

PURIFICATION OF TOCOCHROMANOLS FROM EDIBLE OIL

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ABSTRACT

Enriching vitamin E from its natural sources, i.e., edible oils, and in its original, natural homologous matrix is becoming increasingly desirable. Supercritical fluid extraction (SFE) offers several advantages over conventional techniques such as vacuum distillation. In this paper results are presented that were obtained in the course of investigating two different feed materials, namely crude palm oil (CPO) and a soya oil deodorizer distillate (SODD). CPO contains several tocopherols at a concentration of approx. 500 ppm. The investigated SODD contains approx. 50 wt.-% tocopherols. Thus, this work allows conclusions about the vitamin E extraction over the entire concentration range. Phase equilibrium measurements were conducted in order to obtain basic data in respect to gas load and separation factors. This data allowed the theoretical description of the separation process within the scope of a separation analysis, eventually yielding in optimum extraction conditions and column design parameters. The results were subsequently verified experimentally by employing a 6m SFE column in pilot scale. The results show that the vitamin E enrichment is well within the ability of SFE techniques.

I – INTRODUCTION

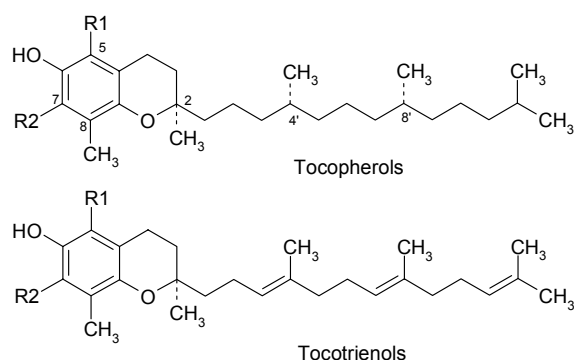


Figure 1: tocopherols

Vitamins are organic agents that are indispensable for maintaining many physical functions. They are essential for the human body and hence have to be ingested with the daily nutrition. A total of eight substances exhibit vitamin E activity in the human and animal body, with varying biological vitality. These eight substances – collectively known as tocopherols – can be divided into two groups: the tocopherols and the tocotrienols, where the trienols possess three unsaturated double bonds in their side chain at the 3', 7', and 11' position. Each group consists of four homologous, designated as α -, β -, γ -, and δ -

, referring to the number and site of methyl groups at the chroman-ring (R1 and R2 in Figure 1). Of these compounds, α -tocopherol is reported to have the highest biological activity [1]. The vitamin E activity has been associated with the antioxidant properties of these components, especially against lipid peroxidation in biological membranes. Hence, it is believed to have anti-tumor properties [2]. Recently, reports were published saying that naturally occurring homologous have a more beneficial effect than the intake of synthetically produced vita-

min E [3]. As a consequence, the demand of natural occurring vitamin E is steadily increasing.

Supercritical fluid extraction (SFE) represents a true alternative technique, especially in treating and processing natural occurring compounds like vitamin E. Besides the advantage of offering a simple technique for obtaining solvent-free products, supercritical fluid extraction competes well with standard techniques such as vacuum distillation for processing of temperature-sensitive components. While temperature of more than 373K are frequently used in vacuum distillation, a typical operating temperature of SFE with CO₂ is 323-333K, which is advantageous for these natural components. The low operating temperature is due to the low critical properties of CO₂ (P_C = 7.38 MPa, T_C = 304.15 K).

In this paper the work conducted on two different feed materials – crude palm oil (CPO) and soya oil deodorizer distillate (SODD) – is presented. The course of action was similar in both cases: performing phase equilibrium measurements in order to obtain reliable data for a theoretical description of the separation, namely a separation analysis, and the verification of the results by performing experiments employing a pilot scale extraction column.

II – MATERIALS AND METHODS

Table 1: composition of feed materials

	CPO [wt.-%]	SODD [wt.-%]
FFA	4.60	2.7
Tocochromanols	0.03	49.7
Squalene	n.a.	3.1
Sterols	~ 0.03	18.8
Glycerides	95.00	n.a.

Table 1 shows the composition of the respective feed materials. As can be seen, the SODD contains a significantly increased content of unsaponifiable matter, especially tocochromanols as the component of interest. In contrast to other major edible oils, palm oil contains tocotrienols as main vitamin E components, whereas tocopherols are the major species present in other oils.

Standard analytical method was gas chromatography. A HP5890A gas chromatograph equipped with an autosampler and FID was used for the analysis. Separation took place on a capillary DB-5 fused silica column from J&W Scientific (30m, 0.1µm, ID 0.25mm). The temperature program started at 343 K and stopped at a final temperature of 623 K. Nitrogen was employed as carrier gas.

