Research Paper

Production of phytosterol esters from soybean oil deodorizer distillates

Carlos F. Torres, Tiziana Fornari, Guzmán Torrelo, F. Javier Señoráns and Guillermo Reglero

Sección Departamental de Ciencias de la Alimentación, Universidad Autónoma de Madrid, Madrid, Spain

Enzymatic esterification and supercritical fluid extraction was used to produce phytosterol esters from soybean oil deodorizer distillates. The raw material was first subjected to a two-step enzymatic reaction; the product obtained mainly comprised fatty acid ethyl esters, tocopherols and phytosterol esters, together with minor amounts of squalene, free fatty acids, free sterols and triacylglycerols. The phytosterol esters were then purified from this mixture using supercritical carbon dioxide. Experimental extractions were carried out in an isothermal countercurrent column (without reflux), with pressures ranging from 200 to 280 bar, temperatures of 45–55 °C and solvent-to-feed ratios from 15 to 35 kg/kg. Using these extraction conditions, the fatty acid esters were completely extracted and, thus, the fractionation of tocopherols and phytosterol esters was studied. At 250 bar, 55 °C and a solvent-to-feed ratio of 35, the phytosterol esters were concentrated in the raffinate up to 82.4 wt-% with satisfactory yield (72%).

Keywords: Carbon dioxide / Enzymatic esterification / Phytosterol esters / Supercritical fluid / Tocopherols

Received: June 6, 2008; accepted: December 9, 2008
DOI 10.1002/ejlt.200800141

1 Introduction

Plant sterols, stanols and their esters have been found to be effective in lowering plasma cholesterol concentrations by inhibiting the absorption of cholesterol from the small intestine [1, 2]. Clinical studies have shown significant reductions in cholesterol levels in a population consuming a high-fat diet fortified with phytosterols [3, 4]. Nevertheless, phytosterols must be ingested in high doses to be effective. Thus, practical applications of phytosterols in foods are limited due to their crystalline property, low solubility in lipid materials and physical reactivity. For example, the solubility of phytosterols in edible oils is very low while their melting points are rather high (about 140–150 °C) [4].

An important goal, then, is to create a more soluble phytosterol-type product which can be given at lower dosages while maintaining a high bio-efficiency. One method to achieve this goal is converting phytosterols to their esterified forms, which are soluble in the oil phase and can be incorporated into fat-based products [5].

Vegetable oil deodorizer distillates (VODD) are a natural source of vitamin E (tocopherols), free sterols and phytosterol esters. Tocopherols and phytosterol esters have been purified from deodorizer distillates by a process including molecular distillation [6, 7]. Chemical modification of the VODD to convert free fatty acids (FFA) to their methyl or ethyl esters has been accomplished, and then short-path distillation of the reaction mixture could be utilized for the elimination of fatty acid esters and thus the purification of tocopherols and phytosterol esters [7, 8].

Additionally, the purification of tocopherols and phytosterols from VODD using supercritical fluid extraction (SFE) has been widely investigated in the last two decades. Several authors have studied the concentration of tocopherols directly from the VODD, i.e. without carrying out any modification pretreatment of the raw material, namely the separation of tocopherols from FFA (see for example Lee et al. [9], Brunner et al. [10], Mendez et al. [11], and King et al. [12]). On the other hand, the chemical modification of the VODD combined with SFE has been recently reported (Fang et al. [13], Nagesha et al. [14]). In this case, esterification and methanalysis of the VODD produced a mixture containing tocopherols, phytosterol esters and fatty acid methyl esters, with the process goal of the SFE process being the elimination of fatty acid methyl esters to concentrate tocopherols and phytosterol esters in the raffinate.
The present work is devoted to study the SFE of an enzymatically modified soybean oil deodorizer distillate (SODD) in order to obtain a raffinate consisting mostly of phytosterol esters. Following the methodology described in previous work [15], the SODD was initially modified by the addition of oleic acid in order to decrease the deodorizer distillate melting point. Then, the oleic acid-modified raw material (OSODD) was subjected to a two-step enzymatic reaction, resulting in a product that contained mainly phytosterol esters, tocopherols and fatty acid ethyl esters. The fatty acid ethyl esters can be eliminated by SFE as demonstrated by others [13, 14]. Thus, the phytosterol esters could be concentrated in the raffinate if the extraction of tocopherols could be achieved, maintaining a reasonable phytosterol ester yield. The SFE separation problem studied in this work is the partition of the phytosterol esters and tocopherols between the top and bottom products of the countercurrent extraction column. Experimental assays were carried out at pressures ranging from 200 to 280 bar, temperatures of 45–55 °C and at solvent-to-feed ratios from 15 to 35.

