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Influence of green solvent extraction on carotenoid yield from shrimp (*Pandalus borealis*) processing waste

Behnaz Razi Parjikolaei *, Rime Bahij El-Houri, Xavier C. Fretté, Knud Villy Christensen

Department of Chemical Engineering, Biotechnology and Environmental Technology, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark

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ABSTRACT

In this work, sunflower oil (SF) and methyl ester of sunflower oil (ME-SF) were introduced as two green solvents for extracting astaxanthin (ASX) from shrimp processing waste. The effects of temperature (25, 45, 70 °C), solvent to waste ratio (3, 6, 9), waste particle size (0.6 and 2.5 mm) and moisture content (0% and 86.8%) as well as stirrer speed (120, 200, 400 rpm) were studied during the process. In all the experiments, ASX yields from extraction with ME-SF were higher than with SF. For both solvents, the highest ASX content using a short extraction time was achieved at a temperature of 70 °C, a solvent to waste ratio of 9 and a stirrer speed of 400 rpm while the waste particle size and moisture content were 0.6 mm and 86.8%, respectively. ME-SF and SF seem promising solvents extracting respectively about 80% and 60% of the total ASX extracted by organic solvents.

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1. Introduction

World production of shrimp, captured and farmed, is about six million tons which makes it the most important internationally traded fishery commodity with an annual value of US$10 billion (Gillett, 2008). During the shrimp processing, depending on the species, size, and shelling procedure, about 40–50% of the raw material weight will be discarded as a non-edible part such as head, tail, and shell (Kandra et al., 2012; Sachindra and Mahendrakar, 2005). These residues may not be of direct value as food, but still contain valuable nutrients and functional compounds such as proteins, chitin, lipids, and carotenoid pigments. The main pigment present is astaxanthin (ASX). ASX with the estimated global market of about US$257 million (März, 2008) is a major carotenoid included in crustacean, salmonids, and other farmed fish feeds (Higuera-Ciapara et al., 2006; Sánchez-Camargo et al., 2011a). Since farmed aquatic animals do not have access to natural sources of ASX to provide the desirable reddish-orange color in these organisms, the total ASX intake must be derived from their feed. In addition to the pigmentation function, ASX has been associated with reduced risk of diseases in humans, such as age-related macular degeneration and ischemic diseases (Naguib, 2000). The effect is attributed to ASX's potent antioxidant activity which is reported to surpass 10 times other carotenoids i.e. β-carotene, lutein, zeaxanthin etc. (Miki, 1991; Naguib, 2000). Due to ASX's potential benefits to human and animals (Boonyaratpalin et al., 2001; Higuera-Ciapara et al., 2006) along with the world demand for natural ASX, shrimp waste carotenoid recently has received worldwide attention as a beneficial alternative to the synthetic carotenoid.

At present, shrimp residues are not utilized optimally, with some even being burned as bio-waste in power plants while recovery of the valuable components not only could improve the economy of the fishing industries but also minimize the pollution potential and environmental impact of shrimp processing. The absence of environmentally friendly, efficient and industrially viable technologies for extraction of the valuable ingredients is one of the main reasons that this biomass is not being used in an optimal manner. Conventional technologies such as extraction with organic solvents (Auerswald and Gäde, 2008; Sachindra et al., 2006; Yvonne, 2007), Soxhlet (Young and Smith, 1958), and ultrasound (Macias-Sanchez et al., 2009; Young and Smith, 1958) are expensive and inflexible and some valuable compounds may undergo a structural change leading to possible loss of functionality or nutritional deterioration. The extraction of ASX depends on the solvent used, its polarity, and the solubility of ASX in the extraction solvents. One promising alternative to traditional methods is using

Abbreviations: ASX, astaxanthin; d_i, is the nominal mesh of the ith sieve (mm); d_{i+1}, is the nominal mesh of the next larger sieve after the ith sieve (mm); HPLC, high performance liquid chromatography; ME-SF, methyl ester of sunflower oil; SF, sunflower oil; WM, wet waste material; w_i, is the mass of the material retained by the ith sieve (g).

* Corresponding author. Tel.: +45 5278 7757.
E-mail address: bep@kbm.sdu.dk (B. Razi Parjikolaei).

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edible oils which offers attractive advantages compared to the conventional methods due to the oil solubility of ASX (Higuera-Ciapara et al., 2006; Li et al., 2014; Li et al., 2013; Sachindra and Mahendrakar, 2005). Extraction using edible oils can fulfill almost all the principles as defined by Chemat et al. (2012) to be considered as a green process e.g. applying environmentally friendly solvents, reducing the energy consumption, and producing the non-denatured extract without contaminants. Further, the oil plays a barrier role against oxygen and consequently retards the oxidation time and degradation rate of the ASX extract (Pu et al., 2010). On the other hand, vegetable oils or fish oil commonly contribute as an energy source in aquaculture feed and the use of pigmented oil in feed formulation thus serves the dual purpose of pigment carrier as well as a source of lipid energy (Pu et al., 2011). Several studies have reported the recovery of ASX using vegetable oils from crustacean processing wastes such as shrimp (Pu et al., 2010; Sachindra and Mahendrakar, 2005; Shahidi and Synowiecki, 1991), snow crab (Shahidi and Synowiecki, 1991) and crawfish (Chen and Meyers, 1982; Omara-Alwala et al., 1985). Among common vegetable oils, SF seems a progressive solvent for extraction of carotenoid while its high viscosity causes low diffusivity and low extraction yield. In spite of the well-known advantages of vegetable oil extraction, such as reduced concern on environment issues, generally low yield compared to conventional methods is a drawback of this method. The reason for the low yield could be the lack of a comprehensive study on the effective extraction parameters (temperature, solvent to biomass ratio, biomass particle size and moisture etc.) and thus not achieving optimal operating condition. However, the high viscosity of the oil is a major problem which results in less diffusivity and consequently lower extraction yield even at higher temperatures. In recent years ultrasound assisted extraction of compounds with antioxidant activities has been widely applied to alleviate this problem (Achat et al., 2012; Li et al., 2013). However, Zhao et al. (2006) observed a negative effect of this process on ASX as they observed degradation of ASX into colorless compounds. Therefore, in order to effectively utilize the shrimp waste, there is a drive to find an alternative solvent which, while it is sustainable, would have lower viscosity and higher solubility for ASX and enhance the extraction yield. Recently, biodiesel has attracted a lot of attention as an alternative to fuel diesels as it is renewable, non-toxic and biodegradable (Hill et al., 2006; Soleimani et al., 2013) while to the best knowledge of the author, applying it as an alternative green solvent has not been reported. Thus, in this study the extractability of ASX by means of low viscous methyl ester of sunflower oil (ME-SF) and high viscous sunflower oil (SF) is investigated. ME-SF is a biodegradable solvent which has a lower viscosity compared to SF and consequently could enhance the diffusivity and solubility of ASX in the solvent.

