

Northern Shrimp (*Pandalus borealis*) Processing Waste: Effect of Supercritical Fluid Extraction Technique on Carotenoid Extract Concentration

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Huge amount of shrimp waste are produced annually in Denmark. This is a natural source of carotenoids and particularly astaxanthin (ASX). In this study, supercritical fluid extraction (SCFE) was used as a green technique and the effects of pressure (200, 300, and 400 bar), temperature (35, 45, and 55 °C), and three green co-solvents (ethanol (EtOH), sunflower oil (SF), and its methyl ester) on ASX yield and total extract yield were investigated. The results showed that high temperature and pressure had a positive influence on ASX yield and total extract yield. Conducting the process at 400 bars and a temperature of 55 °C resulted in ASX yield of about 23 mg/ kg of dried shrimp waste (DW). This amount is comparable to the ASX extraction yield obtained by the conventional organic solvents; however, the total extract yield was significantly lower. Adding 5 % EtOH increased the ASX concentration in the extract almost twice, while the total extract yield did not changed significantly. In addition, by using SF and its methyl ester as alternative co-solvents ASX yields of about 25 and 35 mg/ kg of DW, respectively, were achieved.

1. Introduction

Global shrimp production, captured and cultivated, was estimated to about six million tons with a value of nearly US\$ 10·10⁹ (EL-Qudah, 2008). Among different identified species, Northern shrimp (*Pandalus borealis*) is the most common species found in cold parts of the Atlantic and Pacific Oceans and counts for 31,5511 t (FAO, 2012). In Denmark fishing of *P. borealis* has developed into an international fishery yielding up to 12500 tons annually (Skúladóttir, 1997). Further, Denmark is an intermediate for final processing of the harvests from different countries. In 2009, more than 79,000 t of shrimp were imported to Denmark mainly from Greenland and Canada (www.globefish.org).

During the shrimp processing, depending on the species, size, and shelling procedure, about 40 to 50 % of the raw material weight will be discarded as non-edible parts e.g. head, tail, and shell. These residues still contain valuable nutrients and functional compounds such as ASX (Figure 1). About 90 % of the ASX is produced by chemical synthesis and has an estimated global market of about US\$ 257 10⁶ (März, 2008). It is used as feed additive for salmon and other farmed fish feeds, for human consumption, as well as in cosmetics. Therefore, recovery of this natural component can improve the economy of the fishing industries, and minimize the pollution potential and environmental impact made by shrimp residue.

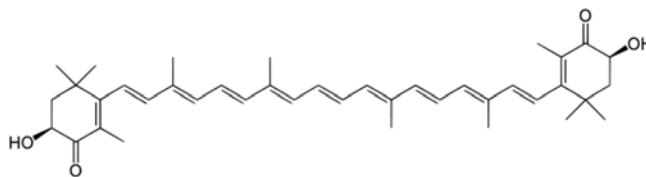


Figure 1: Chemical structure of astaxanthin

Conventional technologies by means of organic solvents are expensive, inflexible, potentially harmful, and time consuming because of required multiple extraction steps. Moreover, applying high temperature especially in the evaporation/concentration step for recovery can have deleterious effects on the structure and functional activities of bioactive compounds. Recent government restrictions on the use of organic solvents combined with the simultaneous increase of consumer demand for natural products has led to a growing interest in applying more environmentally friendly techniques.

Supercritical CO₂ extraction (SC-CO₂) is a promising alternative separation technique in the field of food (Straccia et al., 2012) and nutraceutical applications (Casas et al., 2009). SC-CO₂ is a Generally Recognized As Safe (GRAS) solvent type, cheap and easy to separate from the extract. It can selectively extract desired compounds without leaving toxic residues in the extracts and reduce the risk of degradation of thermo sensitive or easily oxidized compounds like carotenoids. Some researchers have used supercritical fluids successfully in the extraction of carotenoids from different crustaceans waste. For instance, Heu et al. (2003) applied this technique for extraction of ASX from red spotted shrimp (*Farfantepenaeus paulensis*) waste and obtained 20.7 mg of ASX/ kg of freeze-dried waste, accounting for about 40 % of total ASX extracted by organic solvents. The main drawback of using SC-CO₂ is its low polarity, which makes SC-CO₂ less effective when extracting total carotenoids from biomass. This problem can be overcome by employing polar modifiers or co-solvents to change the polarity of the supercritical fluid and consequently increase its solvating power to interact with desired solute. EtOH is the most common organic solvent applied as a modifier (Floreto et al., 2000). However, few studies have described the effect of different non-organic green solvents on ASX extraction particularly from industrial processing residues.

