Food Chemistry 262 (2018) 30–38

Contents lists available at ScienceDirect

Food Chemistry

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

Transglutaminase mediated microencapsulation of sea buckthorn supercritical CO_2 extract in whey protein isolate and valorization in highly value added food products

Liliana Mihalcea, Mihaela Turturică, Vasilica Barbu, Elena Ioniţă, Livia Pătraşcu, Mihaela Cotârleţ, Loredana Dumitraşcu, Iuliana Aprodu, Gabriela Râpeanu, Nicoleta Stănciuc*

Integrated Center for Research, Expertise and Technological Transfer in Food Industry, Faculty of Food Science and Engineering, Dunarea de Jos University of Galati, Romania

ARTICLE INFO

Keywords:
Carotenoids
Sea buckthorn
Transglutaminase
Microencapsulation
Whey proteins

ABSTRACT

Sea buckthorn carotenoids extracted using CO_2 supercritical fluids method were encapsulated within whey proteins isolate by transglutaminase (TG) mediated crosslinking reaction, coacervation and freeze drying. The encapsulation efficiency was 36.23 ± 1.58%, with β-carotene the major carotenoid present in the powder. The confocal analysis revealed that TG-ase mediated cross-linking reaction enhanced the complexes stability to such a manner that a double microencapsulation was performed. The powder showed an antioxidant activity of 2.16 ± 0.14 mMol Trolox/g DW and an antifungal activity against *Penicillium expansum* MIUG M11. Four variants of domestic ice creams were obtained, with a total carotenoids content variation of 1.63 ± 0.03 mg/g D.W. in sample with 2% powder and 6.38 ± 0.04 mg/g D.W. in samples with 4% extract, having satisfactory antioxidant activity. The storage stability test showed a decrease in both total carotenoids content and antioxidant activity in all samples during 21 days at −18 °C. A protective effect of microencapsulation was evidenced.

1. Introduction

Carotenoids are organic pigments naturally found in many fruits and vegetables with 40-carbon molecules and multiple conjugated double bonds (Qian, Decker, Xiao, & McClements, 2012), possessing several functional properties, mainly antioxidant (Caris-Veyrat, 2008) and provitamin A activities (Murillo et al., 2013). β-Carotene is one of the most effective vitamin A precursors with many potential health benefits, e.g. cancer prevention, ulcer inhibitor, life extender, and heart attack inhibitor (Chen et al., 2014). The health-promoting properties of carotenoids are dependent by their bioavailability. Rutkowska and Stolyhwo (2009) considered that carotenoids in natural edible oils could be easily absorbed, and therefore could be directly used for the fortification of traditional food products.

Due to the high concentration of vitamins, such as: vitamin C, B group, E (Korekar, Dolkar, Singh, Srivastava, & Stobdan, 2014) and carotenoids, sea buckthorn (SBT) berries are considered nutraceutical crops with many applications in food industry, pharmaceutical and medicine. The carotenoids in SBT consist of zeaxanthin, β-carotene, β-cryptoxanthin, lutein, lycopene and γ-carotene (Anderson, Olsson, Johansson, & Rumpunen, 2009). For example, the carotenoids content of Carpathians' sea buckthorn (*Hippophae rhamnoides* L., ssp. Carpatica) indicated the presence of the β-carotene (1.9–7.4 mg/100 g DW), β-cryptoxanthin (1.3–1.6 mg/100 g DW), lycopene (1.4–2.3 mg/100 g DW) and zeaxanthin (1.8–2.5 mg/100 g DW), as reported by Pop et al. (2014). Carotenoids as natural pigments are heat stable in systems with minimum oxygen content. However, the use of carotenoids extracts in food products is limited due to their low water-solubility and low thermal stability. Rodríguez-Huezo, Pedroza-Islas, Prado-Barragán, Beristain, and Vernon-Carter (2004) suggested that carotenoids are rather unstable substances, very sensitive to oxygen, light, and heat. In their natural form, they are insoluble in water and slightly soluble in oil at room temperature. The low thermal stability problems of carotenoids can be avoided by emergent technology extraction using supercritical CO_2 (SC CO_2) fluids. This *green* extraction method allows selective separation of bioactive, thermally sensitive components from natural vegetable matrix, in order to obtain extracts that can bring added value when used in food, pharmaceutical and medicinal products. Xu, Gao,

