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Omega–3 encapsulation by PGSS-drying and conventional drying methods. Particle characterization and oxidative stability

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ABSTRACT

Particles from Gas-Saturated Solutions (PGSS)-drying has been used as a green alternative to encapsulate omega–3 polyunsaturated fatty acids (*n*–3 PUFAs) at mild, non-oxidative conditions. PGSS-dried particles have been compared to those obtained by conventional drying methods such as spray-drying and freeze-drying, finding encapsulation efficiencies (EE) up to 98% and spherical morphology for PGSS- and spray-dried particles. Freeze-dried powders showed irregular morphology and EE from 95.8 to 98.6%, depending on the freezing method. Differential scanning calorimetry (DSC) analysis revealed glass-transition and melting peaks of OSA-starch and a cold-crystallization peak corresponding to the encapsulated *n*–3 PUFA concentrate. Compared to conventionally dried powders, PGSS-dried microparticles showed lower primary and secondary oxidation after 28 days of storage at 4 °C. Ascorbic acid addition combined with the mild processing conditions of PGSS-drying yielded particles with a maximum peroxide value of 2.5 meq O_2/kg oil after 28 days of storage at 4 °C.

1. Introduction

An adequate intake of omega–3 polyunsaturated fatty acids (*n*–3 PUFAs) is recommended in healthy diet guidelines due to their important benefits (Ruxton, Reed, Simpson, & Millington, 2004). Long-chain *n*–3 PUFAs, mainly eicosapentaenoic (EPA, 20:5 *n*–3) and docosahexaenoic (DHA, 22:6 *n*–3) acids are eicosanoid precursors, which are immunomodulatory molecules with a key role in the inflammatory response. EPA and DHA are claimed to contribute to the normal brain, eye and cardiovascular functions in adults and help in the normal development of the eyes, the brain and the nervous system in children (EFSA, 2010).

The perceived health benefits of these compounds have created a strong demand for EPA and DHA concentrates in the pharmaceutical and food industries. However, *n*–3 PUFAs are unstable and very prone to oxidation, easily generating lipid hydroperoxides and free radicals under oxidative conditions. These species negatively affect sensory properties, since they can decompose into low-molecular-weight volatile compounds that are perceived as rancid, and what is more, they present potentially cytotoxic, carcinogenic and mutagenic effects (Niki, 2009; Uluata, McClements, & Decker, 2015) For these reasons, *n*–3 PUFA concentrates are often encapsulated in order to protect them from light and oxygen during shelf life; and natural antioxidants such as tocopherols, phospholipids, ascorbic acid, or their mixtures are usually added (Baik et al., 2004; Löliger & Saucy, 1994).

Materials of different nature can be used as *n*–3 PUFA encapsulating agents: proteins such as whey protein isolate, sodium caseinate or gelatin, phospholipids such as lecithin, or polysaccharides such as gum Arabic, carboxymethyl cellulose, maltodextrin, chitosan, or modified starch are some examples of carrier materials for microencapsulation of oils rich in *n*–3 PUFAs (Encina, Vergara, Giménez, Oyarzún-Ampuero, & Robert, 2016). Among them, *n*-octenyl-succinic-anhydride modified starch (OSA-starch) has been chosen in this work because it presents good emulsifying properties and is suitable to encapsulate oils rich in *n*–3 PUFAs, as well as other bioactive compounds such as essential oils and hydrophobic compounds (Carneiro, Tonon, Grosso, & Hubinger, 2013; de Paz, Martín, Bartolomé, Largo, & Cocero, 2014; Drusch, Serfert, Scampicchio, Schmidt-Hansberg, & Schwarz, 2007; Jafari, Assadpoor, He, & Bhandari, 2008; Varona, Martín, & Cocero, 2011).

