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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO
DIVISION DE NUEVAS CREACIONES
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Re: Expediente No. **08.079.059**
Solicitud de patente a nombre de **CAROTECH SDN.BHD.**

Yo, DILIA MARIA RODRIGUEZ D'ALEMAN, identificada como aparece al pie de mi firma, domiciliada en la carrera 11 A No. 86-53, piso 6, obrando como apoderada de la sociedad **CAROTECH SDN.BHD.**, domiciliada en Chemor, Perak, Malasia, solicitante de la patente de la referencia, en respuesta al oficio No. 11026 notificado por fijación en lista el 22 de junio de 2012, nos permitimos informar lo siguiente:

Que presentamos ante ese Despacho copia informal de los siguientes documentos:

- Gast, K., Jungfer, M. Saure, c., and Brunner, G. "Purification of tocochromanols from edible oil" Journal of Supercritical Fluids. Volume 34, 2005, XP002392985. USPRA PRESS.
- Brunner, G. "Fractionation of fats with supercritical carbon dioxide" European Journal of lipid science and Technology. Volume 102, no. 3, 2000, pages 240-244.

Con la presentación de estos documentos damos cabal cumplimiento al requerimiento realizado por esa oficina mediante el Artículo 46 de la Decisión 486 de la Comisión de la Comunidad Andina.

Señor Superintendente,


DILIA MARIA RODRIGUEZ D'ALEMAN
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Purification of tocopherols 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 soy oil deodorizer distillate (SODD). CPO contains several tocopherols at a concentration of approximately 500 ppm. The investigated SODD contains approximately 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 distribution coefficient. 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 6 m SFE column in pilot scale. The results show that the Vitamin E enrichment is well within the ability of SFE techniques.

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Keywords: Supercritical fluid extraction; Vitamin E; Tocopherols; Carbon dioxide; Enrichment

1. Introduction

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 subdivided into two groups: the tocopherols and the tocotrienols, with the -trienols possessing three unsaturated double bonds in their aliphatic side chain at the 3', 7', and 11' position, as shown in Fig. 1. Each group consists of four homologous, designated as α -, β -, γ -, and δ -tocopherol (-trienol), referring to the number and site of methyl groups at the chroman-ring (R1 and R2 in Fig. 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—among others—anti-tumor properties [2]. Recently, reports were published saying that naturally occurring homologous mixture has a more beneficial effect than the intake of synthetically produced Vitamin E [3]. As a consequence, the demand of natural occurring Vitamin E is steadily increasing.

Supercritical fluid extraction (SFE) represents a true alternative technique to conventional processes, especially in treating and processing natural occurring compounds like Vitamin E. An extensive description of the unique properties of supercritical fluids, as well as an overview about SFE applications is given in [4].

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 temperature-sensitive components. While temperatures of more than 373 K are frequently used in

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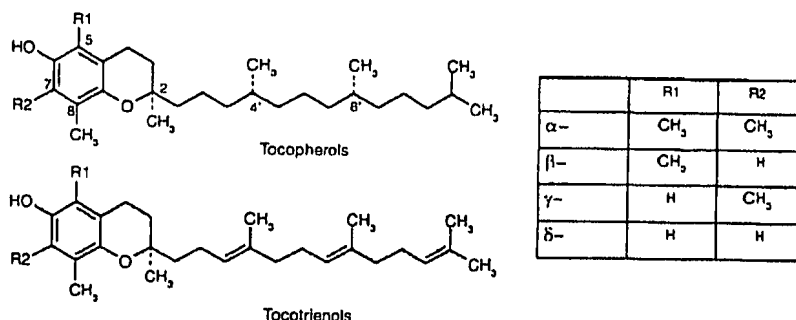


Fig. 1. Chemical structure of tocochromanols.

vacuum distillation, typical SFE operating temperatures with CO₂ are 323–333 K, which have a less detrimental effect on 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 work conducted with two different feed materials—crude palm oil (CPO) and soy oil deodorizer distillate (SODD)—is presented. Palm oil is the major edible oil in the South-East Asian region and only second to soy oil in the world, with Malaysia being the biggest producer of CPO. SODD is a by-product of the soy oil refining. The idea to enrich vitamins from these two feed stocks is hardly new; several authors described the enrichment of provitamin A (β -carotene) from palm oil (e.g., Markom et al. [5]) or its production by-products (e.g., França and Meireles [6], Birtigh et al. [7]) and Vitamin E from soy oil deodorizer distillate (e.g., Nagesha et al. [8], Mendes et al. [9], and Chang et al. [10]) with SFE, some even employing a countercurrent extraction column like Brunner et al. [11]. However, most investigations deal with batch extractions, often conducted in lab-scale apparatuses. Although the quality and importance of these works is not questioned, the next step is a continuous working pilot-scale plant that was employed in this work. Furthermore, the extraction step was modeled with short-cut methods, allowing to obtain design data for a production-scale extraction apparatus.

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, i.e., a separation analysis, and the verification of these results by performing experiments employing a pilot scale supercritical fluid extraction column.

2. Materials and methods

Table 1 shows the composition of the respective feed materials. As a typical crude oil CPO mainly consists of glycerides and free fatty acids (FFA). The Vitamin E content amounts to 300 ppm. 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.

Table 1
Composition of feed materials

	CPO (wt. %)	SODD (wt. %)
FFA	4.6	2.7
Tocochromanols	0.03	49.7
Squalene	n.a.	3.1
Sterols	0.03	18.8
Glycerides	95.0	n.a.

In the SODD matrix the Vitamin E content is significantly increased to approximately 50 wt.%. Other associated components are enriched, too, such as squalene and sterols. Whereas the separation from glycerides is predominant in case of CPO, the separation from phytosterols is prevailing when treating SODD.

Standard analytical method was gas chromatography. An HP5890A gas chromatograph equipped with an autosampler and FID (at 623 K) was used for the analysis. Separation took place on a capillary DB-5 fused silica column from J&W Scientific (30 m, 0.1 μ m, i.d. 0.25 mm). The injector was operated in split mode (1:100) at a temperature of 573 K. 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.

