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Solvent resistant diananofiltration for production of steryl esters enriched extracts

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ABSTRACT

Deodorizer distillate is a by-product from edible oil refining rich in bioactive compounds. However, its use as food additive is not allowed due to the presence of pesticides in relatively high concentrations. This paper discusses the technical feasibility of a solvent resistant membrane-based process for production of steryl esters-enriched extracts, using deodorizer distillates as raw material.

A mass-balance based model was used to predict the profile of species concentration during diananofiltration processing of a hexane-based solution containing 5% (w/w) of deodorizer distillate. This tool enabled the comparison of three commercial SRNF membranes in terms of their discrimination between pesticides and steryl esters. PuraMemS600 from Evonik was identified as the best membrane, showing the best compromise between membrane flux and rejection behavior towards the compounds of interest. This membrane presented a constant rejection of steryl esters (95.5%) and a time-dependent flux, probably associated to swelling effects. Both the rejection and permeability data were used in the simulation of the diananofiltration process, making possible to obtain a good agreement of the model with the experimental data.

The diananofiltration technique investigated in this work showed to be suitable for an efficient removal of pesticides, however, at expense of a significant loss of steryl esters of 42%. An alternative configuration of two-stage diananofiltration was simulated, suggesting an improvement of the efficiency of the process.

1. Introduction

The production of large amounts of wastes by the agro-food industries represents an environmental burden [1]. Demanding legislation concerning waste management has been issued by the European Commission, resulting in a high encouragement to develop and achieve better and improved methods for recovery and purification of value-added compounds from by-products streams. Under this context, the recovery of bioactive compounds has a special interest given the increasing market of functional foods [2]. Deodorizer distillates, a residual stream produced in the vegetable oil refining process, present a high potential for the recovery of added value compounds. Their content in bioactive compounds such sterols, tocopherols and squalene may vary between 2% and 20% [3] although, their pesticide content is too high [4] to allow their use directly as additive in food, pharmaceutical and cosmetic products [5]. Maximum residue levels (MRLs) in crude and refined oils for human consumption are not specifically set but, according to Article 20 of EU Regulation No. 396/2005, they have to be derived from the MRLs established for seeds (0.05 ppm for main lipophilic pesticides) [6]. The concentration of pesticides in deodorizer distillates can be 800–1000 times higher than the actually allowed values [4,7]. Therefore, their valorisation as a food additive rich in bioactive compounds depends from the effective removal of pesticides, which remains challenging.

Solvent resistant nanofiltration (SRNF) is a potential alternative technology for recovery of bioactive compounds, being remarkably simpler than traditional processes such as distillation, solvent extraction and crystallization which are labor-intensive, time consuming and energetically intensive. Additionally, it enables the purification, fractionation and concentration of natural extracts, without requiring high process temperatures, which typically damage their valuable compounds. SRNF has been applied with relative success in the pharmaceutical industry, such as in the purification of an active pharmaceutical ingredient (API) [8,9], catalyst recycling [10], removal of genotoxins from APIs [11], continuous solvent exchange [12] and solvent recycling [13,14]. The use of SRNF in oil refining industry has been discussed, although, mainly from the point of view of solvent recycling and oil recovery [15–17]. The present work discusses a different perspective through the use of SRNF in the valorisation of by-products. Specifically, we propose a solvent

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resistant membrane-based process to produce an extract rich in steryl esters and free of pesticides, from deodorizer distillates.

The use of steryl esters as food additive has been intensified mainly after its recognition by the European Food Safety Authority (EFSA) as compounds with anti-cholesterol activity. The Scientific Panel decided that the maximum phytosterols intake should not exceed 3 g/day and the content of phytosterols in the ingredients cannot be higher than 30% [18,19]. However, the regulation express the importance of toxicology analysis to the final product (including pesticides) to guaranty its safety.

Steryl esters can be produced by enzymatic reaction between free sterols and free fatty acids from deodorizer distillates. Their production is advantageous in terms of bioactivity and incorporation in fat-based products, since steryl esters are more bioactive than free sterols [20] and more soluble in the oil phase. Moreover, the molecular weight of steryl esters (650–800 g mol^{-1}) enables their separation from pesticides (150–400 g mol^{-1}), if the technology applied is based on size exclusion. The separation capabilities of SRNF membranes are related to size exclusion, but there is also an important impact of the chemical interactions of the solvents with the membrane material (that, for example, may induce swelling).

SRNF is generally employed in a diananofiltration mode for removal of contaminants with low molecular weight (MW) from valuable products with higher molecular weight. This method consists in continuously feeding fresh solvent at the same rate as the permeate is recovered. The present work is focused on the purification of the steryl esters rich aiming the removal of pesticides, thus, steryl esters should be retained by the membrane while pesticides are washed-out in the permeate. One of the main challenges of applying diananofiltration to the present system is the identification of a membrane stable in contact with the processing medium and suitable to perform the required separation. In a previous work [21], the membranes GMT-oNF2 from Borsig/GMT (Germany), PuraMemS600 from Evonik (UK) and 030306F from Solsep (Netherlands) were identified as potential candidates, presenting high rejections for steryl esters (>96%) and relatively low rejections of pesticides in diluted hexane-based solutions (5% w/w). The main objective of this work is to select the most adequate membrane to fulfill the final purpose of removing pesticides from steryl esters-enriched deodorizer distillates and demonstrate that diananofiltration is a suitable technique to achieve that objective. This work should provide the basis for the scaling-up of this process, through demonstration of the concept of producing extracts rich in steryl esters, free of pesticides.