2 Materials and methods

2.1 Sample and reagents

The SODD was donated by a Spanish oil company; carbon dioxide N38 (99.98%) was purchased from AL Air Liquide España S.A. (Madrid, Spain). All solvents used were of HPLC grade from Lab-Scan (Dublin, Ireland). The lipase from Candida rugosa was purchased from Sigma (St. Louis, MO, USA). The lipase from Candida antarctica (Novozyme 435) was kindly donated by Novozymes (Bagsvaerd, Denmark).

2.2 Two-step enzymatic modification of the SODD

Step 1: Sterol esterification reaction: OSODD was mixed with 10 wt-% of lipase from Candida rugosa and incubated in an orbital shaker (200 rpm) at 35 °C. Samples (100 µL) were withdrawn periodically (0, 0.5, 1, 2, 3, 5, 10, and 24 h). The reaction was allowed to proceed for 24 h.

Step 2: Ethyl esterification reaction: The product mixture from the sterol esterification reaction was mixed with 10 wt-% of ethanol, 5 wt-% of immobilized Candida antarctica lipase (Novozym 435), and 8 wt-% of hexadecane (internal standard), unless otherwise stated. The mixture was incubated in an orbital shaker (200 rpm) at 35 °C. Samples (100 µL) were withdrawn periodically. The reaction was allowed to proceed for 24 h.

2.2 SFE equipment and extraction method

Figure 1 shows a flow diagram of the countercurrent-SFE (CC-SFE) system employed in this study. The countercurrent extraction column (316 stainless steel) is 100 cm high and 12 mm in internal diameter, and is packed with Fenske rings (3 × 0.5 mm). The CC-SFE device also comprises two separator cells (S1 and S2) of 270 mL capacity each (where a cascade decompression takes place) and a cryogenic trap at atmospheric pressure. Both CO2 and liquid feed sample were preheated at the exit of their respective pumps (Dosapro Milton Roy) before introduction into the extraction column. All units were electrically thermostatted.

During the extraction, a continuous flow of CO2 was introduced into the column through the bottom. When the operating pressure and temperature were reached, the liquid sample was pumped (100 mL/h) from the top during the entire extraction time (60 min).
The first separator was maintained at 150–160 bar and 55 °C while the second separator cell was maintained at low pressure (20 bar) and temperature (15–20 °C). The raffinate and liquid fractions collected in the separators were weighed and analyzed. The material balance closed in all experiments within 7.4%.

2.3 Analysis

2.3.1 HPLC analysis

The composition analyses of the neutral lipids were effected on a KROMASIL silica 60 column (250 mm × 4.6 mm; Análisis Vinicos, Tomelloso, Spain) coupled to a CTO 10A VP 2 oven, a LC-10AD VP pump, a gradient module FCV-10AL VP, a DGU-14A degasser, and an evaporative light scattering detector ELSD-LT from Shimadzu (IZASA, Spain). The ELSD conditions were 2.2 bar, 35 °C, and gain 3. The flow rate was 2 mL/min. A splitter valve was used after the column and only 50% of the mobile phase was directed through the detector. The column temperature was maintained at 35 °C. The mobile phase utilized has previously been reported by Torres et al. [16].

2.3.2 Gas chromatography

For the analysis of fatty acid ethyl esters, 1 mL of the diluted sample was injected into an Agilent (Avondale, PA) gas chromatograph (6890N Network GC System) coupled to an autosampler (Agilent 7683B). The capillary column was a 30-m HP-88 (Avondale, PA) (0.25 mm i.d.). The temperatures of the injector and detector were 220 and 250 °C, respectively. The temperature program was as follows: starting at 100 °C and then heating to 180 °C at 20 °C/min, followed by heating from 180 to 220 °C at 15 °C/min. The final temperature (220 °C) was held for 30 min. Identification of the various FFA was based on a PUFA No. 3 standard (#4-7085) obtained from Supelco.

