EXTRACTION OF BIOACTIVE COMPOUNDS FROM COTTON FRYING OIL BY SUPERCRITICAL CO_2 FOR USE IN BIOPROCESS

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ABSTRACT

Phytosterols are bioactive compounds and provide important health benefits: in particular, cholesterol reduction. In addition, they can be transformed by microbial action into steroids, which are of great value for the production of drugs. From an environmental and commercial point of view, one of the most appropriate techniques to extract phytosterols from plant matrices may be supercritical fluid extraction (SFE) using carbon dioxide (CO_2). The use of residual frying oil as a matrix for extracting phytosterols can bring another economic advantage, as it is a residue that certainly has a lower cost than virgin oil. In addition to the environmental benefit, this waste ends up being transformed into a product with high added value. A small concentration of β-sitosterol (0.043 g/100g of oil) was found in cotton waste frying oil (CWFO). Different amounts of oil were mixed into cassava flour to form a dough (20 g) that facilitated the extraction of bioactive compounds from CRFO. A small amount of extract was obtained through SFE and a variety of fatty acids were found in the CWFO sample. Therefore, this study aims to extract the bioactive compounds, mainly the phytosterols present in the o residual frying oil of by supercritical fluid extraction.

Keywords: Phytosterols. Cotton oil. Frying oil. Unsaponifiable Substances.

1 INTRODUCTION

Phytosterols are triterpenes with a chemical structure similar to cholesterol[1], with more than 200 similar components[2], the most abundant in nature being β- sitosterol, campesterol and stigmasterol[3]. These compounds are normally found in foods rich in lipids, such as peanuts, sesame seeds, fruits and grains and, mainly, in vegetable oils, such as soybean, canola and sunflower oils[4]. In addition, they are precursors to the synthesis of steroid hormones and are involved in plant defense mechanisms. In particular, phytosterols have received much attention due to their ability to reduce serum cholesterol levels in humans[5] (Hicks & Moreau, 2001) resulting in significant reductions in the risk of heart disease. Phytosterols can also be transformed by microbial action into steroids, which are of great value for the production of drugs.

There are different methods of extracting phytosterols from oily matrices, such as mechanical, organic solvent[6], enzyme-assisted aqueous and supercritical extraction of (SFE), among others. SFE is an efficient alternative to the use of organic solvents for extracting functional and nutraceutical components from natural sources[7]. Among supercritical fluids (SFs), carbon dioxide (CO2) is widely used because supercritical CO2 (SC-CO_2) provides some advantages over conventional extraction processes. Several research papers have been carried out on extraction and fractionation of phytosterols using SC-CO_2 from various plant sources effective technology for the recovery of phytosterols.

A very important aspect is the use of waste such as residual frying oil to generate high-value products such as steroids, which can be produced through the bioconversion of phytosterols, via a biotechnological process, using a suitable culture medium and microorganisms.. According to the Environmental Management Program (PGA) (2012)[8], of the Federal Public Ministry, one liter of used cooking oil can contaminate one million liters of water. Improper disposal of residual frying oils in the sewage network leads to an increase in expenses with the maintenance of these networks and the obstruction of the system. One way to minimize the effects generated by this irregular disposal of thermally processed oils would be to use them to extract phytosterols, which are substances with high added value. Therefore, the objective of this study was to extract the bioactive compounds present in WFO by SFE and carry out the characterization of the extract to evaluate its ability to serve as a substrate for Y. lipolytica. Since the extract obtained by the classical method (chemical reaction) presented a small amount of phytosterols (β-sitosterol) and maintained a lipid profile similar to virgin cottonseed oil.

MATERIAL & METHODS

Obtention of cotton waste frying oil (CWFO)

The oil was obtained through a donation from a restaurant chain in the state of Rio de Janeiro, Brazil.

CWFO sample preparation for characterization

A saponification reaction was carried out on CWFO to separate the saponifiable (fatty acids) and unsaponifiable fractions (total amount of substances dissolved in oils and fats that, after saponification with alkalis, are insoluble in aqueous solution, but soluble in common solvents of fats, including phytosterols). The two fractions were analyzed separately in two different methods using gas chromatography, the saponifiable fraction for fatty acid analysis and the unsaponifiable fraction for phytosterol analysis20. Titratable acidity and saponification index analyzes of the CWFO.

Analysis of fatty acid composition by gas chromatography (GC)

Free fatty acids were methylated and analized in a GC-2010 gas chromatograph (Shimadzu, Japan)[9] that was used for all analyses, and the split/splitless injector was operated with a split ratio of 1:30. A moderately polar capillary column (polyethylene glycol; Omegawax-320, 30 m, 0.32 mm internal diameter, 0.25 µm film thickness; Sigma-Aldrich, São Paulo, Brazil) was used to separate the methyl esters from acids. fatty acids, using He as carrier gas (25.0 cm/s). The temperatures of the injector and detector were set at 260 and 280 ◦C, respectively. The column oven temperature was held at 40 ◦C for 3 min, then programmed at 6.5 ◦C/min to 180 ◦C and held for 3 min, then temperature programmed at 2.0 ◦C/min at 210 ◦C and kept for 15 min. Identification of fatty acid methyl esters (FAME) was done by comparison with relative retention times of commercial standards. Equivalent Chain Lengths and the mathematical method were described by Torres et al (2002)[10].

Identification analysis of phytosterols in CWFO

The quantification of β-sitosterol in the oil was determined by GC on a GC-2010 gas chromatograph (Shimadzu, Japan) equipped with a split/splitless inlet, on a DB-5HT capillary column (15 m × 0.32 mm ID and 0.10 µm film thickness) and a flame ionization detector (FID). The temperature of the sample injector and flame ionization detector was 320 °C and 350 °C, respectively. The chromatographic column temperature started at 210°C for 2 min and was heated to 320°C at a speed of 10°C/min and maintained for 15 min. Subsequently, the temperature was heated to 350 °C at a speed of 10 °C/min.

