The Journal of Supercritical Fluids 133 (2018) 94–102

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

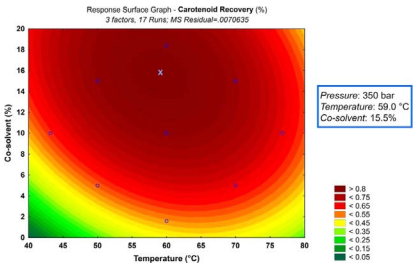
journal homepage: www.elsevier.com/locate/supflu

Optimisation and modelling of supercritical CO_2 extraction process of carotenoids from carrot peels

Micael de Andrade Lima, Dimitris Charalampopoulos, Afroditi Chatzifragkou*

Department of Food and Nutritional Sciences, University of Reading, Whiteknights, PO Box 226, Reading, RG6 6AP, UK

GRAPHICAL ABSTRACT



ARTICLE INFO

Keywords:
Carotenoids
Supercritical CO_2
Carrot peels
Modelling
Recovery

ABSTRACT

This work aimed to assess and optimise the extraction of carotenoids from carrot peels by supercritical CO_2 (S-CO_2), utilising ethanol as co-solvent. The evaluated variables were temperature, pressure and co-solvent concentration. According to the validated model, the optimal conditions for maximum mass yield (5.31%, d.b.) were found at 58.5 °C, 306 bar and 14.3% of ethanol, and at 59.0 °C, 349 bar and 15.5% ethanol for carotenoid recovery (86.1%). Kinetic experiments showed that 97% of the total extractable carotenoid content was recovered after only 30 min, whereas model fitting confirmed the fast extraction trend and desorbing nature of carotenoids from the sample matrix. The process is potentially scalable, as demonstrated by runs performed with a 10-fold initial sample size, which led to even higher recoveries (96.2%), indicating that S-CO_2 can be as efficient as a conventional solvent extraction for recovering high value compounds from vegetable by-products.

1. Introduction

Due to the increasingly high volumes of waste generated by the food processing industry, developing and establishing waste management practices is paramount. Fruit, vegetables and their by-products are known to contain a variety of valuable compounds including carbohydrates (e.g. dietary fibre, oligosaccharides), aromatic compounds and phytochemicals (e.g., polyphenols, glucosinolates, carotenoids) [1].

Carrots are one of the most consumed vegetables with over 37 million tonnes produced every year worldwide [2]. Such a vast production results in proportionally large amounts of waste, as during carrot processing around 11% of the initial mass is lost, mainly in the form of peels, tubers and attached flesh. Carrots are enriched with phytochemicals of high importance, such as carotenoids and phenolic compounds [3] that could be extracted and utilised as natural additives (e.g. colourants) for food and pharmaceutical applications. In particular, carotenoids are ubiquitous compounds in vegetables and constitute essential nutrients in the human diet, exerting antioxidant and potentially cancer-preventive properties [4–6].

The extraction of phytochemicals from vegetable matrices is commonly carried out with the aid of conventional chemical solvents, due to their ease of use, efficiency, relatively low cost and wide applicability

* Corresponding author.
E-mail address: a.chatzifragkou@reading.ac.uk (A. Chatzifragkou).

http://dx.doi.org/10.1016/j.supflu.2017.09.028
Received 11 July 2017; Received in revised form 26 September 2017; Accepted 27 September 2017
Available online 28 September 2017
0896-8446/

[7]. The mechanism of extraction rests on the differences in the solubility of the matrix (insoluble residue) and the compounds of interest which, by having high affinity with the liquid solvent phase, promptly diffuse into it. However, conventional solvents require several hours to achieve satisfactory recoveries. Also, the end solutions are often dilute and therefore, an additional concentration step is needed, which could result in degradation or bioactivity losses of the components of interest. In the case of carotenoids, different solvents such as acetone, methanol, hexane and tetrahydrofuran (THF) are commonly used due to the non-polar nature of such phytochemicals [8]. A clear disadvantage is the toxicity of many of these solvents that renders their handling and disposal a hazard for handlers and the environment.

With the growing environmental concern and the advent of green technologies, new methods for extracting these classes of phytochemicals have been suggested and are currently being studied, including microwave and ultrasound assisted extraction, subcritical water extraction, enzyme-aided extraction and supercritical fluid extraction (SFE) [9–12]. SFE technology employs mainly CO_2 and is regarded as a fast, efficient and "clean" method for the extraction of natural products from biomass matrices, such as fruit and vegetables. The extraction of compounds happens similarly to conventional solvents, but fluids in supercritical state possess gas-like properties of diffusion, viscosity and surface tension, as well as liquid-like densities and solvation powers. These properties combined render S-CO_2 ideal for extracting compounds in a shorter time with higher yields, when compared to conventional liquid-state solvents [13]. Supercritical CO_2 results in the recovery of compounds without toxic residues, no degradation of the active components, which can be then recovered in high purity. It is currently used commercially for the decaffeination of coffee and tea [14,15], the extraction of flavours from plant leaves, lipids from milk and fish oils, alcohol from beverages [16] and specialty bioactives for cosmetic applications, such as antiaging creams [13].

