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Semi-continuous extraction/purification of lipids by means of supercritical fluids

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Abstract

A method for semi-continuous extraction using supercritical carbon dioxide has been developed. In this technique, two streams, a solution of the material that will be fractionated and supercritical carbon dioxide, are coaxially introduced to a nozzle where a spray is formed. Extensive physical contact between the supercritical fluid and the feed solution is achieved in the spray thus facilitating extraction. The performance of the extractor is illustrated by the extraction of triacylglycerols (TGs) from an acetone extract of oat bran. In the extractor, the TGs are dissolved in the CO_2 and removed from the precipitation unit with the stream of CO_2. The target product, which is digalactosyldiacylglycerol (DGDG) is trapped in the precipitation unit. Through-put is related to the desired purity of the product. A DGDG purity of 95.4% was obtained at a solvent/feed ratio (S/F) of 48, the rate of production was then about 1 g/h. At a S/F of 200 the purity of the DGDG was 97%. © 1997 Elsevier Science B.V.

Keywords: Supercritical fluid extraction; Lipids; Carbon dioxide; Digalactosyldiacylglycerols

1. Introduction

Supercritical fluids have, for many years, been employed for selective preparative extractions. There are a number of well established fields of application, e.g., decaffeination, production of concentrated hops extracts and the extraction of scents and essential oils [1]. During recent years, there has been an increasing interest in the application of techniques involving supercritical fluids for the extraction of oils and fats [2].

For extractions, supercritical fluids provide several advantages over organic solvents. First, the low viscosity and higher diffusivity of supercritical fluids improve mass transfer in solid and liquid matrices and decrease the overall extraction time. Second, since SFE can be performed at moderate temperatures, thermal degradation of thermally labile compounds can often be avoided. Third, the solvating properties of supercritical media can be readily and accurately modified by adjusting the pressure or temperature, this provides excellent possibilities for selective extractions. Fourth, the supercritical medium can be easily removed from the extraction products, thus minimizing the need for cleanup. Fifth, SFE is environmentally friendly, it does not generate chemical waste.

Extractions can be performed according to two different principles, in a batch-wise mode or in continuous operation. The batch-wise mode of operation has been used extensively [3–8]. This technique provides excellent possibilities to achieve highly selective extractions of target substances. The selectivity can be optimized through a number of variables. These include mode of operation, i.e., static or dynamic conditions or combinations of the two

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modes, choice of extraction medium, pressure and temperature. Finally, there is a possibility to combine the extraction with a chemical modification of the target substances in order to further enhance selectivity. Such reactions can conveniently be performed by means of immobilized biocatalysts [9,10].

In continuously operating systems, a general problem is how to provide sufficient contact between feed and extraction medium. The contact between the phases can be maximized when these are fed in a countercurrent mode to an extraction cell containing some packing material [11–13]. In such a system, a raffinate can be removed from the bottom of the cell, and fractions of the extracted material can be collected in a series of separators with decreasing extraction medium densities. For the deoiling of raw lecithin, Stahl and co-workers applied a special technique known as jet extraction where spray particles, formed in a jet, are extracted [14,15]. This technique was later further developed by Eggers and Wagner [16–18]. Also these authors used the method for the deoiling of high viscous products such as soy lecithin. Mixing with the extraction medium is particularly difficult for such products, and different designs of the mixing device were evaluated. In a continuously operating SFE system, not only the feed should be continuous but also the raffinate should be continuously transported out of the extraction vessel. Systems where the latter requirement is not fulfilled, as in the present system, may be called semi-continuous.

The preparation of finely divided powders by means of rapid expansion of supercritical solutions (RESS) is an important area of supercritical fluid application. Several systems have been developed for that purpose [19,20]. The aim of the present work is to apply a system, similar to those applied for the preparation of fine particles, to the semicontinuous extraction of a seed oil which is dissolved in an organic solvent. The solvent is present in order to improve mixing in the jet extractor.

Digalactosyldiacylglycerol (DGDG) is one of the major lipids in the cells of higher plants. The DGDG is amphiphilic, and it can form lamellar crystalline phases [21]. DGDG can therefore be used to prepare lipid aggregates such as liposomes and oil-in-water emulsions. Liposomes are of interest for nutritional, cosmetological and pharmaceutical applications. An extensive review on glycolipids was published recently [22]. Extraction and characterization of glycoglycerolipids by means of liquid chromatography has earlier been reported from our laboratory [23,24]. In the present work, supercritical fluids are used for extraction and characterization of DGDG.