3. Phase equilibrium measurements

The knowledge of the phase behavior of the investigated system is crucial for designing a separation strategy, for obtaining optimum SFE operating conditions, and for modeling the extraction process (as discussed in more detail in Section 5). The measurements were conducted according to the static-analytical method employing an apparatus shown in Fig. 2. The equilibrium autoclave has an inner volume of approximately 1 dm³ and is equipped with a magnetic stirrer. It is connected to a second autoclave that serves as a compensation vessel (inner volume approximately 0.7 dm³). Both the

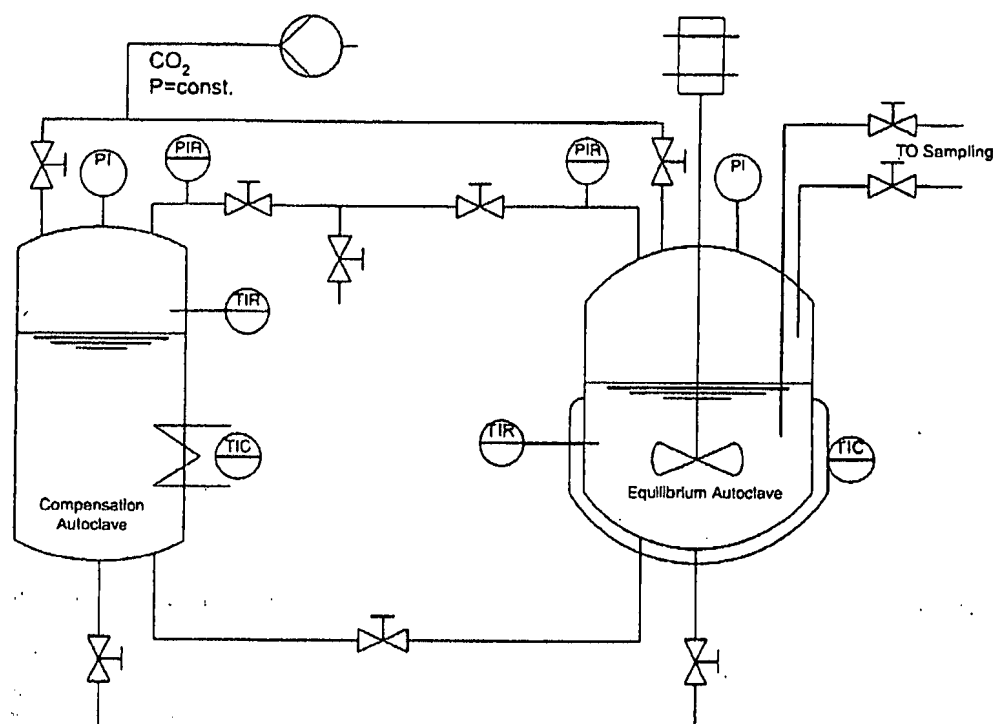


Fig. 2. Setup of equilibrium apparatus.

compensation autoclave and the high pressure CO₂ pump that ensures a constant CO₂ pressure prevent pressure fluctuations during sampling. Sampling lines in the gaseous and the liquid phase enable to draw samples from both phases. In order to obtain an amount of solute sufficient enough for GC analysis, relatively high gas volumes (2–20 dm³) need to be sampled due to the rather low solubility in compressed CO₂. With this setup, pressure fluctuations could be lowered to less than 0.1 MPa during sampling. Without the above-described compensation, pressure loss would be inevitable during sampling resulting in a disturbed equilibrium. All necessary components of the apparatus were electrically heated and insulated; temperature fluctuations were less than 0.5 K.

In Fig. 3 the employed sampling setup is shown. The solute precipitated inside a cool trap filled with crushed ice. Gas volumes from 0.1 (liquid phase samples) to 20 dm³ (gas phase samples) need to be read off with a high precision. Thus, two different ways of determination were implemented. Small volumes were detected in a burette system, where the gas displaces water inside the burettes. Larger volumes were detected with a gas meter. After sufficient mixing (usually overnight) and settling of the phases (1–3 h) at least three samples were drawn from the respective phases. If necessary, more samples were taken until the mean value of the solubilities showed less than 5% error.

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 :

$$K_i = \frac{y_i}{x_i} \quad (1)$$

Hence, a substance enriched in the gaseous phase has a distribution coefficient being bigger than one; a substance that is enriched in the liquid phase exhibits a K_i -value being smaller than one. A distribution coefficient being close to one indicates a component that is neither enriched in the gaseous nor in the liquid phase. Consequently, in a countercurrent separation column, substances with $K_i > 1$ can be obtained as top phase product, whereas components with $K_i < 1$ will be found in the bottom phase of the column. A component having a distribution coefficient of $K_i \approx 1$ at certain P and T cannot be enriched in an extraction column under these conditions. The CO₂ used as solvent in these investigations is withdrawn from the system after the separation; thus, it is neglected when calculating the solubilities y_i and x_i , as well as in K_i . The values are hence calculated solvent-free.

With this information the separation can be analyzed as it is described below. The measured mutual solubilities of both feed materials and CO₂ are given in Table 2. A comparison

