J. of Supercritical Fluids 82 (2013) 177–182

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

journal homepage: www.elsevier.com/locate/supflu

Production of a lycopene-enriched fraction from tomato pomace using supercritical carbon dioxide

G. Perretti [a], A. Troilo [a], E. Bravi [a,*], O. Marconi [a], F. Galgano [b], P. Fantozzi [a]

[a] Department of Economics and Food Sciences, Section of Food Technology and Biotechnology, University of Perugia, Via S. Costanzo n.c.n., 06126 Perugia, Italy

[b] School of Agricultural, Forestry, Food and Environmental Sciences, University of Basilicata, Via dell'Ateneo Lucano, 10, 85100 Potenza, Italy

ARTICLE INFO

Article history:
Received 28 February 2013
Received in revised form 10 June 2013
Accepted 16 July 2013

Keywords:
Supercritical carbon dioxide
Fractionation
Lycopene
Tomato pomace
Ethanol–oil extract

ABSTRACT

In recent years there has been a growing interest in functional foods because they may provide beneficial effects on human health. Moreover, the increasing interest of consumers in functional foods has brought about a rise in demand for ingredients obtained using technologies perceived to be natural and safe. In this study, supercritical fluid technology was used in order to obtain lycopene from an extract of dried tomato pomace using sunflower oil and ethanol. After the supercritical fluid fractionation, four fractions were collected, three separated fraction (SF) from the separator (after 30, 60 and 120 min of fractionation) and one residual fraction (RF) from the bottom of column (after 120 min). The concentration of lycopene was studied in the different fractions obtained under different pressures (10 and 30 MPa), CO_2 flow rates (5 and 15 kg h^{-1}), and heights of loading (top and bottom). The quantitative determination of lycopene was performed by HPLC coupled with a diode array detector. The effects of the different extraction parameters, as well as their interactions, were investigated using a full factorial design with three factors and two levels; the optimal conditions were calculated through response surface methodology. A statistically significant difference in lycopene content in the four fractions was obtained.

1. Introduction

Carotenoids are important dietary compounds that act as antioxidants and precursors of vitamin A. They have also been reported to be anti-carcinogenic agents, prevent cardiovascular diseases and regulate the immune system [1]. Lycopene, a C40 polyisoprenoid compound containing 13 double bonds, is the most abundant carotenoid, accounting for approximately 80–90% of the total pigment of ripe tomatoes. Specifically lycopene imparts the characteristic red hue of tomatoes [2] and, with its 11 conjugated and 2 non-conjugated double bonds, lycopene was found to be a more efficient antioxidant (singlet oxygen quencher) than β-carotene, α-carotene, and α-tocopherol [3]. Moreover, in vivo and in vitro clinical studies have reported lycopene has a protective effect on the growth of tumor cells, against cardiovascular disease [4] and cancer [2]. The lycopene content of any one tomato varies considerably, reflecting the influence of variety (genetic factors), maturity, and both the agronomic and environmental conditions of growing [5–8]. Tomato is the major source of lycopene in the human diet [9]. Watermelon, apricot, papaya, pink grapefruit and rosehip are also dietary sources, but with lower contents [10–12].

The importance of this natural carotenoid as a coloring and antioxidant agent in the food industry has increased in recent years [13] alongside a general trend toward the use of natural compounds in foods rather than synthetic ingredients [1]. Moreover, the increasing interest in functional foods and the current concern for safety in food products has increased interest in green and reliable extraction techniques, instead of the conventional organic solvent extraction processes.

Commercial processing of tomato produces a large amount of waste at various stages. Tomato pomace is the major waste product remaining from the pulper. Pomace contains 33% wet seed, 27% wet skin and 40% wet pulp while dried pomace contains 44% seed and 56% pulp plus skin [14]. The skin in pomace could be utilized for extracting lycopene [14–17]. However, fresh skin has a high moisture content that makes it susceptible to microbial proliferation and spoilage, therefore the best practice would be to preserve the skin by drying it and then using it for lycopene extraction [18].