Oat was selected as a source of DGDG, since oat has a relatively high concentration of DGDG [25]. Further, the fatty acid moieties of DGDG from oat were mainly palmitic, oleic and linoleic. Only minor amounts of linolenic acid residues have been reported [26], this is an advantage with respect to the stability of the oat DGDG.

In this paper, batch-wise and semi-continuous methods for the extraction of DGDG from an oat oil are described and compared. The composition and properties of the DGDG fraction as an emulsifier were evaluated.

2. Experimental

2.1. Materials

The oat bran was purchased from Kungsörnen (Järna, Sweden). All solvents were of p.a. quality.

2.2. Apparatus for batch-wise extraction

The extraction system consisted of a Suprex SFE/50 supercritical fluid extractor (Suprex, Pittsburgh, PA, USA), when using modified carbon dioxide a Varian 8500 syringe pump was employed. The six-port switching valve, N6W, was from Valco (Houston, TX, USA). The extraction cell was an empty LC column 80×6 mm I.D., DuPont (Wilmington, DE, USA) and a fused-silica capillary, 30 cm×25 μm I.D., Polymicro Technologies, (Phoenix, AZ, USA) was used as restrictor. The set-up is schematically shown in Fig. 1.

2.3. Apparatus for semi-continuous extraction

The extraction system is shown in Fig. 2. It consisted of two Gilson 307, Gilson (Villiers Le Bel, France) piston pumps; one was for the oil solution and one for the carbon dioxide. The latter was equipped with a cooling system, pump head tempera-

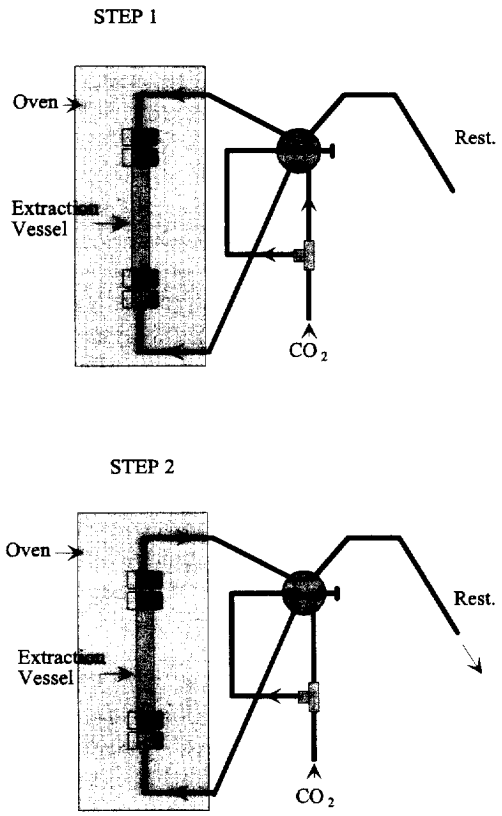

Fig. 1. Schematic diagram of set-up for batch-wise extraction. Step 1: introduction of CO_2 to the extraction cell; step 2: extraction.

ture was 1°C. The dimensions of the home made precipitation unit were 20 mm I.D. and height 39 mm, volume 12 ml. In order to facilitate fraction collection, the unit was equipped with a stainless steel insert, 26 mm×13 mm I.D., O.D. 19 mm at the upper end and 16 mm at the lower end, Fig. 2. Further, the ends of the insert were covered with stainless steel frits, diameter 12.7 mm, thickness 1.57 mm, porosity 5 μm. In this system, the fluid is introduced tangentially into the chamber and at a high velocity. Due to centrifugal forces, the solid particles or liquid drops tend to stay on the outer wall and the fluid exits in the middle, thus we have a cyclone type of separation [27]. For the inlet and outlet connections to the precipitation unit, two Valco unions, ZU1, which had been drilled through to an I.D. of 1.65 mm were used. The nozzle consisted of two stainless steel tubes, the inner tube was 0.5 mm