3 Results and discussion

The compositions of the original SODD, the OSODD and the enzymatically modified OSODD (EM-OSODD) are given in Table 1. As mentioned previously, Candida rugosa lipase was used in the first step to produce the esterification of free sterols from the OSODD using FFA. Then, the ethyl esterification of the remaining FFA and acylglycerol was conducted employing Candida antarctica lipase. As can be concluded from the compositions reported in Table 1, this lipase effectively discriminates against phytosterol esters [17], and FFA and acylglycerols could be converted into their corresponding fatty acid ethyl esters without affecting the phytosterol esters.

The EM-OSODD was utilized for the selective enrichment of phytosterol esters via CC-SFE.

Taking into account previous work reported in the literature [13, 14], the extraction of fatty acid esters from enzymatically esterified VODD can be achieved at 18 MPa and 60 °C [13] or at lower pressures (16 MPa) and using a temperature gradient of 40–75 °C [14]. As mentioned before, not only fatty acid esters but also tocopherols have to be extracted from the EM-SODD obtained in this work in order to concentrate and purify phytosterol esters in the raffinate. Thus, pressures higher than 20 MPa were explored at a fixed temperature of 55 °C (no temperature gradient was utilized).

The different extraction conditions, i.e. temperature, pressure and solvent-to-feed (S/F) ratio, employed in the experimental SFE assays are given in Table 2. Also given in the table are the raffinate composition and the phytosterol ester yield (mass of phytosterol esters in raffinate/mass of phytosterol esters in feed) obtained. As can be observed in the table, squalene, fatty acid ethyl esters and FFA were almost completely extracted in all experimental assays reported in Table 2. Additionally, high pressures greatly favor the extraction of tocopherols, and phytosterol esters can be concentrated in the raffinate with satisfactory yields.

Figure 2 shows the variation of phytosterol ester and tocopherol weight fractions obtained at constant temperature and solvent-to-feed ratio. As the phytosterol ester weight fraction increases with increasing pressure, the tocopherol weight fraction decreases, while the concentrations of free sterol and triacylglycerol remain almost constant (see Table 2) in the raffinates.

Additionally, the phytosterol ester purity could be increased from 77.9 to 82.4 wt-% by increasing the gas load, as can be observed in experiments 3 and 6 in Table 2 (solvent-to-feed ratio increased from 25 to 35). This effect was also observed at 200 bar and 55 °C, by increasing the solvent-to-feed ratio from 15 to 25. A minor effect of temperature on phytosterol ester purity and yield was observed in the (narrow) experimental range explored.

The top product was fractionated by a cascade decompression into two different fractions (named S1 and S2). The results of the fractionation are shown in Table 3. Fraction S1

Table 1. Composition (wt-%) of the original SODD, the OSODD and the EM-OSODD.

<table>
<thead>
<tr>
<th></th>
<th>SODD</th>
<th>OSODD</th>
<th>EM-OSODD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocarbons</td>
<td>4.5</td>
<td>3.2</td>
<td>3.3</td>
</tr>
<tr>
<td>Phytosterol esters</td>
<td>7.3</td>
<td>6.2</td>
<td>38.6</td>
</tr>
<tr>
<td>Tocopherols</td>
<td>20.1</td>
<td>16.3</td>
<td>17.5</td>
</tr>
<tr>
<td>Acylglycerols</td>
<td>21.1</td>
<td>12.1</td>
<td>4.2</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>19.8</td>
<td>40.3</td>
<td>4.1</td>
</tr>
<tr>
<td>Sterols</td>
<td>27.1</td>
<td>21.4</td>
<td>2.3</td>
</tr>
<tr>
<td>Fatty acid ethyl esters</td>
<td>–</td>
<td></td>
<td>30.0</td>
</tr>
</tbody>
</table>
Table 2. Composition and phytosterol ester yield obtained in the raffinate product of the CC-SFE assays carried out at the different extraction conditions.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>SQ</td>
<td>FAEE</td>
</tr>
<tr>
<td>1</td>
<td>200</td>
<td>25</td>
<td>55</td>
<td>0.39</td>
<td>0.10</td>
</tr>
<tr>
<td>2</td>
<td>225</td>
<td>25</td>
<td>55</td>
<td>0.00</td>
<td>0.06</td>
</tr>
<tr>
<td>3</td>
<td>250</td>
<td>25</td>
<td>55</td>
<td>0.00</td>
<td>0.09</td>
</tr>
<tr>
<td>4</td>
<td>280</td>
<td>25</td>
<td>55</td>
<td>0.00</td>
<td>0.09</td>
</tr>
<tr>
<td>5</td>
<td>200</td>
<td>15</td>
<td>55</td>
<td>0.59</td>
<td>0.30</td>
</tr>
<tr>
<td>6</td>
<td>250</td>
<td>35</td>
<td>55</td>
<td>0.00</td>
<td>0.10</td>
</tr>
<tr>
<td>7</td>
<td>225</td>
<td>25</td>
<td>45</td>
<td>0.00</td>
<td>0.14</td>
</tr>
</tbody>
</table>