Different encapsulation techniques can be used to encapsulate *n*–3 PUFAs, such as emulsification, spray-drying, freeze-drying, coacervation, *in situ* polymerization, extrusion, or fluidized-bed coating (Bakry et al., 2016). Among these, the most widely used technique in the food and pharmaceutical industries is spray-drying, followed by freeze-drying. Freeze-drying is often applied to thermolabile and easily oxidizable compounds due to the protective low temperatures and vacuum

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conditions involved in the process. Its main drawback is the energy consumption, linked to the low temperature and high vacuum conditions as well as the long residence times required to completely dry the product, which in turn translate into high processing costs. On the contrary, spray-drying is a low-cost microencapsulation technology which operates in a relatively simple and continuous way, thus it is commonly used at industrial scale (Bakry et al., 2016).

Prior to the drying step, the non-soluble *n*–3 PUFAs need to be dispersed into the encapsulating agent solution, obtaining an oil-in-water (O/W) emulsion. Several methods can be used to prepare O/W emulsions, such as conventional emulsification (colloid milling, high speed blending and high-pressure homogenization), ultrasound (US) assisted emulsification, membrane emulsification, and micro-channel emulsification (Chatterjee & Judeh, 2015). Among them, US-assisted emulsification has grown in importance among the pharmaceutical, cosmetic, and food industries, thanks to its versatility and the possibility of obtaining high quality food products with enhanced functional properties (Abbas, Hayat, Karangwa, Bashari, & Zhang, 2013). US-assisted emulsification can be applied to improve stability and bioavailability of the dispersed bioactive compounds and, in particular, it can be used to obtain O/W emulsions with nanometric droplet size and narrow size distribution. Typically, US-assisted emulsification consists on applying low-frequency sound waves of 20–100 kHz through a metallic sonotrode immersed in the liquid medium, in order to generate disruptive forces that break down the macroscopic phases to nanosize droplets. The nano-scale emulsions obtained present interesting functional properties and enhanced stability against oxidation (Abbas et al., 2013).

Supercritical fluids, and particularly supercritical carbon dioxide (SC-CO_2), are a convenient medium to produce particles loaded with bioactive compounds. Carbon dioxide is an inert, non-toxic solvent, and is completely released from the product as a gas once back to atmospheric conditions. Besides, the accessibility of the supercritical state of carbon dioxide (T_C = 31.1 °C; p_C = 73.8 bar) and its advantageous physical properties (high density and diffusivity, and low viscosity) make SC-CO_2 the solvent of choice in many particle formation processes (Türk, 2014). Among the several available techniques, the Particles from Gas Saturated Solutions (PGSS) process overcomes the problems of solubility limitations and high gas consumption of other particle formation methods using SC-CO_2 (Türk, 2014). This technique can be used for drying aqueous solutions, dispersions or, as in this work, O/W emulsions, in the so-called PGSS-drying process (Türk, 2014).

Basically, the PGSS-drying technique consists on mixing an aqueous solution with supercritical carbon dioxide upon saturation, and subsequently expanding the gas-saturated solution down to atmospheric pressure through a nozzle. This technique can be used as an alternative to conventional spray-drying, achieving a more efficient atomization due to the sudden vaporization of the dissolved CO_2 and the expansion of gas bubbles in the solution during depressurization from supercritical to atmospheric conditions. Both effects improve the atomization of the sprayed solution forming small droplets, thus reducing the particle size of the dried powder and enhancing the drying process (Martín & Weidner, 2010; Weidner, 2009). Besides, and because of the intense and deep cooling caused by the Joule-Thomson effect, it is possible to dry the product at low temperature (40–80 °C) (de Paz, Martín, & Cocero, 2012; Weidner, 2009). The mild-temperature conditions, combined with the intrinsically inert atmosphere due to oxygen displacement, prevent, or at least delay, oxidative degradation of the encapsulated bioactive compounds (de Paz et al., 2012; Weidner, 2009). Operating conditions in the spray tower, particularly temperature and gas-to-product ratio (GPR), must be taken into account in order to operate above the dew line of the carbon dioxide-water system (Martín & Weidner, 2010), and ensure the complete drying of particles.

