Manual for astaxanthin production from locally sourced red prawn residues

Extraction procedures

Abstract

Bythos has produced a manual for the extraction of astaxanthin from red prawn heads and shells – residues sourced from a local sea food processing plant

STEBICEF – Dipartimento di scienze e tecnologie biologiche, chimiche e farmaceutiche dell'Università di Palermo

December 2020

Dipartimento STEBICEF Sez. Biologia animale

Via Archirafi, 18 90123 – Palermo Italia – Tel. +39 091 23891804

www.BythosEU.com @BythosEU Page: BythosEU bythoseu

Manual for Astaxanthin extraction from locally sourced red shrimp waste

1. Preface

It is known in literature that crustacean waste is rich in a variety of compounds that have a high value in various industrial applications. Examples of these compounds are chitin, astaxanthin, proteins (or enzymes), lipids (or oils) e some minerals (Auerswald & Gäde, 2005, 2008; Küçükgülmez, 2018; Taher et al., 2019).

Among these, astaxanthin has played a very important role in various applications and researches. It is a carotenoid; it is the main pigment found in many aquatic animals and cannot be synthesized by animals but must be obtained through food (Britton 1995). It has several essential properties and its lack in the diet can lead for example to external discoloration of crustaceans (Howell and Matthews 1991) or can influence the pigmentation in fish (Wathne et al., 1998). In its unsaturated form, is bound to a carotenoprotein named crustacyanin (which prevents the oxidation of astaxanthin, Ciaci et al., 2002), clean free radicals and for this reason is of interest for several pharmacological applications (e.g. anti-cancer agent, treatment for Alzheimer's disease and Parkinson's disease, skin photo-protector).

In Sicily the red shrimp is commercially the most important crustacean for which a considerable amount of waste is produced during the year. For this reason, it would become very important to give a value and a valid utility to the considerable amount of waste obtained from the "fish food" industries. In a Blue Economy perspective this will lead to the production of important compounds in both human and animal fields (Auerswald and Gäde, 2008) with the indispensable reduction in the quantity of waste produced and in the economic costs to dispose of it.

2. Materials and methods

2.1 Sample preparation

The astaxanthin extractions were carried out on the waste (heads) of red shrimp of Mazara del Vallo. The waste used in particular was obtained from a manufacturing company in the sector.

After having carried out the normal processing of the shrimp, the manufacturing company collected the heads which were transported to the Stebicef Department of the University of Palermo and kept at -20 ° C until the moment of analysis.

Before the astaxanthin extraction the heads had to be processed to obtain the exoskeleton flour.

In particular 5 heads (with organic part have a weight of 10.06 g ± 1.68) must be placed to thaw at room temperature.

After defrosting, the heads were cleaned of organic elements to obtain only the exoskeleton (Figure 1), the organic material will be scraped off with using a scalpel and the exoskeletons were cleaned using running water or distilled water.

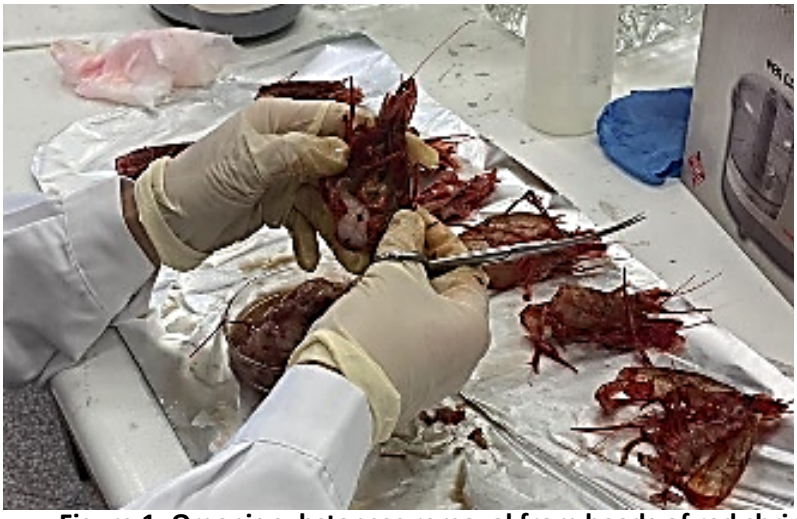

Figure 1: Organic substances removal from heads of red shrimp.

Then, the exoskeletons were rinsed in distilled water once and left to dry in a stove for 2 nights at 60 ° C (Figure 2).