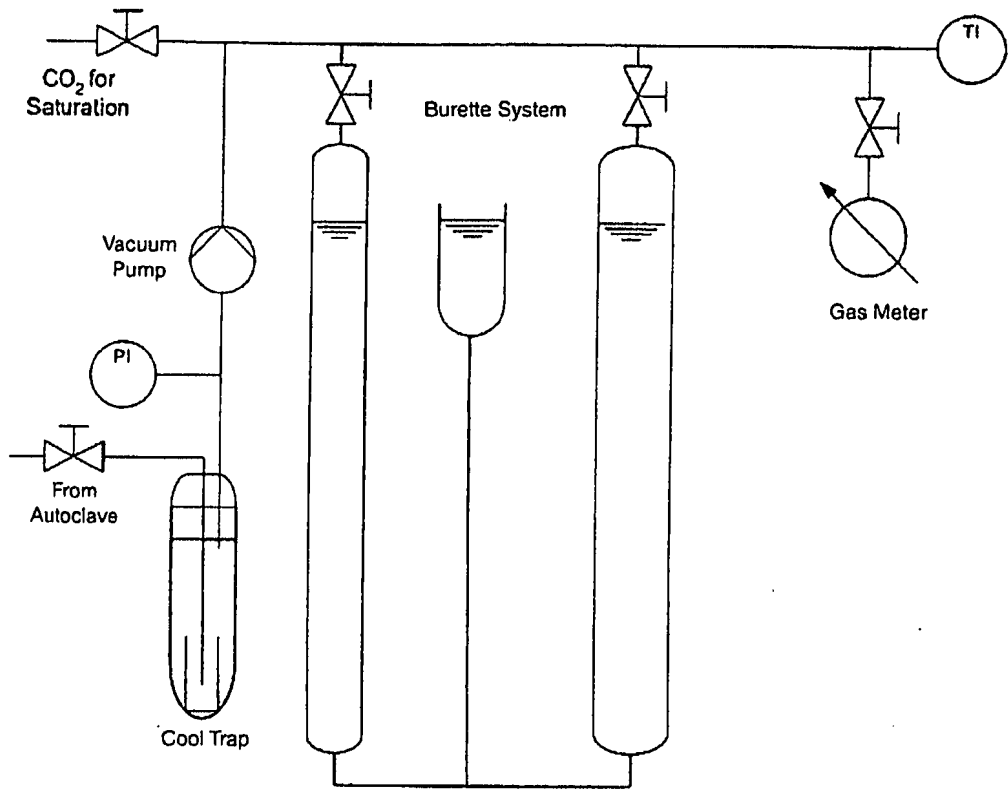


Fig. 3. Sketch of sampling unit.

Table 2
Solubilities of feed materials in CO₂

Oil	<i>T</i> (K)	<i>P</i> (MPa)	Density (kg/m ³) [14]	Solubility (mass fraction)	
				Liquid phase <i>x</i> _{CO₂}	Gaseous phase <i>y</i> _{CO₂}
CPO	310	20.0	856.3	0.2879	0.9952
		25.0	893.3	0.3029	0.9922
		30.0	922.0	0.3221	0.9893
	340	20.0	679.7	0.2349	0.9980
		25.0	752.7	0.2679	0.9953
		30.0	801.2	0.3027	0.9928
	370	20.0	496.1	0.2119	0.9993
		25.0	603.1	0.2569	0.9975
		30.0	674.8	0.2984	0.9933
SODD	343	16.0	549.1	0.1875	0.9966
		20.0	660.0	0.2033	0.9922
		23.0	711.2	0.2127	0.9894
	353	20.0	594.8	0.1980	0.9945
		23.0	655.7	0.2157	0.9913
		26.0	700.7	0.2204	0.9877
	363	23.0	601.5	0.2248	0.9960
		26.0	652.4	0.2203	0.9899
		29.5	698.2	0.2355	0.9866

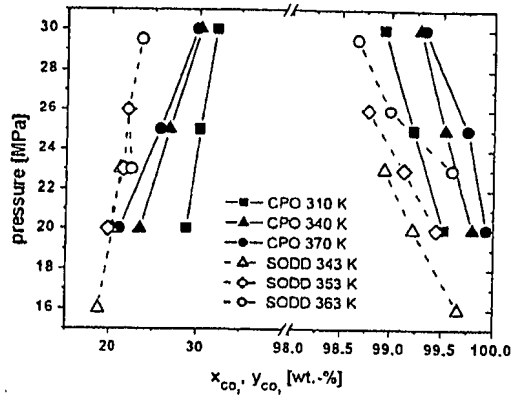


Fig. 4. Mutual solubilities of CPO and SODD with CO₂.

of the solubility of both materials in CO₂ as a function of pressure and temperature is shown in Fig. 4.

As can be seen from this figure, mutual solubility generally increases with pressure. In 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 with the solubility decreasing 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 temperatures and pressures is higher in case of SODD. This behavior is obvious if the composition of both materials is taken into account. 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. This fact is also shown in Figs. 5 and 6.

In Fig. 5 the distribution coefficients of the components of interest in this work are shown. The distribution coefficients were calculated from the raw data shown in Table 3. Free fatty acids (FFA) and tocopherols exhibit a much

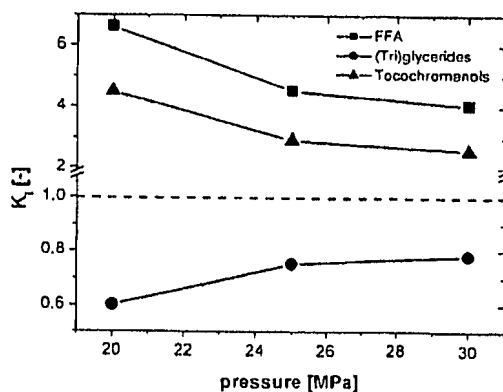


Fig. 5. Measured distribution coefficients for CPO at 340 K.

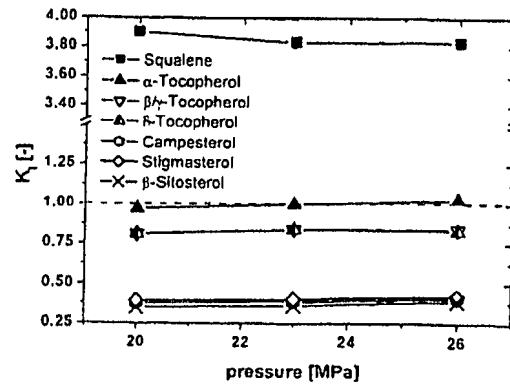


Fig. 6. Measured distribution coefficients for SODD at 353 K.

higher solubility in CO₂ than the triglycerides. Hence, these components are enriched in the gaseous phase, expressed by a distribution coefficient being higher than one. Additionally, the K_i values are rather high, suggesting a somewhat 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 in 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 [12].

The common denominator in both approaches is that subsequently the tocopherols have to be separated from other non-glyceride components present in edible oils. This separation was subject for the investigations performed with SODD. In this feed material, tocopherols are the main compo-

Table 3
Composition of liquid and gaseous phase in phase equilibrium measurements of CPO

T (K)	P (MPa)	Liquid phase x_i (wt.%)			Gaseous phase y_i (wt.%)		
		FFA	TG	Tocos (ppm)	FFA	TG	Tocos (ppm)
310	20.0	6.16	93.84	276	25.36	74.64	1540
	25.0	6.59	93.41	282	27.15	72.85	1335
	30.0	6.67	93.33	n.a.	26.84	73.16	n.a.
340	20.0	6.67	93.33	589	44.15	55.85	2654
	25.0	6.63	93.37	558	30.11	69.89	1639
	30.0	6.65	93.35	583	26.93	73.04	1498
370	20.0	6.73	93.27	499	75.25	24.75	1381
	25.0	7.09	92.91	n.a.	53.32	46.68	n.a.
	30.0	6.66	93.34	163	39.55	60.45	265

Table 4
Composition of liquid and gaseous phase in phase equilibrium measurements of SODD

