Evaluation of different disruption processes efficiency on encysted *Haematococcus pluvialis* cells for astaxanthin recovery and bioavailability

M M Mendes-Pinto[1], M F de J-Raposo[1], J Bowen[2], A Young[2] and R Morais[1]

1- Escola Superior de Biotecnologia - Universidade Católica Portuguesa

Rua Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal

2- John Moores University, Liverpool L3 3AF, UK

ABSTRACT

The microalga *Haematococcus pluvialis* is one of the most important sources of astaxanthin for the aquaculture industry. However, due to a very hard cellulosic cell wall, astaxanthin extraction and its bioavailability in intact encysted cells is low.

Different physical and chemical processes, which allow the disruption of the encysted cells of *H. pluvialis* in terms of astaxanthin recovery, were evaluated as well as the facility of its extraction from the cells.

Key words: *Haematococcus*, microalgae, processing, bioavailability, astaxanthin.

INTRODUCTION

Haematococcus pluvialis is a unicellular microalga, and one of the most important natural sources of astaxanthin (Sommer *et al.*, 1991), which is produced when cultured in nutrient deficient media and / or under high light intensities or high temperatures (Fan *et al.*, 1994; Droop, 1954).

Astaxanthin (3,3'-dihydroxy-β,β'-carotene-4,4'-dione) is a reddish pigment with antioxidant activity, being an important component in the aquaculture industry (Choubert & Heinrich, 1993). *Haematococcus* can accumulate up to 1% of dry weight astaxanthin, mainly in the form of the 3S,3'S enantiometer, mono and di-esters (Grung *et al.*, 1990)

However, due to the very hard cellulosic cell wall, astaxanthin extraction and its bioavailability in intact encysted cells is low.

The purpose of this work was to evaluate different physical and chemical processes which allow the disruption of *H. pluvialis* encysted cells, in terms of astaxanthin recovery and the facility of its extraction from the cells.

The astaxanthin quantification, employing spectrophotometry and chromatographic techniques (TLC and HPLC), was used as an indirect method to evaluate the efficiency of each process.

The direct effect of those processes on the cell wall disruption was analysed by scanning electron microscopy (SEM).

MATERIAL AND METHODS

Microalgae: *Haematococcus pluvialis* (34/7 strain of CCAP - Culture Collection of Algae and Protozoa, Cumbria - UK) was grown and carotenised in Bold Basal Medium mod., and the biomass was first obtained by sedimentation of the culture.

Physical and chemical treatments: After sedimentation and further centrifugation, the carotenised biomass was submitted to the following processes: ❶ autoclave 30 min, 121 °C, 1 atm; ❷ HCl 0.1 M, 15 min and 30 min; ❸ NaOH 0.1 M, 15 min and 30 min; ❹ enzymatic treatment with a mixture of 0.1% protease K and 0.5 % driselase in a phosphate buffer, pH 5.8, 30 °C, during one hour; ❺ spray drying, inlet 180°C, outlet 115°C; and ❻ mechanical disruption, with a cell homogeniser developed for this purpose.

After the treatments, all samples (30 mg each, in triplicate) including the control, were submitted to the astaxanthin extraction procedure.

Astaxanthin extraction: The astaxanthin extraction was performed using a solvent mixture of ethanol:diethylether:n-hexane, 1:1:1 (v/v/v), all laboratory grade (Merck).

After the addition of some glass beads to the test tubes, triplicates of 30 mg samples were submitted to a combination of vigorous vortex for 1 min and ultra-sounds for 15 min. After this, all samples were centrifuged for 5 min at 5000 rpm and evaporated under a steady stream of nitrogen.

Comparative bioavailability into acetone: Simple extraction of astaxanthin, from *H. pluvialis* cells previously centrifuged, into 5ml acetone, was carried out overnight (16 hours) at room temperature, for all the treatments, including the control.

Spectrophotometry: The spectra were recorded in a double beam UV-Vis spectrophotometer (Shimadzu 1601). For quantification of astaxanthin, the extracts originated from the solvent mixture extraction were redissolved in 5ml n-hexane (extinction coefficient 2100, Abs max 470nm). Those obtained from the extraction of astaxanthin into acetone, were analysed directly in aceton (extinction coefficient 2100, Abs max 476nm).