SQ, Squalene; FAEE, fatty acid ethyl esters; PE, phytosterol esters; TOC, tocopherols; FFA, free fatty acids; S, free sterols; TAG, triacylglycerols.

Table 3. Composition of the top product fractions obtained in the S1 and S2 separators of the CC-SFE. S1 conditions: 160 bar and 55 °C; S2 conditions: 20 bar and 55 °C.

<table>
<thead>
<tr>
<th>EM-SODD</th>
<th>S1 separator unit</th>
<th>S2 separator unit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ext.1</td>
<td>Ext.2</td>
</tr>
<tr>
<td>FAEE</td>
<td>30.1</td>
<td>10.2</td>
</tr>
<tr>
<td>PE</td>
<td>38.7</td>
<td>34.1</td>
</tr>
<tr>
<td>TOC</td>
<td>17.4</td>
<td>36.2</td>
</tr>
<tr>
<td>S</td>
<td>2.3</td>
<td>5.7</td>
</tr>
<tr>
<td>TAG</td>
<td>4.2</td>
<td>5.2</td>
</tr>
</tbody>
</table>

EM-SODD, Enzymatically modified soybean deodorizer distillate; FAEE, fatty acid ethyl esters; PE, phytosterol esters; TOC, tocopherols; S, free sterols; TAG, triacylglycerols.

was obtained at 160 bar and 55 °C and contained ca. 40 wt-% of tocopherols. The enrichment factor of tocopherols and free sterols in S1 was ca. 2 and 3, respectively. It should also be noted that a similar composition of phytosterol esters and triacylglycerols, with respect to that of the starting material, was obtained in S1. On the contrary, the percentage of fatty acid ethyl esters was ca. 10% (three times lower than that of the starting material). Regarding the recovery, the total weight of fraction S1 never exceed 18 g, which represents less than 20% of the total material introduced into the column. At 280 bar and 55 °C, ca. 60% of total tocopherols introduced into the column were collected in S1. It should also be indicated that ca. 90% of the total fatty acid ethyl esters introduced into the column were recovered in S2. These results indicate that it is possible to fractionate the top product to recover an extract enriched in tocopherols and sterols.

Phytosterol esters from SODD were produced in this work by combining enzymatic modification of the original raw material and countercurrent CO₂-SFE. Figure 3 shows a scheme of the process developed, indicating operational conditions and product compositions. After the two-step enzymatic esterification, the EM-OSODD contained around 38 wt-% of phytosterol esters. The experimental SFE carried
Figure 3. Schematic representation of the process developed to produce phytosterol esters from SODD.

out in our laboratory demonstrated that phytosterol esters can be concentrated in the raffinate at up to 82 wt.% (with 72% yield) working at 250 bar, 55 °C and a solvent-to-feed ratio of 35. Additionally, fractionation of the extract allowed 60% of tocopherol recovery in the first separator and 90% of fatty acid ethyl esters in the second separator.

Acknowledgments

The authors gratefully acknowledge the financial support from the Comunidad Autónoma de Madrid (ALIBIRD, project S-505/AGR-0153) and the Ministerio de Ciencia y Tecnología, projects AGL2008-05655/ALI and 25506 FUN-C-FOOD (CONSOLIDER-INGENIO 2010), Spain. T. F. and C. T. would like to acknowledge the postdoctoral contract (Programa Ramón y Cajal) given by the Ministerio de Ciencia y Tecnología and the Universidad Autónoma de Madrid, Spain.

Conflict of interest statement

The authors have declared no conflict of interest.

References