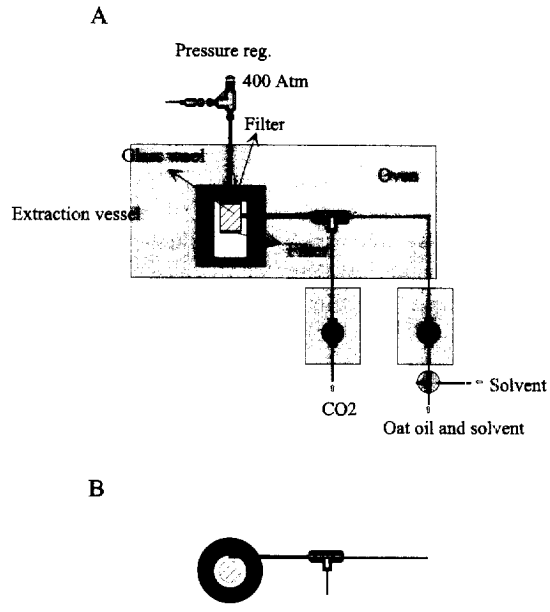

Fig. 2. (A) Schematic diagram of set-up for semi-continuous extraction: (B) the precipitation unit seen from above.

O.D. and 0.3 mm I.D. and the other tube was 1.59 mm (1/16 in. [1 in.=2.54 cm)] O.D. and 1.10 mm I.D. At the nozzle the I.D. of the outer tube was decreased to about 0.52 mm. In order to maintain the pressure in the system a Nupro (Willoughby, OH, USA) R3A Series externally adjustable relief valve was used as a restrictor.

2.4. Liquid extraction

Oat bran, 1000 g, was refluxed with 1.5 l acetone for 1 h. The procedure was performed three times and the acetone, 4.5 l, was centrifuged and evaporated.

2.5. Optimization procedure

The performance of the extraction was evaluated using a fractional factorial design 2^{4-1}, with four variables and five experiments in the center point using Codex, Sum-It System (Solna, Sweden). For each experiment 2 ml oat oil was dissolved in a mixture of acetone–*n*-hexane (1:1, v/v), to the appropriate concentration. The presence of *n*-hexane was necessary for the solvation of higher oil con-

centrations. Solvent-to-feed ratio was calculated as flow of CO_2/flow of neat oil.

2.6. Supercritical fluid chromatography

The chromatographic system consisted of a Lee Scientific (Salt Lake City, UT, USA) 600 Series SFC system. Detection was with a miniaturized evaporative light scattering detector [28,29]. Columns were prepared from glass lined metal tubing, 100×0.9 mm I.D. and 1.59 mm O.D., SGE (Ringwood, Victoria, Australia). The columns were packed with a diol-modified silica, LiChrosorb Diol 5 μm, Merck (Darmstadt, Germany). The mobile phase consisted of 19 or 14 mol% methanol in SFC grade carbon dioxide, AGA (Lidingö, Sweden). The mixture was prepared in the pump as described earlier [30].

2.7. Determination of surface activity

Surface activity at the sunflower oil–water interface: both the interfacial tension and the interfacial elasticity were measured with a dynamic drop tensiometer [31]. Emulsifying activity: emulsions of 40% water in oil were prepared as described earlier [32], except that no solid fat was added. Initial drop size after 24 h was determined in order to judge the capacity of the emulsifier to produce fine and stable emulsions.

3. Results and discussion

3.1. Batch-wise extraction

In the early stages of the work, direct extraction of oat bran was performed in an extractor in a batch-wise mode. First, triacylglycerols were removed by means of extraction with neat supercritical carbon dioxide. Second, DGDG was extracted by a mixture of CO_2–acetone (80:20). A main drawback of such a procedure is the relatively low through-put. To increase through-put, large extraction cells may be used, but since the extraction pressure must be high, such cells must be thick-walled and they thereby become relatively expensive and, when operating at a laboratory scale, cumbrous. In order to increase the through-put while maintaining a relatively small extraction cell volume, an oil was first produced which subsequently was purified by SFE. The liquid extraction was with refluxing acetone thus taking advantage of the selectivity of that solvent. In the SFE procedure, the TGs were extracted with CO_2 and the product was left in the extractor, Fig. 1.

3.2. Semi-continuous extraction

In the present system, semi-continuous extraction is performed in a spray. In the spray, the oil components having the lowest polarities, TG and monogalactosyl-diacylglycerols (MGDG) are dissolved in the supercritical carbon dioxide and transported out of the precipitation unit. The design of the nozzle, where the spray is formed, is a critical part of the system. Thus, in order to avoid precipitation of DGDG in the nozzle, which could take place at low solvent/feed ratios (S/F), the feed line was longer, 0.5 mm, than the solvent line, Fig. 2. Further, to avoid precipitation of the large amounts of extracted TG, the pressure regulator, Fig. 2, had to be heated to about 45°C. If desirable, the TGs could be collected, and for that purpose, a separator and a second pressure regulator should be attached at the outlet. The non-soluble part of the oil precipitates in the precipitation unit. In the present system, the volume of this unit is relatively small, 12 ml, and a break-through of the product may take place after several hours of operation. However, this can be easily avoided by the application of a larger unit and/or a collection tube attached to the unit.