T (K)	P (MPa)	Liquid phase x_i (wt. %)							Gaseous phase y_i (wt. %)						
		Squalene	δ -Tocopherol	β - γ -Tocopherol	α -Tocopherol	Campesterol	Stigmasterol	β -Sitosterol	Squalene	δ -Tocopherol	β - γ -Tocopherol	α -Tocopherol	Campesterol	Stigmasterol	β -Sitosterol
343	16.0	3.13	17.46	37.11	3.72	4.67	3.17	7.17	11.81	11.09	23.45	2.93	1.12	0.79	1.57
	20.0	3.07	17.44	37.09	3.71	4.66	3.16	7.19	11.35	12.30	26.37	3.28	1.41	1.00	2.03
	23.0	2.94	17.44	37.17	3.69	4.69	3.18	7.23	11.08	12.81	27.76	3.50	1.54	1.11	2.25
353	20.0	2.67	17.69	37.56	3.73	4.85	3.30	7.52	10.40	14.40	30.43	3.56	1.74	1.23	2.50
	23.0	2.60	17.67	37.52	3.73	4.87	3.30	7.52	9.96	14.77	31.45	3.72	1.86	1.31	2.69
	26.0	2.57	17.68	37.59	3.71	4.86	3.29	7.52	9.85	14.70	31.78	3.81	1.89	1.33	2.74
363	23.0	2.48	17.75	37.81	3.75	4.94	3.35	7.60	10.06	15.50	32.52	3.68	1.84	1.27	2.58
	26.0	1.96	17.63	37.30	3.64	5.21	3.54	8.09	7.79	17.06	36.31	4.05	2.41	1.68	3.50
	29.5	1.93	17.59	37.44	3.64	5.26	3.56	8.13	7.41	17.01	36.38	4.08	2.46	1.72	3.63

nent and have to be separated from other unsaponifiable substances present in edible oils, e.g., squalene and sterols.

Fig. 6 shows the distribution coefficients of these components. The phase compositions of both phases are shown in Table 4. As can be seen, of these compounds squalene exhibits the best solubility in supercritical carbon dioxide. All phytosterols have a rather low solubility in CO₂ 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 CO₂ than the other homologous. This behavior could be attributed to the molecular structure of α -tocopherol. Since it has three methyl groups at its chroman ring (R1, R2 = CH₃ in Fig. 1) the polar OH-group is better shielded compared to the other homologous, which enhances the solubility in the unpolar solvent carbon dioxide. Pereira et al. [13] also found a higher solubility of α -tocopherol than for the δ -specie in the respective binary mixtures.

4. Extraction experiments

SFE experiments were conducted in a pilot scale plant (Fig. 7). The core of this apparatus is an extraction column having an effective separation height of 6 m equipped with a Sulzer EX gauze packing. Half a meter at each end of the column are left packing-free allowing for phase separation. The inner diameter of the column is 17.5 mm and it 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 mode of operation 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 extract precipitates in the separator by reducing the pressure to 5–6 MPa. Windows at the bottom of the separator and at the bottom of the column enable the operator to visually observe the production of extract and raffinate, respectively. A window at the middle of the column allows for observing the flow regime inside the column and is important for noticing flooding of the column. The gaseous solvent is regenerated inside the separator and can thus be recycled. The mass flow of the recycled carbon dioxide stream is measured by a mass flow meter (Rheonik, Germany) and controlled by an air-driven regulation valve (Kammerer, Germany). The column pressure is also regulated by a similar regulation valve situated in the line leading from the columns top to the separator (extract line). It closes when the pressure inside the column is below the set point. If the pressure exceeds the set point the pressure is released to the separator. All necessary components are heated electrically with the exception of the feed container and the feed pump. These two parts are connected to a thermostat working with water. The feed pump and the reflux pump are both piston pumps; their flow rates are regulated via their respective number of revolutions, be-

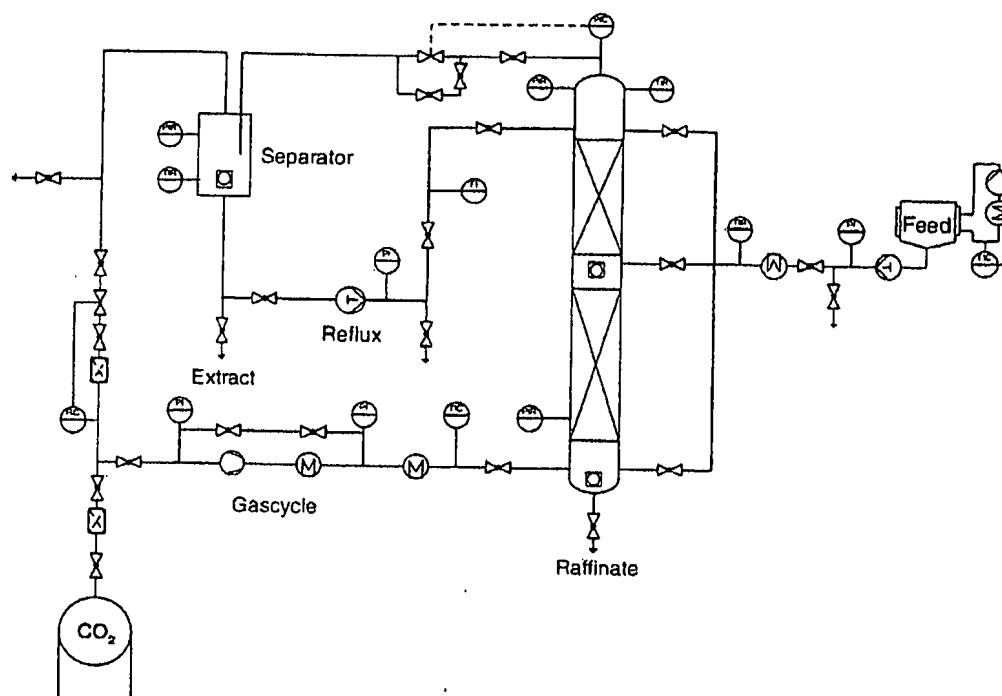


Fig. 7. SFE pilot scale plant.

cause operating them with a small stroke proved to be difficult with some feed materials.

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 tocopherols could be obtained, meaning a 10-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 deacidification.