Thin-layer chromatography: Precoated silica gel H60 plates 20x20 cm (Merck, Darmstadt, FRG) were used for the separation of carotenoids. Solvent system: acetone:n-hexane, 3:7 (v/v) (Kobayashi *et al.*, 1991), and a pre-run in 2.5% (w/v) solution of citric acid in methanol (Andrew J. Young, 1999). After the pre-run, the TLC plates were dried under warm air, at 40°C, during 10 min.

The qualitative analysis of TLC plates was performed using an imaging densitometer (Model GS-700, BIO-RAD).

HPLC: Samples were analysed by normal phase HPLC (Hewlett Packard) using a UV detector (450.8, 470.8 nm) and a Lichrosorb column (12.5 cm x 4.6 cm), 5µm, pre-coated with 1% H_3PO_4 in methanol (v/v) before used. Mobile phase n-hexane:acetone, 1:1 (v/v).

SEM: *Haematococcus* biomass, submitted to the different treatments, was analysed by Scanning Electron Microscopy (Hitachi, Ltd), after being lyophilised and coated with a thin gold-palladium film (10-15nm).

RESULTS AND DISCUSSION

- when the appropriate solvent mixture is used, the best results were obtained with autoclave, spray-dryer and mechanical disruption processing (figure 1). This was confirmed by HPLC results (not shown) and TLC analysis (TLC I)
- the highest recovery of astaxanthin (into acetone) from intact cells was observed with autoclave and mechanical disruption processing. These results were supported by TLC analysis (TLC II). However, the pigment amount in all treatments was lower than the one obtained with the solvent mixture;

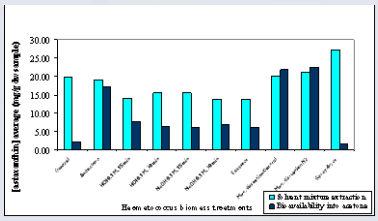
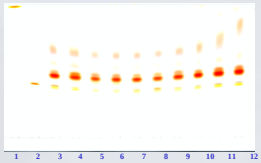


Figure 1. Astaxanthin content (mg/g dw biomass) with solvent mixture extraction and bioavailability into acetone.



TLC II. Chromatogram of bioavailability into acetone extracts, ressuspended in 500µl n-hexane. Applied volume of sample 15µl; standards: β-carotene (2.5µg) and astaxanthin (3S,3'S - 2.5µg).

TLC I. Chromatogram of solvent mixture extract, ressuspended in 500µl n-hexane. Applied volume of sample 15µl; standards: β-carotene (2.5µg) and astaxanthin (3S,3'S - 2.5µg).

For both the plates, tracks correspond to :

1- β-carotene
2- astaxanthin
3- control
4- autoclave
5- HCl 0.1M, 15 min
6- HCl 0.1M, 30 min
7- NaOH 0.1M, 15 min
8- NaOH 0.1M, 30 min
9- enzymes
10- spray-dryer
11- mechanical disruption (control)
12 - mechanical disruption under a stream of nitrogen.

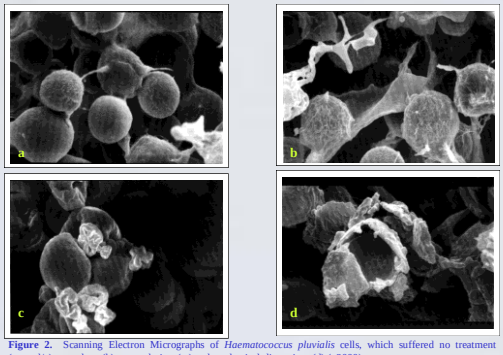


Figure 2. Scanning Electron Micrographs of *Haematococcus pluvialis* cells, which suffered no treatment (control/a), autoclave (b), spray-drying (c) and mechanical disruption (d) (x2000).

- The micrographs (figure 2) show us the different effects of each treatment on the cells, like the cell wall disruption in (d), the clustering (c), or the roughening (b) of the cells, allowing the way out of all the contents and, mainly, the carotenoids.

CONCLUSION

- We can conclude that the best extraction and so far the most effective process is the mechanical disruption.

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