Lycopene extract from tomatoes is commonly obtained using organic solvents such as ethanol, acetone, petroleum ether, hexane, benzene, chloroform [19–26]. A mixture of hexane with acetone and ethanol or methanol is preferred [8,26,27] because (i) other components such as diethyl ether and tetrahydrofuran may contain peroxides that react with carotenoids [27], (ii) recovery rates with other mixtures (including ethyl acetate) are very low [26], and (iii) the stability of lycopene extracts obtained with hexane/acetone or

* Corresponding author. Tel.: +39 075 5857923; fax: +39 075 5857922.
E-mail addresses: e.bravi@unipg.it, e_bravi@yahoo.it (E. Bravi).

0896-8446/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.supflu.2013.07.011

hexane/ethanol is higher than that of extracts obtained with other organic solvents [25].

In this study, we explored the use of supercritical fluid fractionation (SFF) in order to obtain various lycopene-enriched fractions from an extract of dried tomato pomace in sunflower oil and ethanol, in column and in separator and as residual fractions (RFs) and separated fractions (SFs), respectively. Furthermore we studied the interaction between supercritical carbon dioxide, ethanol, oil and lycopene mixture and the parameters of the experimental design.

The majority of existing studies using SC-CO_2 in a fractionation process concern the enrichment of eicosapentaenoic and docosahexaenoic acid [29–31] and the separation of ω-3 fatty acids from a mixture of fatty acid ethyl or methyl esters. In these studies, fractions concentrated with eicosapentaenoic acid and docosahexaenoic acid esters could be obtained. Perretti et al. [30] investigated fractionation of fish oil fatty acid ethyl esters with the aim of obtaining a lipid fraction enriched in ω-3 fatty acids and with a suitable eicosapentaenoic/docosahexaenoic acids ratio. The results obtained highlighted the possibility of modifying the original fatty acid ethyl esters concentration by optimizing the extraction conditions in terms of pressure, temperature, and supercritical carbon dioxide flow rate. More recently, Lopez et al. [32] found that SC-CO_2 was selective in fractionating the triacylglycerols containing the fatty acids eicosapentaenoic acid and docosahexaenoic acid under the conditions of 306 and 313 K at 20 MPa, thus proving the technical viability of fractionating fish oils with lower contents of these fatty acids in the triacylglycerol molecules. Moreover, Perretti et al. [33] showed that under similar conditions the process was able to fractionate ethanol from aqueous solutions. Therefore supercritical fluid fractionation (SFF) appears to be a useful tool for removing ethanol and changing the composition of triacylglycerol molecules and lycopene content in order to obtain high value functional products.

Isolation of fat-soluble components requires the fractionation of liquid mixtures into two or more fractions. Typically two pumps deliver the liquid solution and SC-CO_2 to the packed column. The packing is an inert material characterized by a large specific surface designed to promote the contact between the liquid and the supercritical fluid. SC-CO_2 as a rule flows along the column from the bottom to the top, whereas, the liquid solution is usually added to the top. However, to test different interface effects it is also possible to feed the liquid at an intermediate position along the column and to recycle part of the fluid phase exiting at the top [28]. The process is based on the different solubility of the compounds to be separated in the SC-CO_2. The ideal case is obtained when the compounds to be extracted are soluble only in SC-CO_2 [28].

Pressure and temperature are critical parameters of the process and have to be accurately chosen to maximize the extraction process.

One variation of this processing scheme can be the adoption of a temperature profile along the column with the aim of optimizing the separation temperature with respect to the composition of the mixtures at different levels inside the column.

The fractionation controlled by the relative solubility in SC-CO_2 of the various compounds is the thermodynamic limitation of the process. Mass transfer between the two phases represents the kinetic limitation. The distance from the equilibrium conditions is the driving force for the separation along the column [30].

