ-Tocotrienol

Figure 2. Tocotrienol´s chemical structure

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. An economic analysis showed that this is a feasible process for the industrial extraction of tocols [6-8]. In other studies [9], carotens and vitamin E enriched oils have been obtained from fresh palm-pressed mesocarp fiber using supercritical CO_2. Several researchers [10-13] have been studying supercritical counter current 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 [14, 15]. They showed that adsorption of tocols on silica gel is an exothermic process. The values of ΔH and ΔG are relatively large, which indicates that the prevailing mechanism during the process is chemisorption. The adsorption process is well described by the Langmuir isotherm, and thus the tocols adsorption mostly create a monolayer on the silica particles. Efforts to use supercritical fluid chromatography to isolate carotens and vitamin E isomers have also been presented [11-13]. 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.

In this paper we describe our efforts for developing a process to separate vitamin E isomers from palm oil, by adsorbing the 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 to existing biodiesel production processes.

2. Experimental apparatus and procedure

2.1 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 Corporation of America (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.

2.2 Equipment

Figure 3 shows a schematic of the experimental apparatus that was used for adsorption of tocols on silica gel. The adsorption vessel was a magnetically stirred 250 mL three-necked glass vessel, which was covered with an opaque metallic tape to prevent the adsorbing mixture from degradation due to light exposure. One of the necks is the entrance of an inert gas (CO_2) and a second one was coupled to a 1/8" tubing and is the exit of the gas. Before the inert gas arrives to the adsorption vessel it passes through a coil immersed in the isothermal bath. A piece of tubing was introduced to the vessel through the central neck to collect samples of the liquid by using vacuum without stopping the run.

The isothermal water bath is equipped with a type-K thermocouple, a temperature controller (DISAN, Model BS 1400) and a cartridge resistance. The water bath is connected to a magnetic bomb that circulates this fluid so that the temperature in the bath is homogeneous. The water bath and thus the adsorption temperature are controlled to ± 0.3 ℃.

Figure 4 shows a schematic representation of the supercritical fluid desorption apparatus that was built by our research group and was used in this research. This is a laboratory scale apparatus that was designed and built in a previous work [16] but that was modified in this work for using a bigger extractor (100 cm^3). 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, carbon dioxide 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.

2.3 Experimental procedure

Crude palm oil was obtained from a local company (Hacienda La Cabaña, Colombia) and was filtrated with a vacuum system to eliminate dispersed solids, such as crystals of 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 ℃, 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 [17]. This procedure guarantees the elimination of glycerin, methanol and NaOH from the produced FAMEs.