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 temperature of the sterols. If the concentration exceeds 50 wt.%, the products become too viscous and clog the packing mesh and the column. Higher temperatures were regarded as unhelpful due to the increased thermal strain that entails degradation of

the products. The tocopherols, 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 was collected and introduced as feed a second time. With this sterol-free feed material, the separation of squalene and tocopherols was now possible. Starting from a tocopherol 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.

5. Separation analyses

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), respectively. In this work, a short-cut method according to Ponchon–Savarit was used, implementing a Jänecke load diagram (gas load

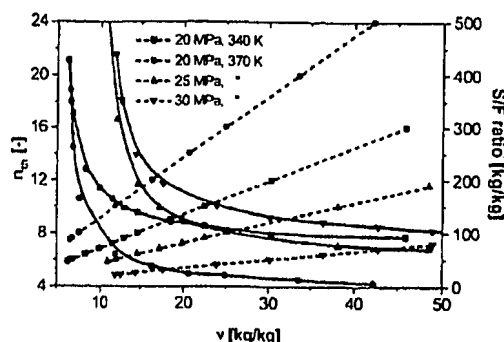


Fig. 8. Separation analysis of the separation of FFA and tocopherols from CPO (95 wt.% purity in top phase, 99.5 wt.% purity in raffinate).

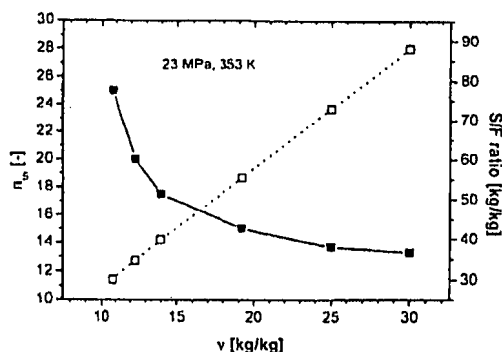


Fig. 9. Separation analysis of the separation of squalene and tocopherols/sterols from SODD (99 wt.% squalene in extract, 1 wt.% squalene in raffinate).

over composition). Fig. 8 (separation analysis for CPO) and Fig. 9 (SODD) show two different arrays of curves. The solid lines represent the dependence of the theoretical number of separation stages to the reflux ratio; dashed lines show 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 obtained from the phase equilibrium measurements.

Generally, an increased reflux ratio causes the necessary number of separation stages to decrease. A higher reflux ratio is thus 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 contrary effects has to be found.

Regarding the separation of FFA and tocopherols (summarized as highly-volatile components, HVC) from CPO (Fig. 8), 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 $v = (2-3)v_{min}$. 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 Fig. 9 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.

6. 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 short-cut 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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Fractionation of fats with supercritical carbon dioxide

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1 Introduction: Fractionation by countercurrent extraction

Separation of complex mixtures of edible oil related substances can be effectively achieved by countercurrent multistage processing with supercritical gases. The supercritical fluid, in most cases carbon dioxide, is applied as a solvent or as a mass separating agent in supercritical fluid extraction (SFE). The process is an extraction with two separation cascades – a stripping section and an enriching section. Reflux of the extract is applied at the top. The process also is comparable to fractional distillation, in particular if a binary solvent-free approach is applied to SFE.

Countercurrent separation using supercritical gases as solvents offers the opportunity for applying many theoretical stages in separation problems. Some fractionations are possible during the extraction process from solids and the downstream precipitation of the extract. This will not be covered here, because this type of fractionation is limited to a very few theoretical stages.

While separating a binary system is comparably easily and well understood, separating compounds from multi-component mixtures is more complex and can only be demonstrated on real cases.

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2 Target compounds of fats and oils with respect to countercurrent separation

In edible oil processing a great number of components may be of importance. The major groups of these compounds are: fatty acid esters, squalene, free fatty acids, carotenes, tocochromanols, mono- and diglycerides, sterols, sterolesters, triglycerides, and others. Solubility of compounds in supercritical carbon dioxide is determined by molecular weight and by molecular interactions. Since carbon dioxide is a relatively nonpolar molecule (no dipol moment, but a quadruple moment) it dissolves nonpolar molecules best. Therefore, molecular weight gives a hint whether separation with supercritical carbon dioxide may be possible within groups of similar chemical compounds and among nonpolar molecules of different chemical structures.

Investigations on the separation of two or more of the above mentioned compounds have been carried out by numerous groups using supercritical fluids, mainly carbon dioxide as a solvent.

Separation of the free fatty acids and the mono-, di- and triglycerides was explored. From crude oils the separation of the free fatty acids (de-acidification), the carotenes, and the tocochromanols have been investigated, from distillates, removal of fatty acid esters, free fatty acids, and the enrichment of squalene, tocochromanols, and sterols have been treated. Oils have been modified (transesterified) to be able to remove the fatty acid esters more easily in order to obtain enriched fractions of squalene, tocochromanols, carotenes, and sterols. Similar, fish-oils have been treated in order to separate and enrich the unsaturated fatty acids (as esters) in particular the n-3-polyunsaturated fatty acids EPA (C 20:5), DHA (C 22:6) and DPA (C22:5).

Some of these separations have been developed to an industrial applicable process yielding products of defined specifications and have been demonstrated on an industrial basis, and have been economically evaluated. In the following, the design procedure will be reviewed, some examples reported, and conditions for an economic operation discussed.

3 Fundamentals of countercurrent SFE. Evaluation by experiments and modelling

Separation of complex mixtures of edible oil related substances can be achieved effectively by countercurrent multistage processing with supercritical gases. Carried out in a continuous mode, the separation is a binary one. In a column or any other multistage separation device with two separation cascades, one compound (or group

of compounds) can be removed to a certain specification and at a given yield. For a cleaning process of a compound from a multicomponent mixture with compounds less and more soluble in supercritical carbon dioxide there have to be at least two runs in a two cascade column (as is the case for fractional distillation).

The following aspects have to be considered for designing a separation process:

- Separation performance or determination of number of theoretical stages; determination of phase equilibria, short-cut methods; multicomponent process simulation; separation analysis;
- Mass transfer: determination of HETP (height equivalent of a theoretical plate), HTU (height of a transfer unit);
- Capacity of mass transfer equipment: determination of pressure drop, flooding limits;
- Column diameter or size of the separation device;
- Solvent cycle: product precipitation from solvent, regeneration of solvent, and solubility enhancement.