The aim of this work was to evaluate the use carrot peels as starting material for the extraction of carotenoids by supercritical CO_2. Although previous studies dealing with the extraction of carrot flesh or other vegetable matrices by SFE have been performed [17,18], these were more focused on statistical approach rather than on the variable effects of process kinetics and extract characterisation.

The efficiency of the process was evaluated with respect to global mass yield (%, ratio between total extracted mass and the amount of initial sample) and carotenoid recovery. A 2^3 Central Composite Design of Experiments (DoE) was carried out to optimise the process conditions and the extraction kinetics, whereas the fit of different models to the data was assessed. Also, experiments using a 10-fold initial sample size were performed to attest the scalability potential of the process. Finally, the obtained extracts were compositionally characterised and insights on their potential applications were given.

2. Materials and methods

2.1. Sample preparation

Samples of Nantes carrots (*Daucus carota*) grown in the UK and harvested in February 2015, were purchased from a local supermarket chain in Reading (UK). The carrots were washed and manually peeled. In our experiments, the peels represented 9.5% of the total vegetable mass loss, a figure quite close to the losses occurred during industrial processing of carrots (11%). The samples of peels and flesh were frozen at −20 °C for 36–48 h, freeze dried (VirTis SP Scientific, UK) for 72 h, milled with a grinder and sieved to exclude particles with diameter greater than 750 μm. The final samples presented a mean particle size of 205 μm (70 mesh) for peels and 245 μm (60 mesh) for flesh. The samples were then stored in containers away from light and kept at −20 °C until further analysis.

2.2. Total carotenoid content (TCC) determination and identification

Carotenoids were analysed according to a protocol optimised for carrot matrices [19]. Briefly, 1.0–2.0 g of sample were weighed and added into 6 mL of methanol. After vigorous mixing, samples were centrifuged for 5 min at 2500 × *g* and the supernatant was separated; a new extraction was performed twice with 8 mL of a mixture of hexane and acetone (1:1). Subsequently, the organic solvent fractions were combined, 25 mL of saturated NaCl were added, and the mixture was shaken in a separator funnel. After phase separation, the lower, water-phase was re-extracted with 8 mL of hexane and the resulting supernatant was combined with the first. The combined fractions were evaporated under nitrogen stream and re-dissolved in methanol prior to High Pressure Liquid Chromatography (HPLC) analysis. An Agilent Infinity 1260 series HPLC system was used, coupled with a 1260 DAD detector (Agilent Technologies, UK). An YMC-C30 silica-based reversed-phase column (250 × 4.6 mm) was used for the separation of carotenoids in a gradient method consisting of (A) methanol/MTBE/water (82:16:2) and (B) methanol/MTBE/water (23:75:2) as mobile phase. The gradient started at 100% of A. Solvent B was then increased to 50% (0-45 min) and further increased to 100% (46–55 min), with this condition being held for 5 min, totalling 60 min per run. The injection volume was 100 μL and the flow rate was kept constant at 1.0 mL/min. For carotenoid identification and quantification, previously-built calibration curves of external commercial standards (*α*-carotene, *β*-carotene and lutein; Sigma-Aldrich) were used. All detected peaks were analysed at 450 nm.

2.3. S-CO_2 extraction parameters and optimisation of experimental conditions

Samples of carrot peels were subjected to S-CO_2 extraction in a S-CO_2 rig (SciMed, UK). The apparatus consisted of a recirculating chiller, CO_2 line, solvent and co-solvent pumps, heat exchanger, 200 mL extraction vessel, automated backpressure regulator (ABPR), collection vessel and a controlling computer. For every run, 5.0 g of dried peel samples were thoroughly mixed with 95 g of inert glass beads (Sigma-Aldrich, UK) to ensure bed homogenisation, placed in the extraction vessel and submitted to a CO_2 flow rate of 15 g/min. Ethanol was used as co-solvent and extraction time was fixed at 80 min.

In order to optimise the process, a non-factorial 2^3 Central Composite Design of Experiments (DoE) with three factors at three levels was employed. The three independent variables assessed in the study were temperature (*T*, at 50, 60 and 70 °C), pressure (*P*, at 150, 250 and 350 bar) and co-solvent concentration (*EtOH*, at 5, 10 and 15% v/v). The dependent variables (or responses) assessed were global mass yield *Y*, defined as the % (g/g) of mass recovered in the extracts with relation to the initial mass load (5.0 g), and total carotenoid content (TCC) recovery *C-REC*, defined as a percentage (%, mg/mg) of the initial TCC. Fourteen different experiments including the low, high and axial points of all the parameters were conducted along with a central point replicated three times to calculate experimental errors, totalling 17 runs. At the end of every run, the extracts obtained in ethanol, were evaporated to dryness in a rotavapor (RE 120, Büchi, UK), weighed and re-dissolved in methanol for TCC analysis as described above.

Response Surface Methodology (RSM) was used to represent the model obtained in the form of a 3D graph. All terms in the model equation were tested statistically by the F-test at a 95% interval of confidence. The values of the determination coefficient (R^2) and the coefficient of variance (*CV*, %) were also used to confirm the quality of the fitted polynomial model. Lastly, after identifying the critical points by localising the graph global maximum (point where the derivative of the curve is zero), additional triplicate experiments were performed at these critical conditions in order to determine the validity of the optimised conditions. The average values of the experiments were compared to the predicted values given by the model to confirm its