In this study, an experimental laboratory set-up has been used to investigate the effects of pressure (200, 300, and 400 bar), temperature (35, 45, and 55 °C), and different green co-solvents (EtOH, sunflower oil, and methyl ester of sunflower oil (ME-SF)) on super critical fluid extraction of carotenoids from shrimp processing waste. The results are discussed using traditional organic solvent extraction for comparison.

2. Materials and Methods

2.1 Raw material

The waste residue contained Northern shrimp (*P. 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. Samples were dried by means of a tray dryer (Armfield, United Kingdom) at an air temperature and velocity of 40 °C and 1.2 m/s, respectively. The samples were homogenized to maintain the constant particle size (about 600 µm) in all the experiments. The powder samples were stored in darkness until the extraction procedure was carried out.

2.2 Solvent extraction

A traditional solvent extraction was applied based on the method optimized by Sachindra et al. (2006). Five grams of the dried sample were weighed and extracted using 25 mL of mixed hexane and isopropanol (Hex: IPA, 60:40 v/v) (VWR Prolabo, Herlev, Denmark). The extraction was repeated four times until no further pigment was extracted by the solvent. In each step the extract was washed with an equal amount (100 mL in total) of 0.1 % NaCl solution in order to separate the phases and remove traces of IPA. 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 Supercritical fluid extraction

Extraction process was carried out in an apparatus supplied by Thar Technology Inc. (Pittsburgh, PA, USA, model SF100), represented schematically in Figure 2. The apparatus included: a reservoir for pressurized CO₂ (99.9 % purity; Abello-Linde, Spain), a thermostatic bath kept at 5 °C, a 100 mL extraction vessel equipped with a thermostatic jacket, two pumps with the maximum flow rate of 50 g/min (one for CO₂ and another for co-solvent), a backpressure regulator valve, and a cyclonic separator to allow periodic discharge of the carotenoids extracted during the process. All parts of the extractor were automatically controlled by computer which allowed a completely stable process for the operating conditions selected. Extract collected in the cyclonic separator were washed by AC, and stored in darkness at -20 °C prior to subsequent analysis. The total extract yield was calculated based on the ratio between total mass of extract and mass of dried sample.

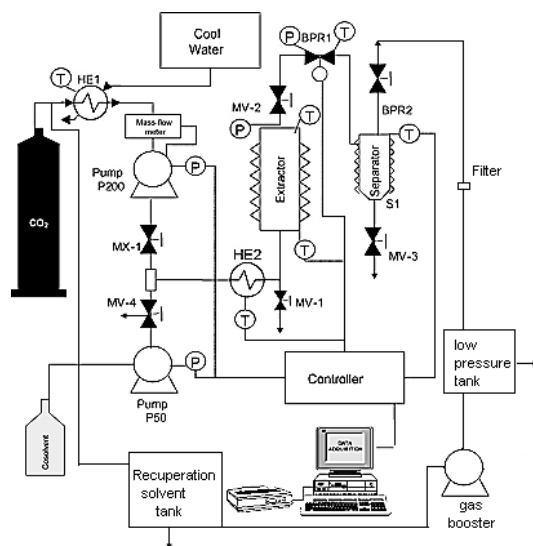


Figure 2: Schematic diagram of supercritical fluid extraction apparatus

In this study, parameters studied were pressure (200 to 400 bar), temperature (35 to 55 °C), and co-solvent (EtOH, SF, and methyl ester of sunflower oil (ME-SF)), at percentage of 0 and 5 %. By considering the economic viability of the process as well as the possible thermal degradation of carotenoids, especially in industrial scale, it is not recommended to increase the pressure and temperature beyond the range studied. At first, all the experiments were carried out with only SC-CO₂ at a flow rate of 20 g/ min for three hours. Afterwards, at the operating conditions giving the highest yields, the effect of different green co-solvents on the SCFE of carotenoids was studied. Finally the results were compared with conventional organic extraction using Hex: IPA, 60:40 v/v.

2.4 Sunflower oil and Methyl ester of sunflower oil

SF (refined, Dansk Supermarked A/S, Denmark) was selected based on the carotenoid yields obtained by Sachindra and Mahendrakar (2005) as well as Mezzomo et al. (2013). 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 minutes. 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). 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)

2.5 Quantification of carotenoids

ASX and its esters are identified as the major carotenoids occurring in the shrimp waste (Mezzomo et al., 2013) and hence the term ASX used in the present work refers to the total carotenoids. A standard curve of ASX (Chiron, Trondheim, Norway) in each solvent (SF, ME-SF, and AC) was determined spectrophotometrically by measuring the absorbance spectra at absorbance maxima (λ_{max}) between 400 and 600 nm, against the particular solvent as blank. Carotenoid contents in the different solvents were measured using the λ_{max} and standard curves and expressed as mg of ASX per kg of DW.