FAME´s 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 ℃, under CO_2 atmosphere and continuous stirring during 1 h [18]. The removal of carotens was quantified by spectrophotometry. A Thermo Scientific Evolution 60 spectrophotometer was used to measure the absorption of FAME´s at 440 nm during the bleaching process [19]. Using this information the bleaching efficiency was determined.
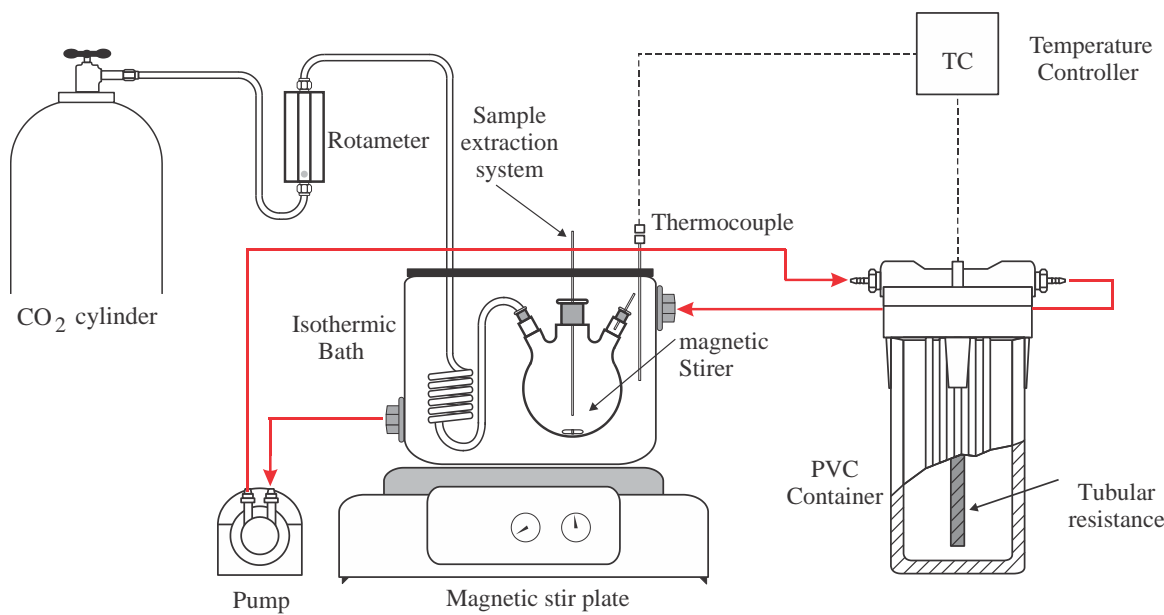
Figure 3. Experimental apparatus used for adsorption of tocols on silica gel.
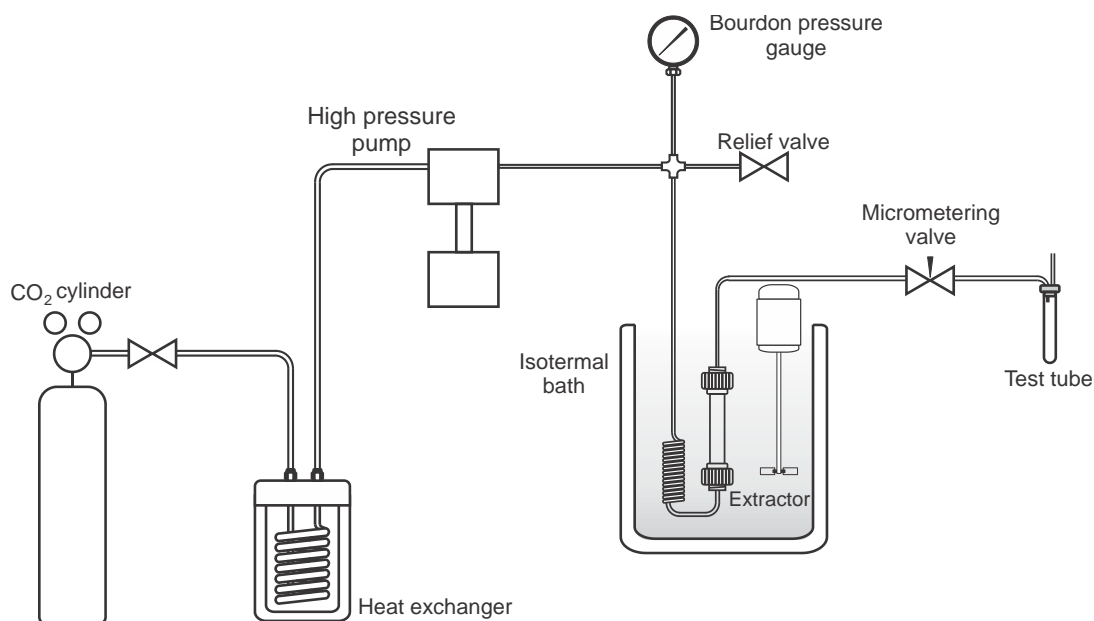
Figure 4. Experimental apparatus that was used for desorption.

The bleached FAMEs were then subjected to adsorption on silica gel. A sample of FAME´s and silica gel in a specific mass ratio (see the section on experimental design below) were loaded into the glass vessel, which then was immersed into the isothermal bath. After the temperature reached a steady value, the inert gas flow rate was set to 2 oz/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 exit 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 [20] for determining their profile of vitamin E isomers.

2.4 Experimental design

An experimental design was prepared 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 FAME´s. The experimental runs were organized according to an augmented $2^2$ factorial experiment in which temperatures between 30 and 60 °C, and FAME`s/silica ratio between 8.33 and 25 mL/g, were used as the 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 experimental plan was prepared 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. The experimental runs were organized according to an augmented 2^2 factorial experiment in which temperatures between 40 and 70 °C, and CO_2 densities between 0.6 and 0.8 g/cm^3, were used as the conditions for the factorial experiment. At each one of the 4 possible combinations of these conditions 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 [21] to calculate the pressure at which the corresponding run was to be made. Conditions of temperature and density were chosen to obtain pressures in the range of the operational capacity of our apparatus.