CO₂ (99.95% purity) for the phase equilibrium measurements and the extraction experiments was supplied by KWD, Germany. CPO was supplied by Deutsche Cargill GmbH, Germany, SODD was obtained from Hoffmann-La Roche, Switzerland.

III – PHASE EQUILIBRIUM MEASUREMENTS

The knowledge of the phase behavior of the investigated system is crucial for finding optimum SFE operating conditions and for modeling the extraction setup for the separation process (discussed in more detail below). The measurements were conducted according to the static-analytical method employing an apparatus that is described in more detail elsewhere [4]. The aim of these experiments was to determine the obtainable gas load (capacity of the solvent) and to analyze the samples gas-chromatographically in order to calculate the distribution coefficients of the major components present in the respective feeds (selectivity of the solvent). Distribution coefficients K_i were calculated as the ratio of one component's concentration in the gaseous phase, y_i , to that in the liquid phase, x_i :

$$K_i = \frac{y_i}{x_i}$$

With this information the separation can be analyzed and is described below. A comparison of the solubility of both feed materials in CO₂ as a function of pressure and temperature is shown in Figure 2.

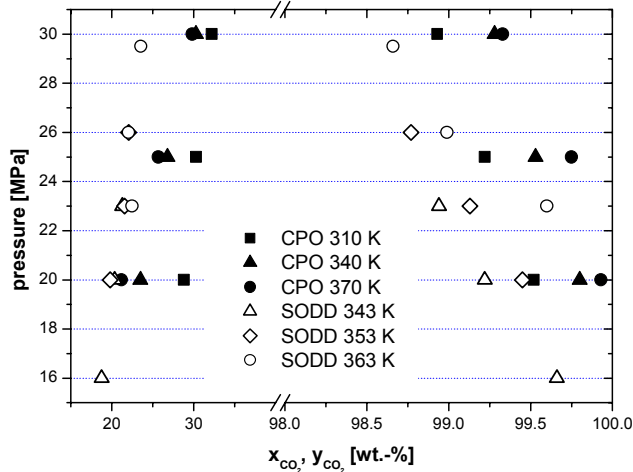


Figure 2: mutual solubilities of CPO and SODD with CO₂

CPO almost only consists of triglycerides that exhibit a rather low solubility in compressed carbon dioxide. The components contained in SODD have a higher solubility in supercritical CO₂. That becomes clear by observing Figures 3 and 4.

In Figure 3 the distribution coefficients for the components important in this work contained in CPO are shown. Free fatty acids (FFA) and tocopherols exhibit a much higher solubility in CO₂ than the triglycerides. Hence, these components are enriched in the gaseous phase, expressed by a distribution coefficient higher than one. Additionally, the K_i values are rather high, suggesting a rather easy separation of these two components from the contained triglycerides. Consequently, the distribution coefficient of the triglycerides is smaller than one, meaning that they stay in the oil phase. As a consequence, the tocopherols as the substance of interest can be extracted as top phase product of a separation column. This separation is thus the first step for enriching vitamin E from natural sources.

Another possible way of treatment should be mentioned briefly. In this method, the triglycerides are subject to an esterification with methanol to form fatty acid methyl esters (FAME). The FAME are easily extractable with CO₂. That means that the tocopherols, together with other unsaponifiable matter (squalene, sterols etc.), are enriched in the bottom phase of an extraction column. This attempt is described in more detail elsewhere [4].

The common denominator in both approaches is that the tocopherols have to be separated from other components present in edible oils. This separation was subject for the investigations performed with SODD. In this feed material, tocopherols are the main component and have to be separated from other substances present in edible oils, namely squalene and sterols.

As can be seen from this figure, mutual solubility increases with pressure. In the case of SODD, the solubility of carbon dioxide in the oil phase is almost independent of temperature. CPO on the other hand dissolves more CO₂ than SODD does and shows a small temperature dependence where the solubility decreases with increasing temperature. However, more important for SFE processes is the solubility of the solute in the gaseous solvent. Here a distinct difference between CPO and SODD can be observed. The solubility at comparable temperature and pressures is higher in case of SODD. This behavior is obvious if the composition of both materials is taken into account.

