Effect of mechanical pretreatment on microalgae for the extraction of carotenoids by using ASE technology

A. Iovine[1,2], P. Casella[1], S. Chianese[2], D. Karatza[2], D. Musmarra[2], T. Marino[3], A. Molino[1]

[1]ENEA, P.zza E. Fermi n. 1, 80055 Portici (NA), Italy
[2]Department of Engineering, University of Campania "L. Vanvitelli", Real Casa dell'Annunziata, Via Roma 29, 81031 Aversa (CE), Italy
[3]Institute on Membrane Technology, National Research Council, ITM-CNR, Via P. Bucci 17/C, 87036 Rende (CS), Italy
Keywords: D. salina, H. pluvialis, S. almeriensis, pretreatment.
Presenting author email: patrizia.casella@enea.it

Introduction

Recently the demand for carotenoids has been high due to their potential applications in food and feed market, for nutraceuticals, pharmaceuticals, and cosmetics manufacture, for their natural colouring properties and principally for their healthy function. Among carotenoids, the most required are β-carotene as precursor of vitamin A (retinol), astaxanthin as powered antioxidant and lutein for its protective effect on eyes health.

Nowadays, β-carotene and lutein are principally produced from vegetables and fruits where they are naturally contained, while astaxanthin is produced through chemical synthesis, but an alternative, promising source of these compounds is represented by microalgae. *Dunaliella salina* microalgae is a natural source of β-carotene that can reach a content of 10% on a dry weight basis. *Haematococcus pluvialis* red phase (HPR) is an appropriate solution for the production of natural astaxanthin, indeed it is able to accumulate large amounts of astaxanthin (1.5-3% astaxanthin on dry basis) (Cerón *et al* 2007). The commercial source of lutein is the marigold (*Tagetes erecta*) plant, but recently, due to several possible applications, other sources are sought, as *Scenedesmus almeriensis* microalgae because its capacity to produce lutein up to 1% d.wt. respect to 0.3% dw. content in marigold (Macías-Sánchez *et al* 2010).

In this context, this study aimed to evaluate the effect of the mechanical pretreatment on *Dunaliella salina*, *Haematococcus pluvialis* red phase and *Scenedesmus almeriensis* in order to improve the extraction of β-carotene, astaxanthin and lutein. Mechanical pretreatment was evaluated changing the ratio between diatomaceous earth and biomass (DE/biom ratio), rotation speed and time.

Materials and methods

D. salina was supplied by Algalimento (Santa Lucía de Tirajana, Gran Canaria) Company, *H. pluvialis* was purchased by the company MICOPERI BLUE GROWTH® (Ravenna, Italy), *S. almeriensis* was acquired by the company AlgaRes Srl (Rome, Italy). Experimental test were done to evaluate the effects of mechanical pretreatment by a Planetary ball mill Retsch PM200 on the freeze-dried powder microalgae *D. salina*, *H. pluvialis*, *S. almeriensis* for the extraction of β-carotene, astaxanthin and lutein which theoretical content is reported in table 1.

Table 1. β-carotene, astaxanthin and lutein theoretical content in *D. salina*, *H. pluvialis*, *S. almeriensis*.

β-carotene (mg/g dry basis ± SD)	astaxanthin (mg/g dry basis ± SD)	lutein (mg/g dry basis ± SD)
34.10 ± 0.7	20.01 ± 1.05	3.04 ± 0.35

Mechanical pre-treatment was firstly tested varying the rotation speed (200 - 600 rpm) and the ratio between diatomaceous earth and biomass (DE/biom ratio) (0.2 – 2.0), for 5 minutes. The second test was done to evaluate the pretreatment time (2.5-10 min) at the best tested conditions. Carotenoids were extracted from untreated and pretreated biomass by ASE 200 (Dionex). β-carotene was extracted using hexane, at a temperature and pressure of 50 °C and 100 bar. Astaxanthin and lutein were extracted at 67 °C and 100 bar using ethanol (Molino *et al* 2018). Each extraction test was carried out for a total time of 20 min (two extraction cycles of 10 min). The liquid extracts were dried through a ZymarkTurboVap evaporator for dry residue quantification. The extracts were saponified using methanolic NaOH (0.05 M) to eliminate chlorophyll's interference and carotenoids were quantified by uHPLC-DAD.

Results and discussion

The pre-treatment resulted very effective for the extraction of carotenoids from *D. salina*, *H. pluvialis* and *S. almeriensis*, obtaining much higher extraction yields than the content extracted in the untreated microalgae as shown in Figure 1 and Table 2. The higher content of β-carotene equal to 33.56 mg/g dry weight was obtained from *D. salina* pretreated for 5 min at 500 rpm with a 0.4 DE/biom ratio (Figure 1a). The extraction performed on untreated *H. pluvialis* allowed to obtain 9.6×10^{-3} mg of astaxanthin/g (Table 2), while the amount of astaxanthin obtained by pretreating the biomass before extraction process was three orders of magnitude larger.