* Corresponding author at: Dunarea de Jos University of Galati, Faculty of Food Science and Engineering, Domnească Street 111, Building E, Room 304, 800201 Galati, Romania.
E-mail address: Nicoleta.Stanciuc@ugal.ro (N. Stănciuc).
URLS: http://www.funfood.ugal.ro, http://www.sia.ugal.ro (N. Stănciuc).

https://doi.org/10.1016/j.foodchem.2018.04.067
Received 29 January 2018; Received in revised form 15 April 2018; Accepted 18 April 2018
Available online 19 April 2018
0308-8146/

Liu, Wang, and Zhao (2008) performed SC-CO_2 extraction of different anatomic parts of the SBT plant (leaves or dried berries) and the optimization process was reported using a extraction system with two separation steps.

Further, the protection and controlled release of carotenoids at the right time and the right place can be achieved by encapsulation (Ezhilarasi, Karthik, Chhanwal, & Anandharamakrishnan, 2013). Microencapsulation of carotenoids in different matrices using various techniques was recently reviewed (Janiszewska-Turak, 2017). Microencapsulation allows the creation of a physical barrier between the core and the wall materials (Dubey, Shami, Bhasker, & Rao, 2009) thus protecting the bioactives. Due to their structural and functional particularities, whey proteins are frequently used as encapsulation and transport agents for different bioactives (Tavares, Croguennec, Carvalhoc, & Bouhallab, 2014). Our research group recently reported results of the microencapsulation process of different biological active compounds, such as: carotenoids from supercritical CO_2 extract (Mihalcea et al., 2017) or hexane:ethanol extract (Aprodu, Ursache, Turturică, Râpeanu, Stănciuc, 2017; Ursache, Dumitrascu, Aprodu, & Stănciuc, 2017, Dumitrașcu, Ursache, Stănciuc, & Aprodu, 2016), anthocyanins from sour cherries (Oancea et al., 2017) or red grapes (Stănciuc et al., 2017) by using whey proteins isolates (WPI) or single β-lactoglobulin as wall materials. These studies indicate that WPI is a promising matrix for the encapsulation of bioactives.

Transglutaminase (TG-ase) is able to catalyze the formation of an isopeptide bond between a free amine group belonging to a protein- or peptide-bound lysine and the acyl group at the end of the side chain of protein or peptide-bound glutamine. Microbial TG-ase has considerable potential to improve the firmness, viscosity, elasticity and water-binding capacity of food products (Kieliszek & Misiewicz, 2014). TG-ase can be used to mediate the encapsulation of a targeted bioactive compound. Mihalcea et al. (2017) recently demonstrated that TG-ase mediated reaction allowed microencapsulation of a higher amount of esters and a lower amount of β-carotene.

Therefore, the aim of this study was to perform a TG-ase mediated microencapsulation process of SBT supercritical CO_2 extract in WPI and acacia gum by coacervation and freeze drying. The obtained powder was analyzed by HPLC for individual carotenoids content, encapsulation efficiency, total carotenoids content, while the microstructure of the particles was analyzed by confocal laser and scanning electron microscopy. The functionality of the powder was demonstrated in terms of antioxidant and antifungal activities and color parameters. In order to demonstrate the functionality of the microencapsulated carotenoids, the obtained powder was added in different ratios in ice cream formula. The ice creams were tested for total carotenoids, antioxidant activity, physico-chemical, rheological and sensorial analysis. Accelerated storage stability of the carotenoids and antioxidant activity was also performed for 21 days at −18 °C. Our results demonstrated the potential applications of both carotenoids from SBT supercritical CO_2 extract and microencapsulated powder in food industry as pigments and bioactive compounds.