2. Materials and methods

2.1. Samples and reagents

Discarded wet tomato pomace from a mixture of plant varieties was obtained from Conserve Italia Soc. Coop. Agricola (Albinia, Italy). Industrial sunflower oil was obtained from Colussigroup (Perugia, Italy).

The lycopene (≥98%) standard was purchased from Extrasynthese (Lyon, France). Methanol (≥99.9%), ethanol (≥99.8%), methyl tert-butyl ether (≥99.9%), butylated hydroxytoluene (≥99%), hexane (≥99%), acetone (≥99.9%) and water (HPLC grade) were purchased from Sigma–Aldrich Chemie GmbH (Steinhein, Germany).

2.2. Lycopene extraction

Lycopene was extracted from tomato pomace powder with a mixture of organic solvents (hexane, acetone, and ethanol) and with ethanol–oil using modified methods from Kaur et al. [34] and Periago et al. [35]. Extraction was performed over 60 min under magnetic stirring with 30:1 solvent:pomace ratio at 323 K in a 500 mL flask covered with aluminum foil to exclude light. The solvents used for lycopene extraction were 300 mL each of hexane, acetone and ethanol (1:1:1, v:v:v) containing 0.05% (w/v) butylhydroxytoluene. After the extraction, the solution was filtered with a 22.09 cm^2 steel mesh (0.5 mm pore size), after which cold distilled water was added and the suspension was shaken. The solution was then allowed to stand 15 min for separation of the polar and non-polar layers. The non-polar layer, containing lycopene, was recovered.

The ethanol–oil extraction of lycopene from tomato pomace was performed as follows. Tomato pomace was dried at 323 K for 24 h to decrease moisture from 75% to 5%. Dried tomato pomace was ground in a laboratory grinder (Osterizer Sunbeam model no. 4153-50) at maximum speed for 30 s. Extraction of lycopene was performed over 120 min using 250 mL of ethanol, 250 mL of sunflower oil and 50 g of tomato pomace powder (5:5:1, v:v:w) under magnetic stirring at 323 K in a 1 L beaker covered with aluminum foil to exclude light. The mixture was filtered with the above-described steel mesh. The recovered mixture was 350 mL and it was composed by 230 mL of lycopene-rich oil and 120 mL of ethanol. The preparation of fresh ethanol–oil extract was repeated for each fractionation run.

2.3. Supercritical fluid fractionation

The supercritical fluid fractionation (SFF) experiments were conducted in triplicate in a Muller Extract Company GmbH (Koburg, Germany) pilot plant (Fig. 1) equipped with a three-stage fractionation column (total length 3 m, diameter 3 cm) with an internal volume of 2 L and packed with stainless steel Raschig rings (10 mm × 10 mm); each column stage was individually thermostated. The separation section of the plant consisted of a 1 L cylindrical separator followed by two cyclonic separators, both set at 303 K and 6 MPa. Each experiment was conducted in one batch with a feed of 350 mL. The three-stage column temperatures were fixed at 313, 323 and 333 K respectively from the bottom to the top. These temperatures were chosen to explore the related densities, to preserve the oil from hydrolysis and oxidation, and to preserve the lycopene. These parameters determined a density gradient in the solvent stream, with SC-CO_2 densities varying along the column. At the end of each end run four fractions were collected, three separated fraction (SF) from the separator (after 30, 60 and 120 min of fractionation) and one residual fraction (RF) from the bottom of column (after 120 min).

The solvent flow rate was measured using a mass flow meter, model RHM 01 (Rheonik, Maisach, Germany), equipped with an electronic transmitter, model RHE 05 (Rheonik, Maisach, Germany), with an accuracy of ±0.5% and a repeatability ≤0.05%. The temperature in the column and in the separator was controlled using four HC5 thermostat (Julabo GmbH, Seelbach, Germany) with an accuracy of ±0.01 K. The pressure transmitter, model ED