The ASX content during 24 h extraction was followed at different temperatures (25, 45, and 70 °C), solvent to waste ratios (3, 6, and 9), particle size (0.6 and 2.5 mm), moisture content (86.7% and 0% of the total weight), and stirrer speed (120, 200, and 400 rpm). The results are compared to traditional organic solvent extraction.

2. Materials and methods

2.1. Raw material

The waste residue contained northern shrimp (*Pandalus borealis*) processing waste including heads, shells, and tails. The residue was provided by Launis Fiskekonserves A/S (Aalbæk, Denmark) and stored at −20 °C prior to experiments. Moisture content was determined by weighing the difference in mass before and after the freeze drying FD4 (Holm & Halby, Brøndby, Denmark) process and expressed in grams per 100 g of total material. All batches were homogenized using a laboratory grinder GM 300 (Retch, Haan, Germany) and then separated by vibrating sieves (10 mm < id > 0.02 mm) (Retsch, Haan, Germany). The dried ground and not ground shrimp particles presented a mean diameter (d_m) of 0.6 and 2.5 mm, respectively, calculated by the ASAE S319.3 method (ASAE S319.3) as from Eq. (1):

$$d_m = \log^{-1}\left[\frac{\sum_{i=1}^{n}(w_i \log(d_i \times d_{i+1})^{\frac{1}{2}})}{\sum_{i=1}^{n}(w_i)}\right] \quad (1)$$

2.2. Traditional solvent extraction

A traditional solvent extraction was applied based on the method optimized by Sachindra et al. (2006) and used by Mezzomo et al. (2013) and Sánchez-Camargo et al. (2011b). Five grams of the wet material sample was weighed and repeatedly extracted using 25 mL of mixed hexane and isopropanol (Hex: IPA, 60:40 v/v) (VWR Prolabo, Herlev, Denmark) until no further pigment was extracted by the solvent (four times). The viscosity of Hex:IPA (60:40 v/v) was 0.88 cp at 20 °C. In order to separate the phases and remove traces of IPA in each step, the extract was washed with an equal amount (100 mL in total) of 0.1% NaCl solution. The supernatant was collected and evaporated under vacuum at 35 °C using a rotary evaporator R-210 (Buchi, Flawil, Switzerland). The resulting carotenoid concentrate was re-dissolved in 4 mL of acetone (AC) before analysis on a spectrophotometer DR 3900 (Hatch, Düsseldorf, Germany). All experiments were performed in dim light.

2.3. SF and ME-SF extraction

SF (refined, Dansk Supermarked A/S, Denmark) was selected based on the carotenoid yields obtained by Sachindra and Mahendrakar (2005) and Mezzomo et al. (2013) who found the highest yield using refined sunflower oil compared to other common vegetable oils i.e. groundnut oil, gingerly oil, mustard oil, soy oil etc.

ME-SF was prepared by transesterification of SF carried out in a closed system with mixing at an agitation speed of 900 rpm for 90 min. Methanol was added in the molar ratio of 6:1 to SF at 60 °C in the presence of sodium hydroxide as a catalyst (1% w/w) (Rashid et al., 2008; Soleimani et al., 2013). ME-SF was separated from glycerol and catalyst using a separating funnel. After the transesterification process, the viscosity of ME-SF and SF was measured to be 6.61 and 60.5 cp respectively at 20 °C (Brookfield DV-II, Stuttgart, Germany).

For practical reasons the study of the effect of different parameters on ASX extraction by SF and ME-SF solvents had to be divided into two sets of experiments using two different batches of shrimp waste residue (Fig. 1). Using batch I, ten grams of the wet homogenized sample was used and experiments were carried out by adding SF and ME-SF in three different ratios to shrimp waste: 3, 6, and 9 at temperatures of 25, 45, and 70 °C. The sample was mixed by mechanical stirrer RZR 2050 (Heidolph, Schwabach, Germany) 200 rpm for 24 h. ASX contents were presented relative to the ASX amount extracted by the organic solvent mixture of Hex:IPA (41.1 mg/kg of wet waste material (WM)) (Fig. 2).

Based on the results obtained with batch I the optimal operating condition and extraction time for SF and ME-SF were chosen. In the second set of experiments, using batch II, the effect of shrimp waste particle size and moisture content as well as stirrer speed on ASX content were investigated (Fig. 1). The experiments were carried out with an extraction time of 3 h while the temper-

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