3. Results and discussion

Pretreatment of the crude palm oil consisted in filtration, methylation and bleaching. As mentioned, filtration removed suspended solids such as crystals of the stearine fraction present in crude palm oil. Methylation produced FAME´s that have viscosities lower than that of crude oil. Lower viscosities increase the mass transfer rate during further steps of bleaching and adsorption. To illustrate, Table 1 shows the viscosity of filtered palm oil and FAME´s as measured with a Brookfield viscometer. Notice that a decrease of an order of magnitude in viscosity is obtained as a result of the methylation of crude palm oil. The bleaching process was conducted to eliminate the carotenes and other pigments that might interfere with the adsorption of tocols on silica gel. The bleaching efficiency that was determined in this research was 93.43% and thus, elimination of that percentage of color-causing compounds was achieved. Figure 5 shows photographs of the raw material in different treatment steps before the adsorption process.

**Table 1.** Filtered palm oil and FAME´s viscosity.

Sample	Viscosity (cP)
Filtered palm oil	34.73 ± 0.25
FAME´s	3.27 ± 0.01

3.1 Adsorption of tocols on silica gel

Figure 6 shows the tocols adsorption from bleached FAME´s at 30 ºC and a FAME´s/silica ratio of 8.33mL/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 were similar to those obtained by other researchers [14, 15, 22], but because in those works the FAMEs were dissolved in hexane, which decreases the viscosity of the liquid, the time for reaching equilibrium was decreased.
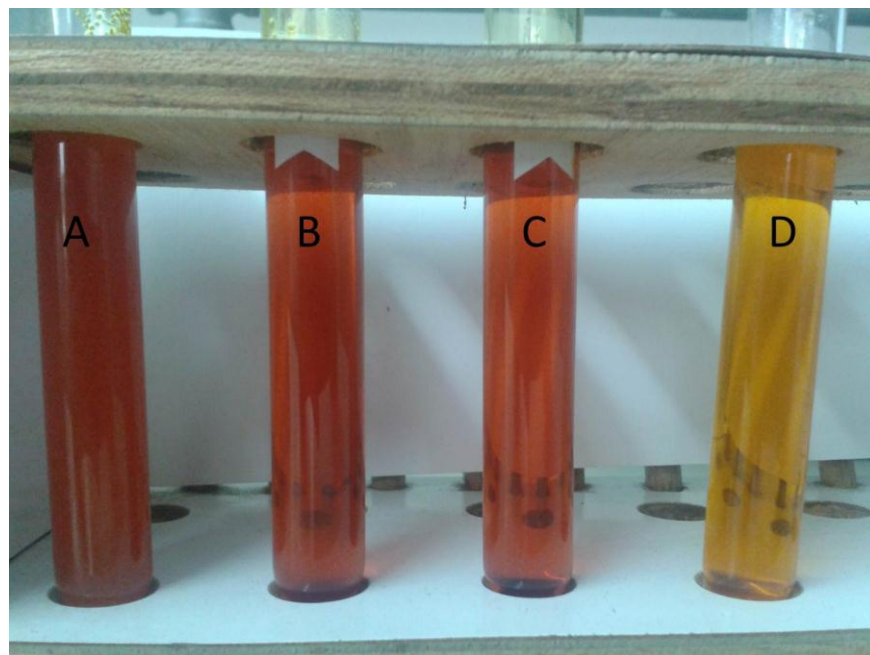
Figure 5. Photographs of the raw material in different pretreatment steps before adsorption process.
(A) Crude palm oil, (B) filtered palm oil, (C) FAME´s, (D) Bleached FAME´s
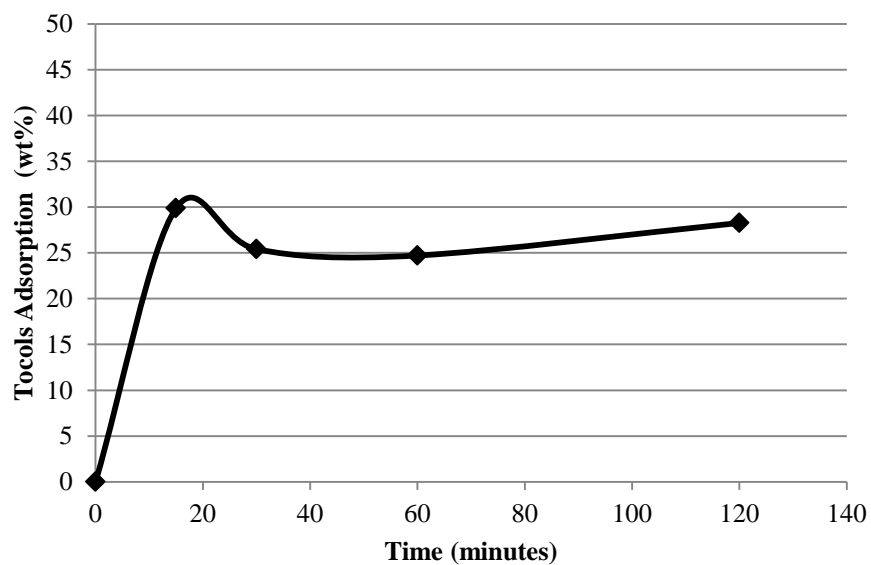
Figure 6. Tocols adsorption from bleached FAME´s at 30 ºC and a FAME´s/silica ratio of 8.33mL/g.

