

## EXTRACTION OF BIOACTIVE COMPOUNDS FROM SHRIMP WASTE

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### RÉSUMÉ

Les déchets du conditionnement industriel des crevettes représentent une bonne source de protéines et de caroténoïdes. L'hydrolyse enzymatique des déchets de crevettes est considérée comme une méthode alternative pour récupérer différents composés bioactifs. Nous avons évalué le degré d'hydrolyse suite au traitement des déchets de crevettes exploités par trois protéases commerciales, Protamex, Flavourzyme et l'Alcalase. En outre, à partir des déchets séchés de crevettes nous avons récupéré l'astaxanthine en utilisant des solvants organiques et le CO<sub>2</sub> supercritique. Le rendement d'extraction par les deux méthodes employées (µg/g) a été comparé par une analyse spectrophotométrique. Parmi les protéases utilisées dans l'hydrolyse enzymatique, le DH (%) le plus élevé a été obtenu par Protamex. L'extraction par le mélange Hexane/isopropanol s'est révélée comme le meilleur solvant pour l'extraction d'astaxanthine.

### ABSTRACT

Industrial shrimp waste is a promising source of protein and carotenoids. The enzymatic hydrolysis of shrimp waste is considered to be an alternative process to recover biological valuable components. We evaluated the degree of hydrolysis following the hydrolysis of shrimp waste carried on by three commercial proteases, Protamex, Flavourzyme and Alcalase.

Furthermore we recovered astaxanthin from dried shrimp waste, using organic solvents and supercritical CO<sub>2</sub>. The extraction yield (µg/g) between the two employed method is here compared via a spectrophotometric analysis. Between the employed proteases, Protamex had the highest DH%. Hexane/isopropanol resulted to be the best solvent for astaxanthin extraction.

### INTRODUCTION

The significant increase of waste materials from industrial processing of fisheries products, is a problem both for environmental sustainability and for companies which are subjected to elevate costs for the disposal of such materials.

Shrimp waste, mainly exoskeleton and cephalothorax, accounts to the 50-70% of the shrimp weight. It is known to contain high biological value components such as protein, chitin and astaxanthin. The amount of these components is related to the species and conditions of processing (Duarte de Holanda & Netto, 2006).

Astaxanthin is employed as a food dye, and in recent years has found wide application in aquaculture and in pharmaceutical and nutraceutical fields (Radzali et al., 2014).

The recovery of the protein fraction by enzymatic hydrolysis from the shrimp waste is widely studied. The hydrolyzed protein may be used as supplement in aquaculture feeds, and moreover may constitute a source of biologically active peptides with

considerable economical potential applications (Duarte de Holanda & Netto, 2006).

### MATERIALS AND METHODS

Deep-water rose shrimp (*Parapenaeus longirostris*) waste (exoskeleton and cephalothorax) were thawed and minced, then dried at 45°C for 40 hours (Figure 1). Two different matrices were obtained: fresh homogenized and dried matrix.

The enzymatic hydrolysis reaction was carried on employing three different commercial proteases, Protamex, Flavourzyme and Alcalase. The degree of hydrolysis (DH%) of each enzyme was determined directly at intervals within 30 minutes (Dumay et al., 2006). The hydrolyzed fractions were recovered and freeze-dried.

Following different trials with organic solvents (Lopez et al., 2004; Sachindra et al., 2006; Paul et al., 2014), the astaxanthin was extracted from the dried matrix with a mixture of hexane/isopropanol 3:2 (v/v). A protocol of extraction by Supercritical Fluid Extraction (SFE) was defined (Figure 2).

The Astaxanthin extracted was quantified spectrophotometrically ( $\mu\text{g/g}$ ) at a wavelength of 468

nm, utilizing the equation shown by Simpson & Haard (1985).



Figure 1 - Steps of processing shrimp waste.



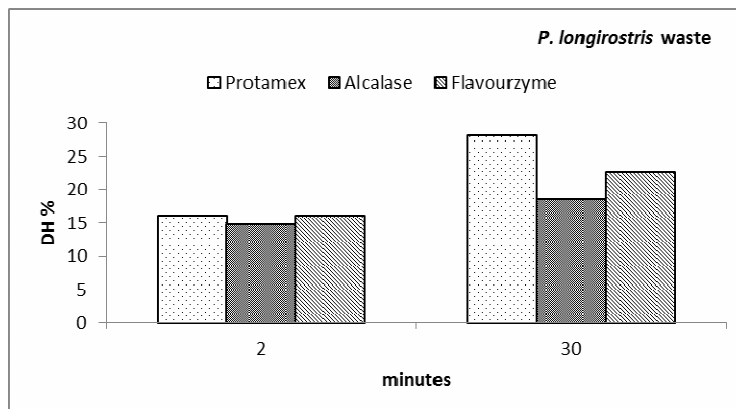
Figure 2 - Innovative technologies used in the astaxanthin extraction from shrimp waste (*P. longirostris*): SFE (Supercritical Fluid Extraction) plant.

## RESULTS

The results for the enzymatic hydrolysis reaction on fresh homogenate waste shrimp show that the maximum values for DH% were reached by using Protamex.

The spectrophotometric quantification reveals that the highest amount of astaxanthin was obtained from the

dried matrix ( $17.50 \pm 0.55 \mu\text{g/g}$ ) with the mixture of hexane/isopropanol. The extraction by SFE resulted in a lower yield ( $6.76 \pm 0.71 \mu\text{g/g}$ ), nevertheless this technique allows to obtain extracts free of any hazard caused by the use of traditional toxic solvents. Hence it results qualitatively suitable for use in nutraceutical, pharmaceutical and cosmetic industry



**Figure 3** – Degree of hydrolysis (%) for fresh homogenate waste shrimp obtained by using Protamex, Alcalase and Flavourzyme.

## DISCUSSION

The degree of hydrolysis obtained with Alcalase is similar to the one reported by Duarte de Holanda & Netto (2006) although the use of Protamex and Flavourzyme resulted in more efficient hydrolysis.

The results on the astaxanthin extraction, with hexane/isopropanol, from dried shrimp waste evidenced a lower yield if compared with similar studies (Sachindra et al., 2006; Paul et al., 2014). On the other hand, the astaxanthin content in SFE extract is within the range reported in the literature (2-20 µg/g) (Sánchez-Camargo et al., 2011; Radzali et al., 2014).

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