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Short Communication

Separation of free fatty acids from deodorizer distillates using choline hydrogen carbonate and supercritical carbon dioxide

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ABSTRACT

One of the main problems of deodorizer distillates is the difficulty in separating free carboxylic fatty acids (FFA) from the remaining added-value components, such as squalene. A two-step novel strategy for the valorization of olive oil deodorizer distillate (OODD) is presented, based on the use of choline hydrogen carbonate and supercritical carbon dioxide ($scCO_2$). In the first step, the FFA present in OODD were neutralized with choline hydrogen carbonate to form choline carboxylates. The choline carboxylates obtained are of interest to the cosmetic industry. Due to their ionic character, they are insoluble in $scCO_2$. Therefore in the second step, the reaction mixture was subjected to extraction with $scCO_2$ at 15 MPa, 313 K and a gas flow rate of 2 ml min^{-1}, yielding an extract with a maximum FFA content of ca. 3% (w/w). A valorization supply chain of OODD is proposed.

1. Introduction

Olive oil deodorizer distillate (OODD) is a low market-value by-product of the olive oil refining process, being the distilled fraction obtained from the deodorization step. It contains up to 40% by weight of squalene, the rest being free fatty acids (FFA), fatty acids alkyl esters (FAAE), and, in small amounts, tocopherols and sterols [1].

The main applications of squalene and its hydrogenated form (squalene) are found in the cosmetic industry, as a moisturizing or emollient agent, and in the pharmaceutical industry, as an adjunctive in vaccines and cancer therapies. Due to the high content in squalene, up to 60% by weight, deep-sea sharks liver oil has been its primary source. However environmental concerns regarding the declining of marine animal population have intensified the search of more sustainable sources [2,3].

The high content in squalene and the low market value of OODD makes it a desirable alternative. Nevertheless the effective and selective removal of the FFA from OODD hinders its implementation as a viable source of high added-value compounds, such as squalene.

Several procedures for the extraction of squalene from vegetable oils have been reported, such as molecular distillation, high-speed counter-current chromatography and supercritical fluid extraction [1,4–6]. Supercritical carbon dioxide ($scCO_2$) extraction remains an attractive process since no additional chemicals are involved and thermally labile compounds are not degraded due to the mild temperatures used. Despite its advantages, the industrial application of this process is problematic due to the extremely similar solubilities of squalene and free fatty acids (FFA) in $scCO_2$ [7].

One approach to tackle this problem is through chemical modification of the FFA. By converting FFA into a different functional group, e.g., fatty acid methyl esters (FAME), the separation factor between the esters and squalene in $scCO_2$ is improved and better separation efficiency can be obtained [5]. Alternative strategies have been recently reported using $scCO_2$ technology in combination with membrane separation, which moderately improve separation efficiency [8].

Another strategy involves the neutralization of the carboxylic groups of the FFA followed by the separation of the unsaponifiable matter from the ionic compounds by liquid–liquid extraction. However this methodology relies on the use of strong bases, such as sodium hydroxide, and large amounts of organic solvents, such as hexane, to solubilize all components and to perform the extraction steps [9,10].

From our previous work [11], we discovered that choline hydrogen carbonate is soluble enough in organic compounds to allow the neutralization of carboxylic acids without the addition of any solvents. This reaction produces a choline salt of the respective carboxylic acid, being water and carbon dioxide the only by-products

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(Fig. 1). Then, separation of the other organic components present in the original mixture from the ionic compounds by $scCO_2$ can be achieved due to the virtual insolubility of must ionic compounds in the supercritical fluid [12].

Moreover, the choline carboxylates formed on the former reaction could have innumerous applications due to the biocompatibility and biodegradability of its components. Choline is fundamental for the normal functioning of all cells and is considered an essential micronutrient for humans [13]. Its combination with fatty acids allows the production of surfactants completely made of materials naturally occurring in the human body. As such these salts are extremely appealing not only to the cosmetic industry but also for drug delivery systems. This kind of surfactants can be easily decomposed both physiologically and environmentally, with studies showing that they are significantly less toxic than common surfactants like sodium dodecylsulphate (SDS) or benzalkonium chloride (BAC). Other advantage of the use of choline as counterion is the substantial reduction of the Krafft point (which is a criterion for the solubility of the surfactant in water) of fatty acids when compared with their alkali counterparts. This means that choline carboxylates are more soluble in water and are capable of forming micelles at room temperature, in contrast to the restricted solubility of homologous alkali soaps [14–16].

Herein, we report the use of choline hydrogen carbonate for capture of free fatty acids by neutralization, in a model mixture (oleic acid:squalene 1:1 (w/w)). The separation of squalene from the reaction mixture by supercritical carbon dioxide, extraction and optimization of the process parameters were also investigated. This methodology was then applied to olive oil deodorizer distillate sample.