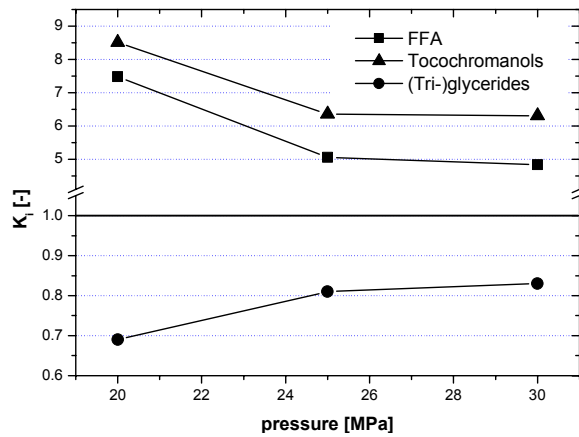


Figure 3: Distribution coefficients for CPO (340 K)

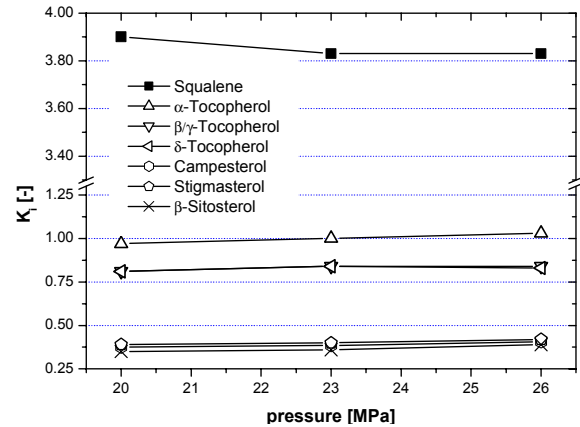


Figure 4: Distribution coefficients for SODD (353 K)

Figure 4 shows the distribution coefficients for these components. As can be seen, of these compounds squalene exhibits the best solubility in supercritical carbon dioxide. All phyto-sterols have a rather low solubility in CO_2 and remain in the oil phase, expressed by a very small K_i value. The solubility of the tocochromanols is intermediate between the other components. Conspicuous is the difference between α -tocopherol and the other species. α -tocopherol exhibits a distinct higher solubility in CO_2 than the other homologous. This behavior could be attributed to the molecular structure of α -tocopherol. Since it has two methyl groups at its chroman ring the polar OH-group is better shielded compared to the other homologous, which enhances the solubility in the unpolar solvent carbon dioxide.

IV – EXTRACTION EXPERIMENTS

SFE experiments were conducted in a pilot scale plant described in more detail in [5]. The core of this apparatus is an extraction column having an effective separation height of 6m equipped with a Sulzer EX gauze packing. 0.5m at each end of the column are left packing-free allowing for phase separation. The inner diameter of the column is 17.5mm and can be operated either in a true stripping mode with the solvent introduced at the bottom and the feed at the top of the column or in reflux mode. In the latter technique the feed is introduced at the middle of the column and part of the extract is reintroduced at the top of the column to ensure countercurrent flow. The gaseous solvent is recycled.

Extraction experiments with CPO were performed at temperatures of 340 and 370 K and at pressures between 20 and 30 MPa. The solvent-to-feed (S/F) ratio was varied in the range from 25 to 65. Operating the column with reflux significantly enhances the quality of the products. Extracts containing 48 wt.-% FFA and 3200 ppm tocochromanols could be obtained, meaning a ten-fold increase in concentration. Furthermore, the raffinate contained only 0.2 wt.-% FFA, thus yielding a product similar to one obtained after conventional palm oil treatment.

Investigations employing SODD as feed material for the extraction were performed at temperatures of 353 and 363 K and at pressures of 23 and 26 MPa. The S/F ratio was varied in the range between 33 and 171, the reflux ratio was set between 1.0 and 38.0. Experiments show that, as expected, squalene is enriched in the top phase product. In fact, it could not be found in the bottom phase. Consequently, it was enriched from 3.1 wt.-% in the feed to 18.8 wt.-% in the top phase product. The sterols were completely enriched in the bottom phase, occasionally to more than 50 wt.-%. Here, some problems arose due to the high melting tem-

perature of the sterols. If the concentration exceeds 50 wt.-%, the products become too viscous and clog the packing mash and the column. Higher temperatures were regarded as unhelpful due to the increased thermal strain that entails degradation of the products. The tocochromanols, as intermediate-soluble components, were neither distinctly enriched in the top nor in the bottom phase product.

In order to simulate a higher extraction column, the top phase product of some experiments were collected and introduced a second time to the SFE column. With this sterol-free feed material, the separation of squalene and tocochromanols was now possible. Starting from a tocochromanol concentration of 48.3 wt.-% an enrichment to 94.4 wt.-% in the bottom product was obtained. This separation was performed at 23 MPa, 353 K, having a S/F ratio of 110 and a reflux ratio of 4.6. The contained squalene could be completely enriched in the top phase product.

The employed column is too short to perform the extractions to the end. In order to describe the separation steps completely, a separation analysis based on a short-cut method was performed for both separations as described in the following section.