Extraction with supercritical fluids (SFE, gas extraction) extends the possibilities of separation processes like distillation, absorption and liquid-liquid extraction to the isolation and purification of components of low volatility. Furthermore, it enables separation of components with very similar properties if used in the countercurrent mode. Process temperatures in gas extraction are determined by the critical temperature of the solvent and not, as is the case of distillation of any kind, by the liquid-vapour transition of the feed mixture. As compared to liquid-liquid extraction, gas extraction enables easily to operate a two-cascade separation column, applying a stripping and an enriching section. This often poses a problem in liquid-liquid extraction where a two-phase region often only can be achieved by applying a second solvent. Combined, these possibilities allow gas extraction to be operated at very moderate temperatures and as a separation process for difficult separations. Countercurrently operated gas extraction can be applied at moderate to low separation factors, in the range of about ≥ 1.2 .

In Fig. 1 the process scheme of a countercurrent gas extraction for the separation of two components into practically pure substances is shown.

The separation column consists of two separation cascades. In the upper one (enriching section) the bottom product compounds are separated from the top product compounds and rejected to the lower section (stripping section). In the stripping section the top product compounds are separated from the bottom product compounds and transported to the enriching section.

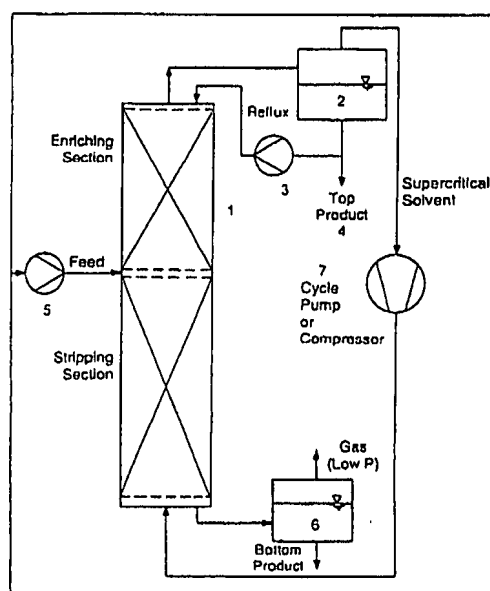


Fig. 1. Scheme of countercurrent multistage processing. Process equipment consists of the separation column (1) where gaseous and liquid phases are contacted countercurrently, a separator at the top for separating solvent and extract (2), devices for feeding reflux to the column (3), for recovering top product (4), for delivering feed to the column (5), for recovering product at the lower end of the column (6), and for recycling the solvent (7).

At the top of the column the separator removes the extract from the solvent. From the extract a specified part is separated and introduced at the top of the column as reflux. The remaining part of the extract is the top product. The solvent is reconditioned (filtered, sometimes liquefied and again evaporated for removing trace substances, pressure and temperature are adjusted) and recycled by the cycle pump as the supercritical solvent at the bottom of the column. The feed is introduced at an intermediate location in the column by the feed pump; for a mixture of two components of about the same concentration, this location is at about the middle of the column.

The separation of two compounds into practically pure substances is possible with multistage countercurrent gas extraction. Two separation cascades are needed for this separation. A separation cascade is a sequence of separation steps. Gaseous or liquid streams enter or leave the cascade only at the ends. No sidestreams or additional feedstreams are allowed. A separation of two components is specified by two values, e.g., the concentration of

a compound at one end of the cascade and the amount recovered of this compound. In order to achieve this specified separation, two separation cascades are needed. The upper one serves to separate the bottom product compound from the top product, and the lower one serves to separate the top product compound from the bottom product. In multistage distillation the separation cascades are known as rectifying and stripping sections. In most cases the two cascades for one separation are unified in one separation column.

The separation of two components is the basic case. This case is not very common in practice, since multicomponent mixtures prevail. But the main features of a multicomponent separation can be clearly shown by a two-component separation. Therefore, it is common practice to try to reduce multicomponent separations to two-component separations.

The procedure for designing a separation process for complex organic natural mixtures at maximum recovery of individual compounds or special fractions comprises the following steps: Determination (in most cases measurements) of phase equilibria. The results obtained are the extent of the two-phase region, the mutual solubilities in the co-existing phases, in particular the solubility in the gaseous phase, the separation factor (selectivity), and, if mixtures of various composition are available, its concentration dependence.

Then, a simplified determination of the number of theoretical plates can be carried out using well-known chemical engineering methods like *McCabe-Thiele* or *Ponchon-Savarit*. The number of theoretical stages is determined assuming a quasi-binary system (key components) or with pseudo-components. These simplified methods provide essential insight into the operation of the separation column or the separation device.

With the separation factor known, the number of theoretical stages can be calculated not only for one set of given operating conditions, but in dependence on reflux ratio, conditions of state, with different separation factors and solubilities, and for different product specifications and yields. The resulting separation analysis provides an overview on limiting operating conditions (minimum number of theoretical stages and minimum reflux ratio), as well as on reasonable process parameters. Important decisions on further experiments, feasibility and favourable operating conditions can be made right from the desk.

Then, separation experiments still have to be carried out in laboratory or small pilot plant columns. They prove the accuracy of the separation factor determined by phase-equilibrium measurements and provide data on the height of a theoretical plate (height of a transfer unit). In addition, material of a different composition to the feed is obtained

for renewed phase-equilibrium measurements which yield the concentration dependence of the separation factor.

Data on pressure drop and flooding limits must be provided for determination of capacity of the separation device. Time-consuming and expensive experiments can be avoided nowadays in most cases. Correlations for limiting flow (flooding) are now available for structured packings as used in SFE.

Simulation of the multicomponent separation and the solvent cycle is carried out for optimisation of number of stages and energy consumption. A cost analysis then provides the basis for further decisions.

4 Systems investigated and processes developed

The following examples provide an overview on the type of separations which have been investigated. The summary illustrates the depth of work, it is not comprehensive.

4.1 Deacidifying (removal of free fatty acids)

The separation factor between free fatty acids and triglycerides is high. At 30 MPa and about 330 K the separation factor is 5. It increases to 8 at 20 MPa. Removal of free fatty acids in a countercurrent column is relatively easy and can be completed with a few theoretical stages and practically no loss in triglycerides. Solubility of the free fatty acids in supercritical carbon dioxide is low (about 1 wt.-% at the conditions given). Since the content of free fatty acids in the crude oils (triglycerides) is relatively low, and only the fatty acids have to be removed via the gaseous phase, deacidification could be a relatively favourable application for countercurrent SFE. There have been some laboratory investigations in which the distribution of the minor components has also been taken into account. To my knowledge no industrial evaluation of the process has so far been carried out or published.