2. Material and methods

2.1. Materials

All solvents were obtained commercially and appropriately purified, if necessary. Carbon dioxide (99.98 %) was from Air Liquide. Squalene (98% by weight) and oleic acid (technical grade, 79.9% by mass; other major fatty acids present included myristic acid (C14:0) at 2.0%, palmitoleic acid (C16:1) at 5.2%, palmitic acid (C16:0) at 5.6%, stearic acid (C18:0) at 1.0%, and linoleic acid (C18:2) at 6.4%) were supplied by Sigma Aldrich. Choline hydrogen carbonate was synthesized following the procedure described in the literature [11]. The olive oil deodorizer distillate used in the experiments was gently provided by Sovena S.A. (Lisbon, Portugal).

2.2. Methods

2.2.1. General FFA neutralization procedure

In all the experiments, choline hydrogen carbonate was added in small excess in regard to the FFA content of the oil mixture, to ensure that all acidic compounds were neutralized.

To a 2.0 g sample of model mixture (oleic acid:squalene 50% (w/w)) or OODD was added between 1.2 and 1.5 equivalents of choline hydrogen carbonate (0.70–0.88 g or 0.52–0.66 g respectively). The reaction mixture was stirred at 50 °C for 1–1.5 h before being transferred to the extraction vessel.

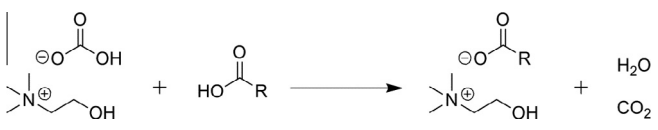

Fig. 1. Neutralization reaction of carboxylic acids by choline hydrogen carbonate.

2.2.2. High pressure extraction experiments

A scheme of the high pressure extraction apparatus used is depicted in Fig. 2. CO_2 was firstly liquefied in a cooling bath (Julabo) and pumped through an HPLC pump (max. 100 ml/min, Knauer 1800) to the desired flow rate measured in MFM01. Before entering the 18 ml extraction vessel CO_2 was heated to the extraction temperature through a heating bath. $ScCO_2$ was then bubbled by a 1/16 in. tube into neutralized OODD mixture previously fed to the extraction vessel. Optimum mixing was assured by a magnetic mixer (400 rpm). Extraction pressure was controlled through the back pressure regulator BPR01. The extract stream was depressurized after BPR01 and the liquid fraction of the stream recovered in a glass trap immersed in an ice bath. Samples were taken in predetermined time intervals by changing the glass trap. Samples were analyzed by GC.

2.2.3. Analytic methods

Qualitative and quantitative analysis was performed to the initial OODD and for each extraction sample recovered.

The total free fatty acid was quantified by titration. An 1 g sample was added to 50 ml of solvent mixture (ethanol:diethyl ether 1:1 (v/v)). Phenolphthalein was used as a pH indicator. Potassium hydroxide 0.1 M in ethanol was added until the solution turned from yellow to pink.

Total free fatty acids, fatty acid alkyl esters and squalene were quantitatively analyzed by gas chromatography in a Thermo Trace GC ULTRA, equipped with a flame ionization detector (FID) and a split injector. The injector and detector temperatures were set at 280 °C. The split flow was set at 134 ml min^{-1}. The analytical column was a TR-Biodiesel (F) from Thermo Unicam. 0.5 μL sample volume was injected by means of an automatic injector TriPlus. Helium was used as carrier at a constant flow of 2 ml min^{-1}. The oven temperature program was 230 °C for 13 min. Peak identification was carried out using known standards (oleic acid and squalene, Sigma–Aldrich). Methyl-heptadecanoate (Fluka) was used as the internal standard (IS). The data were processed with the Chrom-Card software.

The fatty acid profile of both the OODD and extract samples was determined by direct transesterification of the lipids to the corresponding methyl esters and then quantitatively analysed by GC, according with the method of Lepage and Roy with modifications [17]. A 10 mg sample was transmethylated with 2 ml of methanol:acetyl chloride (95:5 (v/v)). The mixture was sealed in a Teflon-lined vial under nitrogen atmosphere and heated at 80 °C for 1 h. The vial contents were then cooled, diluted with 1 ml of water, and extracted with 2 ml of n-hexane. The organic layer was dried over Na_2SO_4. The obtained methyl esters were quantified by GC using the method described before.

3. Results and discussion

The experimental data reported is the average of at least two replicas with a standard deviation of 2.8%.

3.1. Squalene/FFA fractionation from neutralized model mixture

Due to their inherent economical value and to the reported difficulties in their fractionation [1,4–6], the two main compounds in the OODD mixture that require more intensive fractionation studies are squalene and FFA. Thus, fractionation experiments were firstly carried out using a model mixture of 50% (w/w) of squalene and oleic acid. This mixture was subjected to a reaction with choline hydrogen carbonate for 1–1.5 h at 323 K, as previously described. Gas liberation was observed immediately, confirming that neutralization of FFA was effectively taking place, since carbon