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New approaches for the selective extraction of bioactive compounds employing bio-based solvents and pressurized green processes

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ABSTRACT

Solvent selection is a key factor in the development of processes for the extraction of bioactive compounds from natural sources such as algae. The aim of this work was the use of bio-based solvents for the selective extraction of fucoxanthin from *P. tricornutum*, using pressurized technologies. In this regards, the application of Hansen solubility approach reduced the list of candidate solvents for pressurized liquid extraction (PLE) to four: ethyl acetate, ethyl lactate, d-limonene and ethanol. The use of theoretical calculations narrowed the search of suitable solvents, thus making the process greener. Among the bio-based solvents proposed, d-limonene was the most selective, although it was not able to recover all the fucoxanthin present in the biomass unless a continuous extraction aided by supercritical CO_2 was used. The other three solvents tested showed good recoveries of fucoxanthin, but were less selective, following the decreasing order: ethyl acetate > ethyl lactate > ethanol.

1. Introduction

Despite solubility parameter theory has been employed since the first middle of the last century, recently it has received special attention as prior evaluation of the possibility to dissolve a solute in an solvent [1–5]. The solubility theory approach is based on the principle "like dissolves like" and it can be very advantageous to give a first approximation in extraction processes, indicating the most suitable solvent for a given application thus avoiding the selection of impractical experimental conditions. In an effort to improve the applicability of the total solubility parameter, Hansen [6] divided the Hildebrand parameter into three dimensional components, which quantify, individually, the contributions of the dispersion, polar and hydrogen bonding interactions forces [6,7]. The Hansen solubility parameters (HSP) have been widely applied from academic labs to industrial applications and have been employed as a numerical estimation to predict the solubility of many industrial products such as polymers, bio-polymers, drugs, pigments, dyes, and some biological materials in different types of solvents [6]. The estimation of the individual HSP depends greatly upon the availability of data of the target molecule; therefore, when there is no enough information in the databases on the key molecule(s), the group contribution method (GCM) is the most common way employed to predict properties and solubility parameters of molecular structures, using additive rules [8]. On the other hand, due to the growing importance of the sub- and supercritical fluid extraction as green process technologies, several accurately predictions of the HSP in terms of reduced pressure and temperature have been discussed [9–12]. There are few examples in the recent literature in which the estimation of the HSP appears as a useful tool to optimize the pressurized extraction of bioactive metabolites from natural sources [1,4,13–17]. For instance, Srinivas and King [1] estimated HSP of some carotenoids (e.g. β-carotene, curcumin, lutein, violaxanthin and zeaxanthin) present in spices (e.g. black pepper, cayenne, cinnamon, garlic, ginger) in supercritical carbon dioxide (SC-CO_2) at different temperatures and pressures. On the other hand, Chemat's research group have also employed the HSP to evaluate the performance of several alternative solvents, including SC-CO_2, to extract aroma compounds, fat and oils, and carotenoids, among others, from different food matrices, in order to substitute petroleum based solvents (such as hexane and dichloromethane) [5,13,18–20]. In a recent work carried out in our laboratory, we have estimated the HSP of phlorotannins (polyphenols constituted of phloroglucinol monomers with important bioactivities) obtained from a Mediterranean brown algae in different sub-critical green solvents; this theoretical approach was found as a profitable tool to choose a suitable solvent for selective extraction purposes [17].

The Hansen approach, hence, can be exploited in the development of greener selective processes for the extraction of valuable compounds from natural sources, since the use of a theoretical approach reduces the

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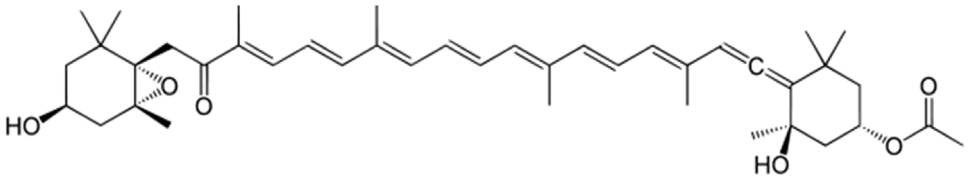

Fig. 1. Structure of fucoxanthin.