2. Materials and methods

2.1. Materials and chemicals

Whey proteins isolate (protein content 95%) was purchased from Fonterra (New Zeeland). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), ethanol, ethyl acetate, hexane and methanol (HPLC grade), β-carotene, lycopene, β-cryptoxanthin, zeaxanthin were obtained from Sigma Aldrich Steinheim, Germany. Commercial microbial TG-ase (ActivaTMTG) isolated from *Streptomyces mobaraensis* was purchased from Ajinomoto Corporation Inc. (Tokyo, Japan). The enzyme activity was 100 U/g protein. For ice cream preparation, UHT whole cow milk (3.5% fat, 3.2% protein, 4.5% lactose reported at 100 g D.W.), UHT whipped cream (35% fat, 3.4% protein at 100 g D.W.), mascarpone (40% fat, 2.1% lactose, 4.2% protein reported at 100 g D.W.) and white sugar were purchased from a local supermarket in Galați, Romania.

Strains of *Aspergillus niger* MIUG M5 and *Penicillium expansum* MIUG M11 were obtained from the Microbial Collection of *Dunărea de Jos* University of Galați (MIUG, Galați, Romania). Molds were individually grown on PDA (Potato Dextrose Agar, Bioxon) slants at 25 °C for 7–15 days until sporulation. The spores were then collected from slants using sterile peptone water (0.1% w/v). The spore count was determined using a haemocytometer and suspensions were adjusted to 10^6 spores/mL.

2.2. Sea buckthorn dried berries

Dried ripe berries of Mara SBT (*Hippophae rhamnoides* L.) were purchased from the Biofarmnet bench, Ialomita city, located in the South region of Romania. According to the trader statement, the fresh SBT berries were selected by leaves, frozen at −20 °C and dried in the discontinuous bed drier at 40 °C until they reached a dry matter content of 8.53 ± 0.26%.

2.3. SC CO_2 extraction of carotenoids

The SC-CO_2 extractions of carotenoids were performed as described by Mihalcea et al. (2017). The extraction parameters were carried out according to Xu et al. (2008) experiments (pressure of 27.6 MPa, temperature of 34.51 °C and extraction time of 82.0 min).

The oil was collected at the end of the extraction process and stored at −18 °C for further analysis.

2.4. Preparation of the encapsulated powders

The method described by Mihalcea et al. (2017) was used for encapsulation of SBT oil extract in WPI and acacia gum by complex coacervation, with some modifications. In brief, 2 g of WPI was dissolved in 100 mL distilled water and subjected to ultrasonication for 10 min at 25 ± 1 °C. Hereafter, in order to facilitate the hydration of the proteins, the solutions were maintained at 40 °C for 20 min over mechanical stirrer (IKA RCT Basic, Germany). Subsequently, 5 mL of SBT oil was added, the mixture was homogenized at 4000 rpm for 30 min to obtain oil in water emulsions. Then, 100 mL 1% (*w/v*) acacia gum solution was added to the emulsion, and allowed to stir for 30 min at 40 °C. Prior to coacervation, the solution was subjected to a cross-linking reaction by using 4 mg/g protein TG-ase, followed by enzymatic reaction at 40 °C for 24 h. In order to promote coacervation, the pH of the solutions was adjusted to 3.75 with 1 N HCl solution at approximately 40 °C, under constant mechanical stirring at 600 rpm. The reaction mixture was then allowed to cool in ice bath under stirring and stored at 7 °C overnight to promote decantation. The coacervates were freeze dried (CHRIST Alpha 1–4 LD plus, Germany) at −42 °C under a pressure of 0.10 mBar for 48 h. Afterwards, the powders were collected and packed in metallized bags, and kept in a freezer at −20 °C until analysis.

2.5. HPLC technique

The chromatographic analysis of the carotenoids from microencapsulated SBT powder was performed according to Mihalcea et al. (2017). The carotenoid extraction was assessed using the following procedure: 100 mg powder was mixed for 1 min with 2 mL NaCl:MeOH (v/v) and then with 10 mL hexane:acetone (v/v). The organic phase was collected and then evaporated at 40 °C. The concentrated extract was redissolved in 1 mL of ethyl acetate. The carotenoids were analyzed at 450 nm on a Lichrosorb RP-18 (5 μm) Hibar RT 125–4 column, using a Thermo Finnigan Surveyor HPLC system (Finnigan Surveyor LC, Thermo Scientific, SUA). The elution solvents were 90% acetonitrile (A)