In this work, an *n*–3 PUFA enriched fish oil has been encapsulated by the alternative and green technology Particles from Gas Saturated Solutions (PGSS)-drying. The main hypothesis of the study is to explore whether or not the potential benefits of supercritical carbon dioxide technologies applied to particle formulation and encapsulation may affect particle properties and oxidative stability of heat-sensitive and easily oxidizable compounds such as *n*–3 PUFAs, compared to other conventional drying methods. This way, the PGSS-dried particles have been compared to those obtained by spray-drying and freeze-drying, which are commonly applied in the pharmaceutical, cosmetic, and food industries to dry aqueous solutions and dispersions. Characterization of the particles obtained by the different drying methods has been performed in terms of particle morphology, residual humidity, and particle size distribution of the reconstituted particles. Besides, encapsulation efficiency and oxidative stability (primary and secondary oxidation) of the encapsulated n–3 PUFA concentrate have been monitored over time in the particles formulated with each of the drying methods. Additionally, an antioxidant (ascorbic acid) has been added to some of the formulations as a strategy to potentially enhance the oxidative stability of the encapsulated *n*–3 PUFA concentrate.

2. Materials and methods

2.1. Materials

n–3 PUFA concentrate from fish oil, Algatrium™ Plus, was kindly donated by Brudy Technology S.L. (Spain). It has been stored at 4 °C in darkness and N_2 atmosphere. Hi-Cap™ 100, an octenyl-succinic-anhydride modified starch (OSA-starch) derived from waxy maize, was provided by Ingredion Inc. (Germany). Carbon dioxide (99.9%) was provided by Air Liquide S.A. (Spain). Ascorbic acid (L(+)-Ascorbic acid, AA) was purchased from Panreac AppliChem (Spain).

37% hydrochloric acid (HCl), diethyl ether, 1-butanol, 2-propanol, methanol, 2-thiobarbituric acid (TBA), and trichloroacetic acid (TCA) were provided by VWR Chemicals (Germany). Hexane, absolute ethanol, Iron(II) sulphate heptahydrate, and ammonium thiocyanate were purchased from Merck KGaA (Germany). 2,2,4-trimethylpentane (isooctane) and barium chloride dihydrate were supplied by Macron Fine Chemicals (France) and Panreac AppliChem (Spain), respectively. Cumene hydroperoxide and 1,1,3,3-tetraethoxypropane (TEP) standards were purchased from Sigma Aldrich (USA).

2.2. Characterization of the n–3 PUFA concentrate

Neutral lipid profile of the *n*–3 PUFA concentrate has been analyzed by normal-phase HPLC (NP-HPLC). Separation was carried out at room temperature in a Lichrospher Diol column (5 mm, 4 mm × 250 mm) and detection was performed by evaporative light scattering (ELSD) (Agilent Technologies 1200 Series, USA) at 35 °C and 3.5 bar. Solvent gradient and calibration procedure have been reported elsewhere (Solaesa, Sanz, Falkeborg, Beltrán, & Guo, 2016).

Fatty acid profile of the *n*–3 PUFA concentrate has been determined according to the AOAC Official Method (AOAC International, 2012) in a Hewlett Packard gas chromatograph (6890N Network GC System) equipped with an auto-sampler (7683B series) and a flame ionization detector (FID). The separation was carried out in a fused silica capillary column (Omegawax-320, 30 m × 0.32 mm i.d.) with helium (1.8 mL/min) as carrier gas. Injection and detection temperatures, as well as ramp conditions have been previously reported (Rebolleda, Rubio, Beltrán, Sanz, & González-San José, 2012). Most of the fatty acids were identified by comparison of their retention times with those of chromatographic standards (Sigma Aldrich). As indicated by the AOAC Official Method (AOAC International, 2012), an internal standard (methyl-tricosanoate, C23:0) was used for quantification purposes.

HPLC with diode array detection (HPLC-DAD) of the *n*–3 PUFA concentrate was carried out in order to detect tocopherol isomeric forms and other vitamin E analogs added to the *n*–3 PUFA concentrate, as their presence was reported by the provider. The analytical method is based on the IUPAC official method (Pocklington & Dieffenbacher,