2.2 Astaxanthin extraction

To obtain astaxanthin firstly the exoskeletons were grinding to pulverized them. I detail, the powder was obtained crushing dried exoskeleton with a mortar and subsequently with electric coffee grinders.

The correct size of the flour grains must not exceed 250-300 µ. In these conditions the powder was suitable for the extraction of astaxanthin, and to evaluate its size it was enough to try to sift it using a flour sieve.

Astaxanthin was extracted using the modified Auerswald & Gäde, 2005 method's by vortexing 200 mg of frozen fine-powdered in 1.5 ml of methanol or ethanol. Subsequently the sample was sonicated on ice for 2 × 20 seconds. Thereafter, the homogenate was centrifuged at 4 000g for 10 minutes at room temperature. The supernatant was transferred into a fresh Eppendorf to analyse the astaxanthin content. Normally the methanol is the better solution to extract this carotenoid because the yield of astaxanthin achieved with this solvent is highest (Auerswald & Gäde, 2005). The supernatant obtained was bright orange (Figure 3). Sample preparation should be done immediately prior to assessment of astaxanthin levels.

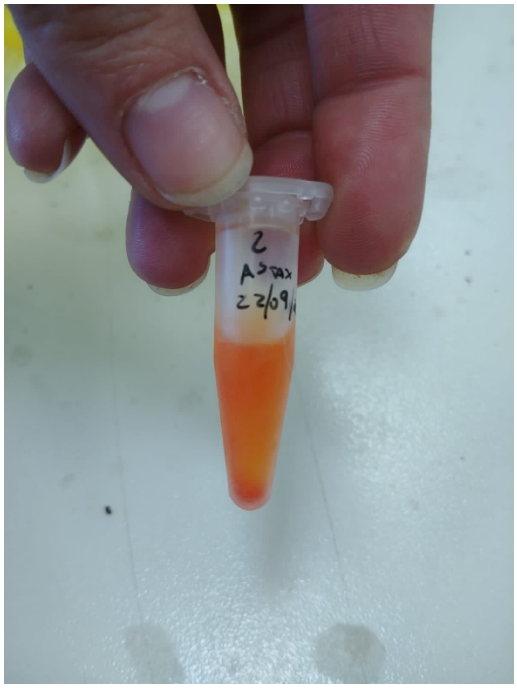

Figure 3: The bright orange supernatant obtained.

2.3 Astaxanthin determination by UHPLC-HESI-MS

Identification and quantification of astaxanthin were carried out by ultra-high performance liquid chromatography, heated electrospray and mass spectrometry (UHPLC-HESI-MS). UHPLC analysis was performed using a Dionex Ultimate 3000 System (Dionex Softron GmbH, Germering, Germany) equipped with an autosampler controlled by Chromeleon 7.2 software.

A UHPLC column (Phenomenex Luna C18(2) 150 x 2 mm, 5 µm) was set for separation of the selected compounds at 20 °C and the injection volume at 1 µl. The mobile phases used were 0.1% formic acid in water (A) and methanol (B). The gradient elution programme was as follows: 0–5 min 90% B; 5–12 min linear increase to 100% B, 12–25 min 100% B, 25-27 min 90% B and 27-30 min coming back to the initial conditions until full stabilisation.

The flow rate was 200µl min-1. MS detection was performed using a Q-Exactive accurate-mass spectrometer (Thermo Scientific, Bremen, Germany). The HESI parameters were set using positive ion mode with spectra acquired over a mass range from 200 to 2000 m/z. The optimum values of HESI-MS parameters were as

follows: auxiliary gas unit flow rate at 15 arbitrary units; capillary temperature at 320 °C; auxiliary gas heater temperature at 150 °C; spray voltage at 4 kV and S lens RF level at 50%. The automatic gain control was set to a maximum injection time of 200 ms. Positive HESI-MS spectra yield the singly protonated ion $[M+H]^+$ 597.39384 m/z ($C_{40}H_{52}O_4$). The total UHPLC-HESI-MS method runtime was 30 min. Detection was based on calculated exact mass and on retention time of astaxanthin reference standard (purity>=97% HPLC, Sigma Aldrich). The detection was evaluated by Quan/Qual browser Xcalibur 3.0 (Thermo Fisher Scientific, San Jose, CA). Linearity of the MS response was verified with solutions containing astaxanthin at four different concentration levels from 0.4 to 1 ppm. Each point of the calibration graph corresponded to the average of five independent injections.