number of experiments with different solvents. In addition, since the extractions are more selective, purification steps are reduced. Therefore, there is a reduction on waste generation, thus making the processes more environmentally friendly. But to develop a green process, additional criteria for solvent selection should be taken into account. Different organizations have established classifications of solvent "greenness" attending to different standards, e.g.: environmental impact, health, safety, life cycle assessment (LCA), waste, etc [21,22]. However, scarce attention has been paid in the solvent selection guides to bio-based solvents until the last years [21,23,24], when their potential as extraction solvents for the development of sustainable processes has started to be explored [2]. The development of green sustainable processes to place in the market products enriched in bioactive compounds obtained from natural sources is one of the challenges that chemical and pharmaceutical industries have to face nowadays. The trend to a "bio-based world" has put the focus on renewable sources such as algae, recognized as rich source of bioactive and high-added value compounds [25,26]. For instance, fucoxanthin is a xanthophyll (an oxygenated carotenoid) with a unique structure, which includes an allenic bond and a 5,6-monoepoxide (see Fig. 1). Fucoxanthin is one of the most abundant carotenoid present in algae, very appreciated for its several biological activities, including antioxidant, antiobesity, antidiabetic, anti-inflammatory, antiphotoaging and anticancer [27]. The commercial production of fucoxanthin has been explored, using *Phaeodactylum tricornutum* (a marine diatom microalga) as biomass [28]. In this regard, pressurized liquid extraction (PLE) using a bio-based solvent such as ethanol has been proposed for the recovery of fucoxanthin and other pigments from *P. tricornutum* [29]. Also, ethanol was reported as optimum solvent for the extraction of total carotenoids from *Neochloris oleoabundans*, after an experimental design in which the bio-based solvent d-limonene was also evaluated [30]. The mixture ethanol:d-limonene has been proposed as an alternative to replace *n*-hexane for the extraction of lipids from microalgae [31]. D-limonene has also been proposed recently as a promising green solvent for the extraction of carotenoids and other bioactive compounds from food [32]. However, in none of these examples the extraction was selective towards a target compound but a family of compounds. The aim of this work was to develop an efficient and selective extraction method of fucoxanthin from *P. tricornutum* by using pressurized bio-based solvents. According to the theoretical predictive assessment of HSP, ethanol, ethyl acetate, ethyl lactate and d-limonene were the selected solvents and their behavior was experimentally evaluated. In addition, SC-CO_2 employing d-limonene as co-solvent was tested. The quantification of fucoxanthin in the obtained extracts was carried by HPLC-DAD-APCI-MS/MS. The selectivity of the processes was measured by the ratio between total carotenoid and total chlorophylls concentration. Besides, in order to improve the fucoxanthin recovery, the study of two different approaches using PLE and SFE was carried out.

2. Theoretical modelling of solubility parameters

2.1. Estimation of HSP at normal conditions by HSPiP software

Hansen solubility parameters are based on the concept that the total cohesive energy density is calculated by the sum of E_D, dispersion energy (related to the Van der Waals forces), E_P, polarity energy (related to dipole moment), and E_H, hydrogen bonding energy. Dividing this by the molar volume gives the square of the total (δ_T^2) solubility parameter as the sum of the squares of the Hansen (D, P, and H components), as defined by Eq. (1) [6]:

$$\delta_t(\mathrm{MPa}^{1/2}) = \sqrt{\delta_D^2 + \delta_P^2 + \delta_H^2} \quad (1)$$

HSPiP® software (Version 5.0, UK) offers to calculate HSPs by Yamamoto − Molecular Break (Y-MB) method, which estimates the parameters directly from the molecular structure in computational form employing a neural network (NN) technique and multiple regressions fits. By means of DIY (Do It Yourself) tool available in the software menu, the HSP of fucoxanthin (solute) was calculated. Once its chemical structure was transformed in its simplified molecular input line syntax (SMILES) notations (CC(=CC=CC=C(C)C=CC=C(C)C(=O)CC12C(CC(CC1(O2)C)O)(C)C)C=CC=C(C) C=

C=C3C(CC(CC3(C)O)OC(=O)C)(C)C), it was subsequently used for HSP calculation employing Y-MB method. After, from the solvent optimizer menu 4 bio-based solvents (d-limonene, ethyl lactate, ethyl acetate and ethanol) were selected employing the *Ra* term as criteria, which refers to the distance of a solvent from the center of the Hansen solubility sphere, given by Eq (2). The solvents were selected in ascending order of *Ra*, where the smaller *Ra* corresponds to the greater affinity between solute and solvent.

$$R_a = \sqrt{4(\delta_{Di}-\delta_{Dj})^2 + (\delta_{Pi}-\delta_{Pj})^2 + (\delta_{Hi}-\delta_{Hj})^2} \quad (2)$$

In the equation 2, subscript *i* refers to the solute and *j* refers to the solvent. To determine whether the bio-based solvent and the solute are miscible, the relative energy difference (RED) number can be calculated using Eq. (3).

$$\mathrm{RED} = R_a/R_o \quad (3)$$

Ro value refers to the radius of interaction of a Hansen solubility sphere; this value must be found experimentally and will be discussed in Section 3.3. As a general guideline: if RED < 1, the molecules are alike and will dissolve each other; if RED = 1, the system is right on the soluble/insoluble border and if RED > 1 the system will not dissolve.

2.2. Estimation of HSP at sub- and supercritical fluid conditions

For the pressurized conditions, Jayasri and Yaseen [33] method can evaluate the temperature dependence of the solute solubility parameter (since pressure does no exert a large influence on the properties of solid) employing Eq. (4), where *Tr* refers to the reduced temperature at room temperature (1) at a given sub- or supercritical temperature (2).

$$\delta_2 = \delta_1\left(\frac{1-T_{r2}}{1-T_{r1}}\right)^{0.34} \quad (4)$$

For this purpose, the estimation of critical data of fucoxanthin was carried out employing Marrero & Gani [34] group contribution method (third-order group), and the Yamamoto-Molecular Break method using its SMILES notation (HSPiP Version 5.0, UK) was employed for the evaluation of the molar volume. For the bio-based solvents, d-limonene, ethyl acetate, ethyl lactate and ethanol were assessed at subcritical conditions considering two temperatures levels, 40 and 100 °C, and keeping the pressure constant at 10.0 MPa. For supercritical (or CO_2-expanded liquid) conditions, CO_2 and mixtures of CO_2 + d-limonene, CO_2 + ethyl acetate, CO_2 + ethyl lactate and CO_2 + ethyl ethanol