3.2.1. Optimization of extraction conditions

The performance of the extraction is dependent of several factors. For optimal performance, it is important to understand the direct and mutual influence of the different variables. The influence of four key variables was therefore investigated by a factorial design experiment. The variables were temperature, concentration of oil in the feed solution, flow-rate of feed solution and flow-rate of carbon dioxide. The pressure was kept at 400 atm, which was maximum what the system allowed. Thereby, a relatively high carbon dioxide density could be maintained even though the temperature was elevated. Optimization was performed for two different aims: maximum purity of the product (DGDG) and maximum pro-

Table 1
Factorial design for optimization of the semi-continuous extraction

Experiment No.	Temperature (°C)	Percent oat oil	LC flow (ml/min)	CO_2 flow (ml/min)	Area% DGDG	Area% DGDG/time	CO_2/oil ratio
12	60	26.5	0.35	7.0	95.0	4.4	75
09	60	26.5	0.35	7.0	95.0	4.4	75
05	50	20.0	0.50	8.0	93.8	4.7	80
02	70	20.0	0.20	8.0	97.0	1.9	200
03	50	33.3	0.20	8.0	93.6	3.1	120
10	60	26.5	0.35	7.0	94.3	4.4	75
13	60	26.5	0.35	7.0	93.9	4.4	75
06	70	20.0	0.50	6.0	94.2	4.7	60
08	70	33.3	0.50	8.0	95.4	8.0	48
04	70	33.3	0.20	6.0	93.6	3.1	90
07	50	33.3	0.50	6.0	85.4	7.1	36
01	50	20.0	0.20	6.0	92.5	1.8	150
11	60	26.5	0.35	7.0	93.3	4.3	75

duction/time unit when highest purity was not a main concern. The results of the optimization are shown in Table 1. It was found that temperature and carbon dioxide flow were the most important variables. Thus high temperature and high CO_2 flow-rate gave the highest purity, 97% DGDG, Fig. 3. Solvent/feed ratio (S/F) is an important factor in continuous extraction, and the highest purity was obtained at S/F of 200.

The highest production of DGDG/time unit was with a high concentration of oil in the feed solution, 33%, a high feed flow-rate, 0.5 ml/min, and a high CO_2 flow-rate which corresponds to a S/F of 48.

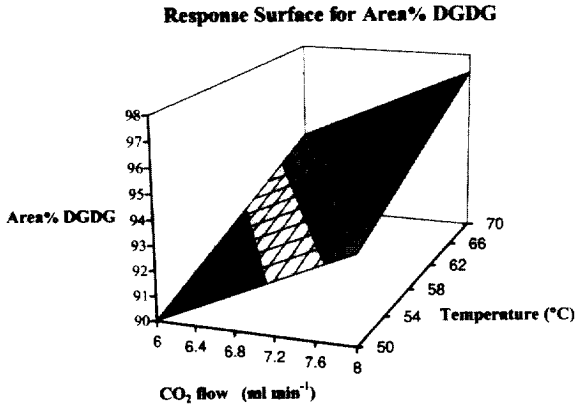

Fig. 3. Three-dimensional plot of DGDG purity (expressed as area%) as a function of CO_2 flow and temperature. Concentration of oat oil in feed solution, 26.5%; feed solution flow-rate, 0.35 ml/min.

The purity of the DGDG produced in this way was 95.4%, Table 1. The rate of production was then about 1 g/h. In the present system, the 33% is the maximum concentration of oil in the feed solution. At higher concentrations, the viscosity becomes too high for efficient extraction. Of course, extraction temperature can be increased, however, then also pressure must be increased if the density of the extraction medium is to be maintained, and in the present set-up pressure was limited to 400 atm. The thermal stability of the target product may also be a restricting factor. Further, the feed flow-rate should not be too high since that would lead to too high concentration of organic solvents in the precipitation unit resulting in an incomplete precipitation of the target product. However, the speed of production can be further increased, in the present system, when applying a higher volumetric flow of CO_2, maintaining the pressure at 400 atm. That would allow an increase in the feed flow-rate while maintaining the S/F ratio at the given level.