2.6 Statistical analysis

All the experiments were repeated two times and values were expressed as the mean \pm the standard error (SE). Two-way analysis of variance (ANOVA) was used for testing the significance of the differences of the studied parameters at the 95 % confidence level except when stated otherwise.

3. Results and Discussion

3.1 Supercritical fluid extraction without co-solvent

As shown in Table 1, at the operating temperatures studied, increasing the pressure had a significant positive effect on ASX yield ($p < 0.1$). This behaviour can be attributed to an increase in the fluid density with pressure, which consequently favours the solubility of the products in SC-CO₂ (Macías-Sánchez et al., 2008). For instance, at 55 °C, by changing the pressure from 200 to 400 bars the ASX yield increased from 3.5 to 23.2 mg/ kg of dry weight (DW).

Additionally, except at low pressure, increasing the temperature had also a positive effect on the ASX yield, albeit not in a significant way (Table 1). The temperature has two different effects on extraction yield. By increasing the temperature the density and consequently solvating power of SC-CO₂ decrease while the solute vapour pressure increases favouring the solubility in SC-CO₂. Therefore, according to the operating condition, one effect would be dominant over the other. For instance, as seen in Table 1, increasing the temperature when the experiments were carried out at the lowest pressure of 200 bars resulted in reduction in ASX yield from around 12 to 3.5 mg/ kg of DW. Macías-Sánchez et al. (2008) also observed a decrease in the carotenoid content of a microalgae strain at the lowest applied pressure of 200 bars when the temperature was 60 °C. This reduction is due to the low density of SC-CO₂ at 200 bars and thus lower solvating power. However, after increasing the pressure to 300 and 400 bars, the density of the solvent increases and can compensate the reduced density of the solvent at temperature of 55 °C. Therefore, ASX yield increases.

Furthermore, a significant positive effect of temperature and pressure on total extract yield was observed. Total extract yield with pure SC-CO₂ ranged between 0.8 and 3.2 %. Thus, 55 °C and 400 bars were chosen as the best operating conditions using SCFE with pure CO₂. Under these conditions the maximum ASX yield and total extract yield obtained were around 23 mg/ kg of DW and 3.2 %, respectively.

Comparing the ASX concentration with the amount extracted by organic solvents it can be concluded that applying SC-CO₂ was as efficient as conventional organic solvents (Table 1). Nevertheless, the total extract yield was reduced 4.5 times extracting by SC-CO₂. The solubility of polar solutes, such as ASX in SC-CO₂ is very low and therefore adding an organic co-solvent may increase the solvent power of CO₂ and consequently the ASX concentration and total extract yield (Díaz-Reinoso et al., 2006).

Table 1: Astaxanthin yield and total extract yield obtained during supercritical fluid extraction process at 20 g/ min CO₂ flow rate for 180 min

Solvent	Pressure (bar)	Temperature (°C)	Astaxanthin yield mg/ kg of DW)	Total extract yield (%)
SC-CO ₂	200	35	11.9 \pm 1.4	1.5
		45	5.8 \pm 0.3	0.8
		55	3.5 \pm 0.2	1.8
	300	35	13.0 \pm 3.6	2.1
		45	11.7 \pm 4.8	1.5
		55	16.7 \pm 3.2	2.5
	400	35	13.4 \pm 0.8	1.9
		45	20.7 \pm 1.9	2.0
		55	23.2 \pm 0.5	3.2
Hex: IPA (60:40 v/v)			22.4	14.4

DW: dry weight

3.2 Supercritical fluid extraction with co-solvent

In this study, considerable amounts of ASX (51 mg/ kg of DW) were extracted at 55 °C and 400 bar by adding 5 % EtOH as a modifier during the SCFE process (Figure 3). This amount is nearly twice compared to the ASX extracted by the organic solvent. However, the total extract yield achieved was 4 % (data not shown).

The alcohol co-solvent induces dipole-dipole interaction and makes a hydrogen bond with polar functional groups of the solutes. Further adding alcohol such as EtOH can increase the solubility of polar solutes by breaking the polar interaction between the solute and solid matrix. Similar behavior was reported by Floreto et al. (2000) who observed higher ASX productivity in the presence of ethanol (20 %) acting as a co-solvent especially at their highest selected temperature of 50 °C.