Extraction of sterols from CWFO by SFE (CO_2)

Different amounts of CWFO were mixed into cassava flour to form a dough that facilitated the extraction of bioactive compounds. This procedure was necessary, as the equipment used is indicated for extracting compounds from solid samples (extraction tubes). The sample that was satisfactory for extraction contained 15 g of oil, 7.5 g of water and 15 g of cassava flour. Oil extraction by SC-CO_2 was performed in an automatic supercritical fluid extractor system (MV-10 ASFE; Waters®, Massachusetts, USA) equipped with a high-pressure pump for CO_2, a chiller, a cosolvent pump, an oven, a back-pres-sure regulator, a temperature controller, and a heat ex-changer. Extraction pressure, temperature, the flow of CO_2, and cosolvent were controlled by ChromScope v1.20 Software Waters®. The solvents used were water and ethanol and the maximum temperature was 70 °C, the CO_2 flow was 3 mL/min for about 4 hours. The samples obtained will be submitted to GC analysis for characterization and identification of phytosterols[11].

Characterization of oils extracted from CWFO by SFE (CO_2)

The extract obtained from CWFO by supercritical CO_2 was analyzed by GC according to the protocols in sections 2.3 for fatty acids quantification.

2 RESULTS & DISCUSSION

Characterization of CWFO

Table 1 contains the fatty acid composition of residual CWFO that was overprocessed and used in this study. According the literature[12] to Gondim-Tomaz et al. (2016), cotton oil has an average of 1.83 to 2.14% stearic acid, 22.7 to 24.8% palmitic acid, 13.4 to 15.8% oleic acid and finally the linoleic acid is 55.6 to 59.0%. Both in the composition of fatty acids and in the values found for CWFO, there is a great similarity with the standards established in the study by Gondim-Tomaz et al. (2016)[12]. Traces of lignoceric acid were found in the CWFO sample (7.75%) analyzed.

Table 1. Fatty acid composition of residual cotton frying oil (CWFO).

Fatty acids	Content (%)
Saturated	
16:0	15.15 ± 0.03
18:0	2.71 ± 0.08
24:0	7.75 ± 0.01
Unsaturated	
18:1	18.73 ± 0.01
Polyunsaturated	
18:2n-6	55.64 ± 0.05

In order to determine whether CWFO still contained phytosterols, gas chromatography analysis was carried out and a small concentration of β-sitosterol was observed (0.043 g/100g of oil), indicating that this oil may still be useful as a source of bioactive compounds with high added value. The acid number observed for CWFO was 56.67 mg KOH g^{-1} and the saponification index found was 210.32 mg KOH g^{-1}. The acid value found for CWFO was much higher than that observed for refined cottonseed oil, according to the literature, which is 0.3 mg KOH g^{-1}. The saponification index found was very close to that observed inliterature[13], which was 189-198 mg KOH g^{-1}. Probably the successive frying processes with excessive heating and release of water from the food abruptly changed the acidity index of the CWFO.

Characterization of oils extracted from CWFO by SFE (CO_2)

The CWFO sample used in the supercritical fluid reactor was compacted in cassava flour, as the equipment used was specific for solid samples. This procedure was carried out to prevent blockages and oil leaks from the supercritical lines. Oleic acid was mostly found in the CWFO 69.86%, followed by stearic acid 24.25% and Heptadecanoic acid 3.30%, traces of seven other acids were also observed in the tested oil. The fatty acid composition was compatible with that expected for cottonseed oil, but linoleic acid was not observed in this analysis[13].

It was possible to observe that the extract obtained through supercritical fluid, which is a much less destructive method than the chemical method (saponification), better preserved the short-chain fatty acids present in the oil. A greater amount of unsaturated fatty acids were preserved after extraction, probably due to the milder conditions used in the supercritical fluid method.

Table 2. Fatty acid composition of residual cotton frying oil (CWFO) extracted by SFE (CO_2).

Fatty acids	Content (mg)	Content (%)
Saturated		
6:0	0.02 ± 0.00	0.07 ± 0.00
11:0	0.16 ± 0.00	0.51 ± 0.42
16:0	0.09 ± 0.007	0.31 ± 0.23
18:0	7.26 ± 0.30	24.25 ± 0.15
20:0	0.13 ± 0.12	0.43 ± 0.03
Unsaturated		
16:1	0.02 ± 0.00	0.07± 0.00
17:1	0.99 ± 0.04	3.30 ± 0.02
18:1	20.92 ± 0.00	69.86 ± 0.22
18:2	0.15 ± 0.01	0.50 ± 0.01
20:3	0.17 ± 0.02	0.56 ± 0.05

The extract obtained will be used in cultivation with Yarrowia lipolytica in order to evaluate the ability of this yeast to metabolize oily residues and transform them into metabolites of high added value such as hormones. Oil tests were carried out with the extract obtained through saponification and the results indicated the possibility of assimilation of bioactive compounds (mainly phytosterols) by yeast. Oil tests were carried out with the extract obtained through saponification and the results indicated the possibility of assimilation of bioactive compounds (mainly phytosterols) by yeast[14].

3 CONCLUSION

The supercritical fluid extraction method was effective in extracting fatty acids from frying oil and superior in obtaining short-chain and unsaturated fatty acids. The frying oil, despite being overprocessed, remained reasonably stable and can therefore be considered a good metabolic inducer for *Y. lipolytica* for the production of substances with high added value.

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