2. Modelling of diananofiltration process

Diananofiltration in a batch mode of operation at constant pressure and fixed feed volume can be described by a mass-balance for each species i in the system:

$$\frac{d[V_f C_{f,i}]}{dt} = -J_v A C_{p,i} \quad (1)$$

where V_f (L) is the volume of the feed solution, J_v (L m^{-2} h^{-1}) is the permeate flux, A (m^2) is the area of the membrane and $C_{f,i}$ and $C_{p,i}$ (g L^{-1}) are the bulk concentrations of species i in the feed and permeate, respectively.

The observed rejection (R_{obs}) is defined as:

$$R_{obs} = 1 - \frac{C_{p,i}}{C_{f,i}} \quad (2)$$

replacing Eq. (2) into Eq. (1) yields:

$$\frac{d[C_{f,i}]}{dt} = -\frac{J_v A}{V_f} C_{f,i}(1 - R_{obs}) = \left[-\frac{V_D}{t} C_{f,i}(1 - R_{obs})\right] \quad (3)$$

where V_D represents the number of diananofiltration volumes defined as the total volume of permeate collected divided by initial system volume. This dimensionless parameter allows different diananofiltration systems to be compared.

Two parameters were defined for quantifying the efficiency of the system for pesticide (P) removal (Eq. (4)) and steryl esters (SE) loss (Eq. (5)), defined as:

$$\text{Removal}_{P_{(t=i)}} = \frac{M_{f_{P(t=i)}}}{M_{f_{P(t=0)}}} \times 100 \quad (4)$$

$$Loss_{SE_{(t=i)}} = \frac{M_{p_{SE(t=i)}}}{M_{f_{SE(t=0)}}} \times 100 \quad (5)$$

where M_{pi} and M_{fi} are the mass of species i in the permeate and feed tanks, respectively.

3. Materials and methods

3.1. Material

3.1.1. Esterified deodorizer distillate

The sunflower deodorizer distillate from Lesieur (France) used in this work was characterized in our laboratory and its composition was presented elsewhere [22]. Sterols and free fatty acids (FFA) were esterified to produce steryl esters, using a lipase from *Candida Rugosa*. The enzymatic reaction was performed under optimised conditions, that were established in a previous work [22].

The esterified deodorizer distillate used in this study is rich in acylglycerides (35%) and free fatty acids (29.8%). It also contains valuable compounds, such as sterols (0.27%), steryl esters (6.6%), tocopherols (1.7%) and squalene (1.1%). Other compounds comprise hydrocarbons, aldehydes, ketones, pesticides, herbicides and oxidized products from breakdown of tocopherols and free phytosterols. The pesticides pirimiphos-methyl, chlorpyriphos-methyl and chlorpyriphos were present at high concentration (16.4, 10.5 and 0.27 mg/kg, respectively), as well as oxidized products and aldehydes, given by the peroxide number and the p-anisidine value (15.9 mEq O_2/kg and 1056, respectively).

3.1.2. Chemicals

Analytical-grade hexane, ethanol, isopropanol, isooctane, acetic acid glacial and potassium iodine were supplied from VWR (Germany). Technical grade chloroform (99%) for GC analysis and food-grade oleic acid with an acid value of 196.0–204 mg KOH/g were purchased from Sigma Aldrich (Belgium).

The derivatizating and silylation agent, N,O-bis (trimethylsilyl) trifluracetamide (BSTFA) containing 1% of trimethylchlorosilane (TCMS) solution (from Fluka) and pyridine (from Sigma–Aldrich) were both obtained from Sigma (France).

All analytical-grade standard substances, squalene (99.3% purity), stigmasterol (97% purity), β-sitosterol (99%purity), campesterol (99% purity), cholesteryl stearate (96% purity), monoglyceride olein (>99%purity), diglyceride olein (99.7% purity) and triglyceride olein (99.6% purity) were purchased from Sigma (Saint Quentin, France). A tocopherol kit consisting of α-, β-, γ- and δ-tocopherols was obtained from Merck (>95% purity).

The internal standard heptadecanol stearate (HDS) was prepared by condensation of heptadecanol and stearoyl chloride, both obtained from Aldrich (Belgium), as described by Verleyen et al. [23].

3.1.3. Membranes

Three PDMS-based commercial SRNF membranes from different manufactures were studied in this work, namely 030306F from Solsep (Netherlands), PuraMem600 from Evonik (U.K.) and GMT-oNF2 from Borsig/GMT Membrantechnik (Germany). These mem-