3.2.2. Extraction yield

Extraction of 1000 g of oat bran, three times, with 1.5 l refluxing acetone gave about 52 g of oil. A fourth extraction gave an additional 2 g of oil. After the final purification step, the yield of DGDG was about 6 g from 1000 g of oat bran. The yield of acetone extraction and the content of DGDG in the oil are in good agreement with published data [25].

3.2.3. Characterization of the product by supercritical fluid chromatography

The product was characterized by analysis using supercritical fluid chromatography. The composition of the oat oil is shown in Fig. 4A, and a chromatogram of a DGDG sample of high purity is shown in Fig. 4B. The content of DGDG is simply calculated as area percent. With the miniaturized evaporative light scattering detector used here, the response of TG is about 6 times higher than that of DGDG, however, since the TG peak is quite small in Fig. 4B, the difference in response is not of major concern here.

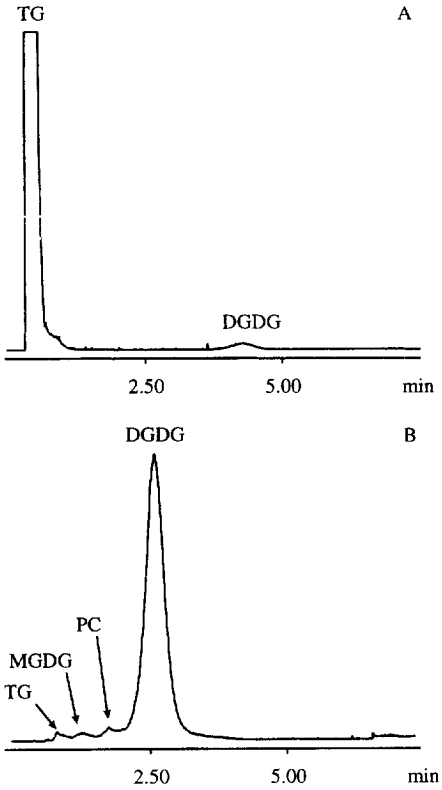

Fig. 4. Sub-critical fluid chromatograms, μ-ELSD. A=oat oil, B=concentrate obtained after extraction of oat oil solution with CO_2. SFC conditions: Column, 100×0.9 mm I.D., packed with diol modified silica, 5 μm. Restrictor, 45 mm×9 μm I.D. at 90°C. Pressure 300 atm, temperature 22°C. Mobile phase: A, CO_2 modified with 14 mol% methanol; B=CO_2 modified with 19 mol% methanol. Extraction conditions: Oat bran extracted with acetone, other conditions as for Exp 02, Table 1. Peaks: TG =triacylglycerols; MGDG=monogalactosyldiacylglycerol; PC= phosphatidylcholine; DGDG=digalactosyldiacylglycerol.

3.2.4. Comparison of HPLC and SFE for purification of DGDG

In a recently presented method for isolation and purification of DGDG, oat bran was first extracted with ethanol, and the extract was purified on silica gel followed by solid-phase extraction. Fractions of galactolipids containing 100 mg of DGDG with a purity of more than 97% were achieved [24]. An additional purification was achieved by separation on a semi-preparative HPLC system with a capacity of about 10 mg/injection [24]. In comparison, the present system is simpler, it has only one separation step. Further, the through-put is higher, mainly as a consequence of the continuous mode of operation.

3.2.5. Characterization of the product as emulsifier

The performance of the raffinate holding 97% DGDG as an emulsifier in emulsions of 40% water in triglyceride oil was examined. This product was found to be very efficient as emulsifier under these conditions. Equilibrium interfacial tensions were as low as $\sigma<6$ mN/m. Dynamic interfacial elasticity was much higher, about 35 mN/m.

4. Conclusions

A method for semi-continuous extraction in a spray has been developed. The design and dimensions of the nozzle where the spray is formed is critical for the performance. Further, the extraction temperature, the concentration of oil in the feed solution, feed solution flow-rate and CO_2 flow-rate have to be kept within certain limits. The present device is effective by virtue of its small dimensions, but it seems that the nozzle can be modified to provide a higher rate of extraction.

The performance of the system has been illustrated with the extraction of an oat oil, however, it seems that the method can be used for a number of applications.

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