Table 2. Adsorption of tocols at equilibrium.

Temperature (°C)	FAME´s/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 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. Stirring rate, inert gas flow rate and other conditions were identical to the ones considered in the experimental design. As a result, a tocols adsorption 60.58% was obtained. The silica gel loaded with this amount of tocols was the used as raw material for the supercritical desorption experiments.

3.2 Supercritical desorption

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 on are significant at 15 and 30% levels of significance, respectively. Note that desorption yield increases with increasing temperature, and also con 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. Of such experimental error, the T3/T ratios reported in Table 3 do not exhibit a clear tendency. It seems like interactions of the two variables affect the T3/T ratio, but the statistical analysis did not confirm this. More experiments have to be conducted to conclude on the effects of the operating variables on the T3/T ratios.

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 7 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 FAME´s, 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. This result is explained by the fact that the affinity of silica is higher for tocols than for FAME´s, carotenes and other compounds contained in the feed material. As a result, the first fractions are richer in these compounds. On the other hand, the polarity of tocotrienols is larger than that of tocopherols, causing a weaker interaction between tocopherols and silica, which makes them elute first. From these results it is observed that supercritical desorption generates a separation of vitamin E isomers.
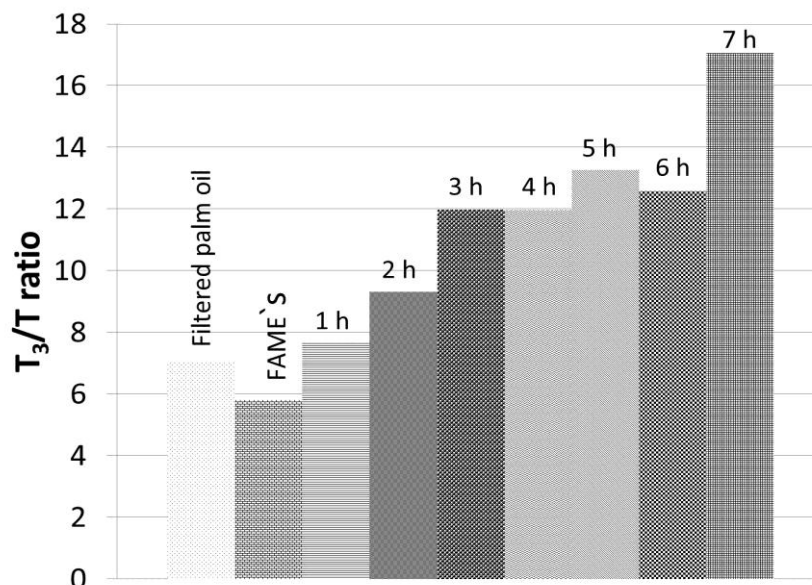
Figure 7. The tocotrienols/tocopherols ratio profile obtained for the desorption run 6 (4500 psi and 70 °C).

4. Conclusions

Supercritical desorption is capable of providing fractions of FAMEs with different tocotrienols/tocopherols ratios and thus is capable of producing a separation of the vitamin E isomers. 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 palm oil.

Acknowledgments

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