Due to the oil solubility of ASX, one promising co-solvent in SCFE processes is edible oil which offers attractive advantages. Apart from being environmentally friendly, oil plays a barrier role against oxygen and consequently retards the oxidation time and degradation rate of the ASX extract (Pu et al., 2011). In the present work, by applying 5 % of SF, no significant increase was observed in ASX yield (25.4 mg/ kg DW) (Figure 3). Nevertheless, it is worth mentioning that the final product can be contributed as an energy source in aquaculture feed serving the dual purpose of pigment carrier as well as a source of lipid energy (Pu et al., 2011). Mezzomo et al. (2013) used 2 and 5 % of SF as a co-solvent and observed a reduction in extracted ASX content compared to using SC-CO₂ alone. On the contrary, Sun and Temelli (2006) found the canola oil as an effective co-solvent in SCFE of carotenoids from carrot. They hypothesized that by penetrating the oil into the plant material matrix, its cellular structure may swell and thus the ability of SC-CO₂ to diffuse in the matrix would be facilitated.

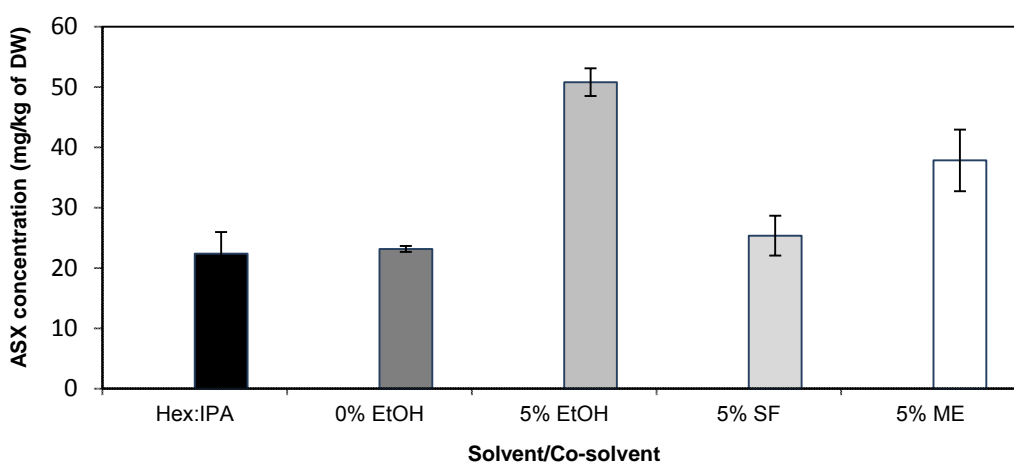


Figure 3: Astaxanthin yield (mg/ kg of dried shrimp waste (DW)) by means of organic solvents and using different co-solvents (under 400 bars, 55°C, flow rate of 20 g/ min for 180 min)

The high viscosity of SF is responsible for low diffusivity and consequently lower extraction yield. To overcome this problem and effectively utilize the shrimp waste, 5 % ME-SF was added. ME-SF is a non-toxic and biodegradable solvent which has lower viscosity (10 times less than SF in the present work). Our previous experiments using ME-SF as a green solvent also showed that under the optimized operating conditions this solvent can extract 80 % of the total ASX extracted by organic solvent (Razi Parjikolaie et al., 2015). As shown in Figure 3, in the present study, by using ME-SF as a co-solvent the ASX yield enhanced to approximately 38 mg/ kg of DW. Considering the recent concern about environmental issues and increasing demand for natural products, SCFE seems an efficient technique for extracting of carotenoids from natural biomass. Using the investigated co-solvents can also improve the ASX yield extracted from shrimp processing wastes. Further, extraction products rich in ASX can be used either directly or as supplementary in human and animal diets.

4. Conclusion

The analysis of the shrimp processing waste has proved this to be a potential source of carotenoids. The extraction of astaxanthin from shrimp waste was investigated using supercritical fluid extraction process and compared with conventional organic solvents method. The optimal conditions regarding the extracted astaxanthin yield and total extract yield were a pressure of 400 bars and 55°C. Under these conditions, supercritical fluid extraction with pure CO₂ gave an ASX yield comparable to that obtained when using a mixture of non-polar/polar solvents (hexane/isopropanol). However, the total extract yield was reduced 4.5 times, from 14.4 to 3.2 %. It was shown that the addition of 5 % ethanol had a significant effect on increasing the extraction of astaxanthin (50.8 mg/ kg of dried shrimp waste), while the total extract yield did not change significantly. Another green co-solvent used in this study was sunflower oil, which extracted the astaxanthin to

the same extent as pure CO₂ and/or organic solvents. However, sunflower oil has commercial potential, where the carotenoid-rich vegetable oil can be used directly in diets of fish and salmon. Methyl ester of sunflower oil on the other hand could extract more ASX, nearly 35 mg/ kg of dried shrimp waste. This makes it a potential green co-solvent replacing the currently used co-solvent during supercritical fluid extraction processes.

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