V – SEPARATION ANALYSIS

A separation analysis eventually yields the optimum operating conditions for a separation column. It correlates the necessary number of theoretical separation stages (n_{th}) and the solvent-to-feed (S/F) ratio to the reflux ratio (v). In this work, a short-cut method according to Ponchot-Savarit was used, implementing a Jänecke load diagram (gas load over composition). The following diagrams show two different arrays of curves. The one having solid lines represent the dependence of the theoretical number of separation stages to the reflux ratio; the one using dashed lines shows the interaction between solvent-to-feed ratio and reflux ratio. Input parameters are feed composition, product purities (to be specified), feed and solvent flux, as well as information about the gas load and separation factor as a function of composition, as gained from the phase equilibrium measurements.

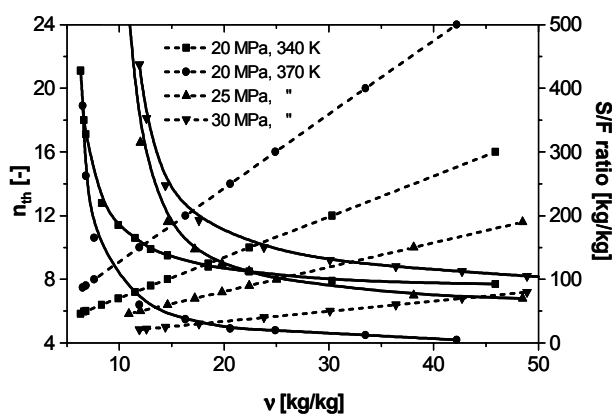


Figure 5: separation analysis for the separation of FFA and tocochromanols from CPO (95 wt.-% purity in top phase, 99.5 wt.-% purity in raffinate)

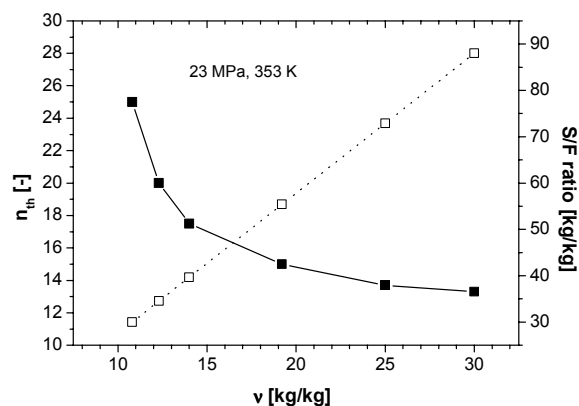


Figure 6: separation analysis for the separation of squalene and tocochromanols/sterols (99% squalene in extract, 1% in raffinate)

As can be seen from the figures, with an increased reflux ratio the necessary number of separation stages decreases. That means that a higher reflux ratio is advantageous for the column design, since it allows for a shorter column. However, a higher reflux ratio entails a higher S/F ratio. That in turn increases operational costs, because the main expenses running a SFE column are the (re-)compression costs of the supercritical solvent. A higher S/F ratio hence means that more solvent has to be conveyed through the plant. Thus, a compromise between these two effects has to be found.

In case of the separation of FFA and tocopherols (summarized as highly-volatile components, HVC) from CPO (Figure 5) it can be observed that at a temperature of 370 K a lower pressure is advantageous in terms of necessary separation stages. Unfortunately, the S/F ratio increases significantly at a given reflux ratio if the pressure is decreased. If a top phase product is desired that contains 95 wt.-% HVC and if the raffinate should only contain 0.1 wt.-% HVC, the separations should take place at 340 K / 20 MPa, 370 K / 25 MPa, or 370 K / 30 MPa. Employing these conditions, a reflux ratio of 10 – 20 and S/F ratios of 50 – 170 would result in a column design that is capable of performing the separation with 6 – 10 separation stages.

For the separation of squalene from sterols and tocopherols in the SODD matrix a separation analysis was performed at 23 MPa and 353 K. Product specifications were a squalene concentration of 99 wt.-% in the top phase product and 1 wt.-% in the bottom product. At these conditions the minimal reflux ratio is 10.4. As a good rule of thumb, the optimum reflux ratio for extractions is $v = 2-3v_{\min}$. This is true for this case, too, and would result in S/F ratios of 60 – 90. Then, 13 – 15 separation stages would be necessary to perform the extraction and obtain the desired products. Not shown in Figure 6 but also interesting is the fact that a reduction in top phase purity to 90 wt.-% would significantly decrease the necessary separation stages to 7 – 9.

CONCLUSIONS

The results suggest that a vitamin E enrichment starting from crude edible oil to very high concentrations is possible by means of supercritical fluid extraction. Correct data from phase equilibrium measurements are crucial for the theoretical description of the separation. These shortcut methods in turn are capable of giving a good impression of optimum operating conditions and column design. Thus, SFE is a true alternative for conventional techniques due to its capability of producing solvent-free products under moderate thermal conditions.

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