4.2 Methyl oleate, squalene

Removal of fatty acid esters from distillates is relatively easy. The solubility of the main components, the free fatty acids, is much lower in supercritical carbon dioxide than that of the esters. The separation of methyl oleate from squalene as a model system was investigated in the laboratory. At 13 MPa and 313 K separation factors of 2 (15% squalene) and 3.5 (70% squalene) have been reported. The separation is relatively easy, yet an evaluation of the process on a real system has not been reported.

4.3 Separation of free fatty acids (FFA)

The separation of free fatty acids by vacuum distillation is a well established procedure. Using supercritical carbon dioxide, the separation can be achieved at much lower temperatures. For a separation of C16- and C18-free fatty acids (99% of C16 in top product, 1% of C16 in bottom product) at $P = 29$ MPa and $T = 373$ K, the separation factor is 1.6. There is a minimum reflux of 3.3 needed, and the minimum number of theoretical stages is 18. A reasonable set of conditions may be: reflux ratio 5.3, number of theoretical stages 30. The solvent to feed ratio (S/F) is 110 which is relatively high. It represents the low solubility of <2 wt.-% of the free fatty acids in supercritical carbon dioxide.

Since the solvent cycle determines process costs, the solvent to feed ratio must be lowered in order to be competitive with conventional vacuum distillation. This can be achieved by carbon dioxide-propane mixtures. Other methods for improving competitive strength are such which allow recycling of the solvent gas at no or low pressure drop (see below).

4.4 Enrichment of tocopherols

Tocopherol mixtures of initially about 55% have been enriched to fractions of about 85 wt.-% of total tocopherol content. Most of the remaining non-tocopherol compounds seem to have a solubility in supercritical carbon dioxide which is within the range of that of the tocopherol isomers. Therefore, a higher concentration can probably only be achieved with chromatographic methods. Since the feed mixture was obtained by unit operations other than SFE, it is possible that artificial compounds have been formed during the processes. Starting from the initial distillates using SFE may lead to higher concentrations than the so far achieved 85%. No industrial evaluation of this process has been carried out.

4.5 Separation of squalene from tocopherols and sterols

Squalene is an interesting target compound from distillates. After removal of the fatty acid esters, the separation problem is between squalene and tocopherols. The mixture may also contain sterols. This separation was thoroughly investigated in our laboratories. Squalene can be separated as top product. In the limited laboratory column (6m packing, *Sulzer EX*) only part of the separation could be experimentally demonstrated. From a quasi binary separation analysis on the basis of these experiments it was concluded that for a 99%-separation of a 10% squalene feed (99% of squalene in top product, 1% of squalene in bottom product) the characteristic data of a sep-

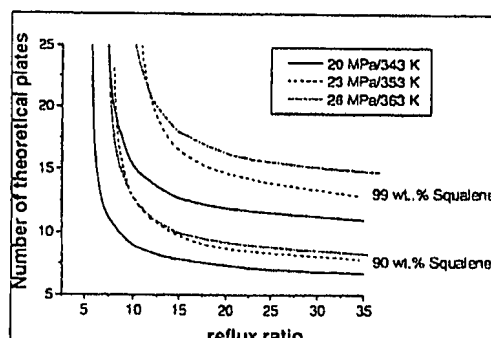


Fig. 2. Analysis of the separation of squalene/tocopherol and sterols: number of theoretical plates versus reflux ratio.

aration are: pressure $P = 26$ MPa, temperature $T = 363$ K, minimum reflux ratio $v_{min} = 9.3$, minimum number of theoretical stages $n_{theor,min} = 15$, solvent to feed ratio $S/F = 30$ kg/kg, number of theoretical stages $n_{theor} = 26$, height of a theoretical stage $HETP = 0.45$ m (*Sulzer EX*), and the height of the column should be 11.6 m (Fig. 2).

The sterols can be enriched in the bottom fraction to about 50%. Then they have to be removed by crystallisation because viscosity of the bottom mixture becomes too high, and solidification may occur. Thus, a highly enriched sterol fraction can be withdrawn.

The process has been extensively investigated on a laboratory scale. The application of carbon dioxide-propane mixture may enhance the performance of the process.

The separation of squalene from an olive oil distillate proved to be possible up to 70% as top product if free fatty acids were present in the feed mixture. At this concentration of squalene, the free fatty acids became enriched in the gaseous phase, while at lower concentrations squalene is enriched in the gaseous phase (if free fatty acids are present). This is no fundamental restriction. The mixture can be further separated in another run or another column.

If the free fatty acids are removed or modified to much more or less volatile components before the separation, then the squalene product can be achieved up to 90% (again with the limited size of the laboratory column). Yet, further enrichment is not as easy as hoped since several compounds seem to have a very similar solubility in supercritical carbon as squalene. Therefore, each case has to be investigated individually.

4.6 Carotenes from crude palm oil (CPO) and from palm-oil-esters

It is an old dream to directly remove carotenes from crude oils. There are two basic restrictions: first, the concentration is low, usually in the range of 500 to 1000 pm, and second the best solvents for carotenes are triglycerides. In terms of selectivity that means that all other components can be readily separated as top product from the crude oil but the carotenes can only be separated as bottom product. That implies that 99% or more of the feed have to be removed as top product. Considering the relative low solubility of triglycerides in supercritical carbon dioxide this is prohibitive.

Therefore, other possibilities were investigated. One is the addition of fatty acid ethyl esters which could act as an entrainer and enhance the volatility of carotenes more than that of triglycerides. Experiments proved that this is not the case. Another possibility is to modify the crude oil to esters (methyl or ethyl esters). The separation of esters from carotenes (and squalene, sterols, and the rest) is easy, as has been discussed above. Then a highly concentrated mixture of carotenes, tocopherols, sterols and other compounds remains which can be treated effectively by countercurrent SFE, as has also been discussed above. The transesterification of crude oils is not far fetched, since the methyl esters are discussed, and partly in use, as biodiesel/fuel.