3. Economic expense plan

On internet it is possible buy 42 mg of pure astaxanthin for an expense between 20 and 30 euros.
In this protocol, on average, about 6 grams of head powder are obtained from 10 gr of heads.

Using 200 mg of powder were obtained 0.95 ppm (mg kg^{-1}) of astaxanthin (the approximately economic expense for one sample in laboratory conditions are resumed in Table 1). Then, using 6 gr of red shrimp powder it is possible to obtain 28.5 mg of astaxanthin.

The cost for the disposal of fish waste is on average ≃5,00 euros per kilo.

Table 1. Description of economic expense plan in laboratory conditions

Materials	Product Price €	Quantity of materials utilized	Product utilized €
Exoskeleton powder	0,00	200 mg (1 sample)	0,00
Methanol or Ethanol (5 L)	37,00	1,5 ml	0,01
Distilled Water (5L)	2,00	500 ml	0,20
Gloves (100 pz)	15,90	2 pz	0,32
	Consumption kWh	**Time of utilization**	
Energy 0,05 € /kWh			

Centrifuge	1650 W	10 min	≃0,025
Coffee grinders	300W	2 minutes	0,002
Stove *	2000W	≃ 50 h	5,00
Vortex	15W	10 sec	<0,0002
Sonicator	1800W	20 sec	<0,0007
Chemical hood	150 W	2 min	0,00002
Work man**	5,00 for hour	1h	5,00
Total cost			

*It is possible delete this instrument because is possible dry the sample at room temperature.

** In the same time interval, it is possible to obtain up to 20 times more astaxanthin by processing multiple samples.

Regarding the table: in the "Materials" column is reported the material used for the experimental steps described in this manual. In the "Product price" column the corresponding price of the product available on the internet or in commercial market (including shipping fees). In the "Product utilized" column, the price corresponding to the quantity of product used in this manual.

In this economic prevision the laboratory instruments used such as Eppendorf, pipettes and other laboratory instruments are not mentioned since they can be replaced by the industry with other suitable materials resistant to temperatures and chemical treatments.

5. Acknowledgements

We would like to thank the red prawn processing company Rosso di Mazara for their kind collaboration in supplying the red prawn residues for the production of this manual.

5.References

Auerswald, L., Gäde, G. 2005. The West Coast rock lobster *Jasus lalandii* as a valuable source for chitin and astaxanthin. African J. Mar. Sci. 27:1, 257-264.

Auerswald, L., Gäde, G. 2008. Simultaneous extraction of chitin and astaxanthin from waste of lobster *Jasus lalandii*, and use of astaxanthin as an aquacultural feed additive. African J. Mar. Sci. 30:1, 34-44.

Britton, G. 1995. Structure and properties of carotenoids in relation to function. FASEB J. 9: 1551–1558.

Ciaci, M., Rizkallah, P. J., Olczak, A., Raftery, J., Chayen, N. E., Zagalsky, P. F. and J. R. Helliwell. 2002. The molecular basis of the coloration mechanism in lobster shell: b- Crustacyanin at 3.2 Å resolution. Proc. Natl. Acad. Sci. U.S.A. 99: 9795–9800.

Küçükgülmez, A. 2018. Extraction of Chitin from Crayfish (*Astacus leptodactylus*) Shell Waste. Alınteri J. Agricult. Sci. 2018, 33(1): 99-104

Howell, B. K. and Matthews A. D. 1991. The carotenoids of wild and blue disease affected farmed tiger shrimp (*Penaeus monodon*, Fabricus). Comp. Biochem. Physiol. 98B: 375–379

Taher, F.A., Ibrahim, S.A., El-Aziz, A.A., Abou El-Nour, M.F., El-Sheikh, M.A., El-Husseiny, N., Mohamed, M.M. 2019. Anti-proliferative effect of chitosan nanoparticles (extracted from crayfish *Procambarus clarkii*, Crustacea: Cambaridae) against MDA-MB-231 and SK-BR-3 human breast cancer cell lines. Int. J. Biol. Macromol. 126: 478–487.

Wathne, E., Bjerkeng, B., Storebakken, T., Vassvik, V., Odland, A.B. 1998. Pigmentation of Atlantic salmon (*Salmo salar*) fed astaxanthin in all meals or in alternating meals. Aquaculture 159, 217-231.