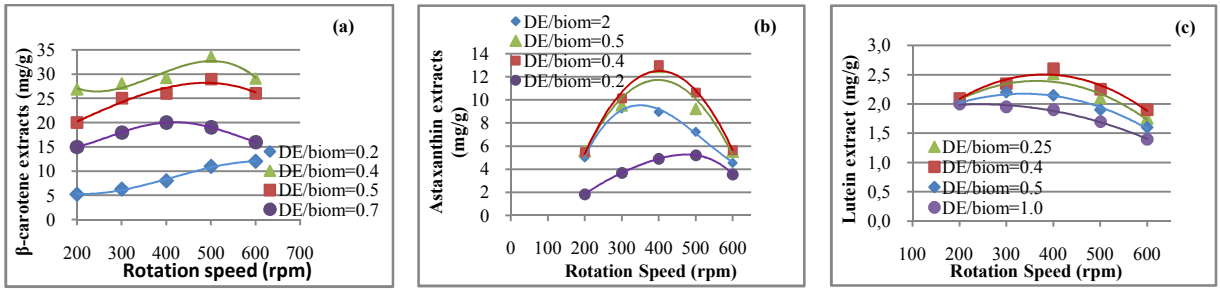

Figure 1. Pre-treatment effect on the extraction of (a) β-carotene under DE/biom ratios equal to 0.2, 0.4, 0.5, 0.7 (5 min, 200 - 600 rpm), (b) astaxanthin under DE/biom ratios equal to 0.2, 0.4, 0.5, 2 (5 min, 200 - 600 rpm), (c) lutein under DE/biomass ratios equal to 0.25, 0.4, 0.5, 1 (5 min, 200 - 600 rpm). Extracts were quantified as mg/g dry weight biomass.

As illustrated in Figure 1(b) the highest value (13.03 mg of astaxanthin/g) was reached at 0.4 DE/biom ratio and 400 rpm. The amount of lutein extracted from the untreated *S. almeriensis* was equal to 2.03 mg/g. After biomass mechanical pretreatment, a slight increase of lutein extraction was obtained, but not in all pretreatment conditions. It is noted that the optimal condition was demonstrated at 0.4 DE/biomass ratio and 400 rpm obtaining 2.6 mg of lutein/g (Figure 1c). The best rotation speed and DE/biomass ratio conditions were used to test time effect as reported in table 2.

Table 2. Pre-treatment time effect on β-carotene, astaxanthin and lutein extraction. Value with asterisk (*) are relative to untreated microalgae.

Time (min)	β-carotene (mg/g)	astaxanthin (mg/g)	lutein (mg/g)
0	2.52*	$9.6x10^{-3}$*	2.03*
2.5	22.35	4.29	2.1
4.0	32	11.25	2.45
5.0	33.56	13.03	2.51
7.5	16.11	11.53	2
10.0	-	9.65	1.6

Table 2 evidenced as the higher content of β-carotene, astaxanthin and lutein was extracted from *D. salina*, *H. pluvialis* and *S. almeriensis* pretreated for 5 minutes.

Conclusion

Microalgae pre-treatment allowed to improve the extraction yield. The best pretreatment conditions were 500 rpm as rotation speed, 0.4 DE/biomass ratio, 5 minutes as pretreatment time to obtain β-carotene from *Dunaliella salina*, and for astaxanthin and lutein from *Haematococcus pluvialis* and *Scenedesmus almeriensis* were 0.4 as DE/biomass ratio, at 400 rpm, for 5 minutes.

References

M.C. Cerón, M.C. García-Malea, J. Rivas, F.G. Acien, J.M. Fernandez, E. Del Río, M.G. Guerrero, E. Molina, Antioxidant activity of Haematococcus pluvialis cells grown in continuous culture as a function of their carotenoid and fatty acid content. Applied Microbiol Biotechnology (2007) 74:1112–1119.

M.D. Macías-Sánchez, J.M. Fernandez-Sevilla, F.G. Acién Fernández, M.C. Cerón García, E. Molina, Supercritical fluid extraction of carotenoids from Scenedesmus almeriensis. Food Chemistry 123 (2010) 928–935.

A. Molino, J. Rimauro, P. Casella, A. Cerbone, V. Larocca, S. Chianese, D. Karatza, S. Mehariya, A. Ferraro, E. Hristoforou, D. Musmarra, Extraction of astaxanthin from microalga Haematococcus pluvialis in red phase by using generally recognized as safe solvents and accelerated extraction. Journal of Biotechnology 283 (2018) 51–61.

Acknowledgements

This research was funded by Bio Based Industries Joint Undertaking under the European Union's Horizon 2020 R&I program under grant agreement No 745695 (VALUEMAG).