4.7 Polyunsaturated fatty acid esters (PUFA) from fish oil

Polyunsaturated fatty acid esters can be separated by countercurrent SFE. The separation can be laid between the naturally occurring carbon numbers, e.g., between C16 and C18, or C18 and C20, or C20 and C22. The target compounds EPA, DHA and DPA can be produced up to concentrations of more than 90% (DHA and DPA in a mixture) which so far could not be achieved by vacuum distillation. A typical set of characteristic data for a C18/C20 separation is: $P = 14.5$ MPa, $T = 333$ K, separation factor = 1.33, $v_{\min} = 5.2$, $n_{\text{theor,min}} = 27$, $n_{\text{theor}} = 45$, $HETP = 0.25$ m (Sulzer CY). The process is fully developed and demonstrated at industrial conditions. An experimentally determined and simulated concentration profile of a C18/C20 separation is shown in Fig. 3.

5 Process cycle, economics and conclusions

Separation of lipophilic compounds in a countercurrent multistage mode using supercritical solvents is very effective. It can be superior to comparable separation processes like vacuum distillation or molecular distillation with respect to the degree of separation which is possible, and

even with respect to economics, as will be discussed in the following.

The cost of the solvent cycle determines operating cost in processes applying an additional solvent as a separating agent. This is also true for SFE. High solvent ratios and a high energy demand due to recompression of the solvent render the total process in most cases to be non competitive if only supercritical carbon dioxide is considered as solvent, and reducing the pressure is the only way to recover the product from the solvent.

There are two ways to lower cycle costs in SFE: Enhance solubility in the gaseous phase, and maintain operating pressure during product recovery.

The first way can be achieved by enhancing pressure or temperature. Enhancing the concentration (solubility) in the gaseous phase this way, has its natural limits in phase equilibria: the critical point of the mixture may be nearby which renders practical operation of a separation device impossible, or temperature is too high for the components. It is therefore better to use an alternate solvent. Pure hydrocarbons like ethane or propane are a choice, but it is found that the separation factor (selectivity) is reduced to unacceptable low values ($\alpha \approx 1$). Another way is adding some propane to supercritical carbon dioxide. In the concentration range of 10 to 20% of propane, the sol-

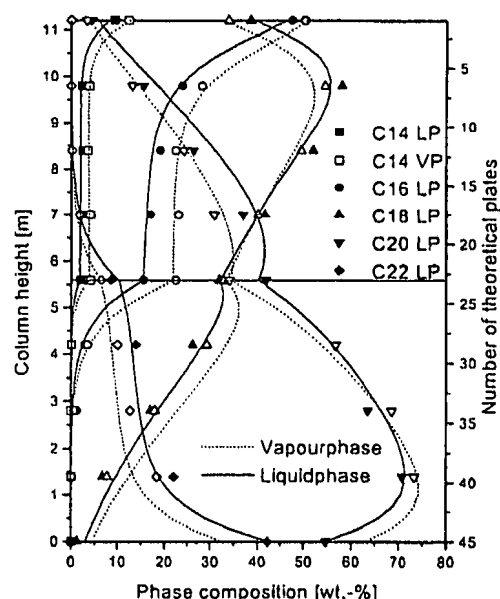


Fig. 3. Experimental concentration profiles and multicomponent process simulation.

ability in the gaseous phase is enhanced appreciably while selectivity is maintained.

Enhancing solubility thus directly reduces solvent cycle costs. It could be shown that in the purification of tocopherols the costs could be reduced by a factor of 10.

The second way is to precipitate the product at operating pressure. This can be achieved by an anti-solvent (N_2 , He, H_2), by membrane separation, by adsorption, and by absorption. Other methods have been discussed, but these are the most obvious ones. In the decaffeination, adsorption on active charcoal was used first, while now absorption in water is preferred. An anti solvent is very effective. The resulting mixture of gases must be separated, preferably by membrane separation. At the high pressures applied in SFE processes, membrane processes have not been demonstrated on an industrial basis. There are very promising laboratory results, but application on an industrial scale has yet to be proven.

A favourable case is when the extract can be adsorbed on an adsorbent and the adsorbate is a product. For such a case it could be shown that gas cycle costs reduce (using pure CO_2 as solvent) by a factor of 4, if a mixture of carbon dioxide and propane is used combined with adsorption, the reduction is by a factor of about 20.

In conclusion, there are modes of operation for supercritical fluid extraction in the countercurrent multistage mode available which reduce costs for the solvent cycle substantially and have been demonstrated on a pilot plant scale. With respect to investment costs, SFE enables a simplified process scheme, since removal of volatile substances is not necessary before beginning the separation of the target compounds. From the known investigations it can be concluded that for lipophilic compounds, as discussed, a separation column of 300 mm diameter could produce 400 tons of product per year. The investment could be around 1.5 mio Euro, and the production costs for such a separation could be about 1.5 Euro per kg of product. Of course, for higher amounts of product the specific costs would be lower, but this is the size for which countercurrent SFE can be taken as developed.

The prospect for separation of lipophilic compounds with supercritical fluids is excellent. A few processes have been developed to commercial application. There is still a wide field for further successful development and introduction to commercialisation.

A compilation of literature can be downloaded from: <http://www.tu-harburg.de/vt2>.

Fractionation of fats by membranes, mechanisms and development opportunities

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Membrane tangential filtration has been used for 30 years in food technology, especially in the cheese-making process, in order to separate the soluble fraction of the milk from the protein phase. Taking into account the size of the pores of the membrane material, several methods have been developed in various food transformations leading to complex fractionation routes and many separation techniques, mainly depending on the size of molecules (Fig. 1).

In such a narrow view, investigating a membrane alternative for the fractionation of fats is an interesting topic. One of the first works carried out on filtration of oils have been done by Bormaz et al. [2] highlighting a discrimination between specific triacylglycerol (TG) groups in butter oil. These authors showed that a threshold was observed on a stainless steel membrane for each group characterised by a given unsaturation degree.

Under a classical approach, the filtering of triglycerides appears troublesome, because of the short extent of molecule size (500–1000 Da), which seems only compatible with nanofiltration. Compared to a polar medium containing well identified macromolecules dispersed in an aqueous solvent phase, a hydrophobic medium made of oils or melted fats, exhibits a *continuum* of properties which are obviously very difficult to separate across a porous material. The discrimination between TG molecules should rely on specific physical properties. It has been said above that the range of molecular size is very short: the length of the carbon chains does not permit discrimination between species, especially in the vegetal oil area, where C16–C18 chains prevail. This may be quite different for animal fats, and especially for butter oil in which the range of molecule size is larger. The particular stereochemical behaviour of the first terms (C4–C8 FA chains) leads to a possible discrimination based on the specific conformations that may affect these molecules in interface interaction [3]. Such a behaviour could be observed through

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