CHEMICAL ENGINEERING TRANSACTIONS

VOL. 52, 2016

A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet

Guest Editors: Petar Sabev Varbanov, Peng-Yen Liew, Jun-Yow Yong, Jiří Jaromír Klemeš, Hon Loong Lam

ISBN 978-88-95608-42-6; **ISSN** 2283-9216

DOI: 10.3303/CET1652174

Life Cycle Analysis of Microalgae Extraction Techniques

Sofia G. Papadaki, Kontastina E. Kyriakopoulou, Magdalini K. Krokida

School of Chemical Engineering, National Technical University of Athens, Zografou Campus, Athens, GR-15700, Greece
spcheng@central.ntua.gr

In this study, a comparative analysis between conventional solvent extraction and innovative green extraction methods, using microwaves and ultrasounds, has been conducted for the recovery of bioactive compounds and especially astaxanthin from *Haematococcus pluvialis* microalga. The isolation of the desired natural compounds as well as the environmental impact of each method has been the criteria for the different processes evaluation. A comparative life cycle analysis was carried out, using proper databases and software, in order to evaluate the selected extraction processes' sustainability. Among the extraction procedures applied, ultrasound assisted extraction was found the most sustainable method so far for astaxanthin extraction, regarding its high yielding, low cost, short time and medium environmental impact. Moreover, microwave assisted extraction showed great potential as a rapid and overall eco-friendly technique suffering though from low yielding due to thermal degradation of carotenoids in long processing times.

1. Introduction

The green algae *Haematococcus pluvialis* (*H. pluvialis*) is a freshwater species of *Chlorophyta* from the family *Haematococcaceae*. During unfavorable growth conditions, *H. pluvialis* initiates carotenogenesis while undergoes morphological transformation from green vegetative cells to deep red (Galvão et al., 2013). The most abundant carotenoid in *H. pluvialis* is astaxanthin that can reach high concentration more than 4 % of dry weight (Miao et al., 2006). This carotenoid possesses various pharmacological activities, including antioxidant activity antitumor effects antidiabetic and anti-inflammatory properties. Astaxanthin-rich Haematococcus has already been marketed as a dietary supplement for human consumption. The health benefit of this product is mainly due to its strong antioxidant activity, which is 100 times more than a-tocopherol. Moreover, astaxanthin has an antioxidant action up to 500 times that of vitamin E and is the most stable antioxidant and never turns into a pro-oxidant. In addition, pro-vitamin A, a more effective antioxidant that other carotenoids, decelerate age-related macular degeneration, immunomodulatory effects, etc. (Guerin et al., 2003). The range of shades from astaxanthin includes red as well as orange. Therefore, it can replace synthetic ones such as Tartrazine, Sunset Yellow etc. Moreover, the significant antioxidant activity that astaxanthin presents can sufficiently compete and replace synthetic antioxidants such as BHA, BHT (Kyriakopoulou et al., 2013). For the effective utilization of astaxanthin, the nutrient should be extracted from the microalga. The increasing legislative restrictions on the presence of organic solvents in food products coupled to their negative effects on the nutritional and functional properties of compounds such as carotenoids have driven the search for "greener" alternatives than acetone, which is commonly used to extract valuable lipophilic compounds from microalgae. In addition, nowadays, conventional extraction methods tend to be replaced due to the several disadvantages such as energy usage, solvent consumption, time, etc. Ultrasound Assisted Extraction (UAE) and Microwave Assisted Extraction (MAE) are two proposed green extraction method for the recovery of astaxanthin.

In this study, a comparative analysis between conventional solvent extraction and innovative green extraction methods, using microwaves and ultrasounds, has been conducted for the recovery of astaxanthin from *H. pluvialis*. The isolation of the desired natural compounds as well as the environmental impact of each method has been the criteria for the different processes evaluation. A comparative life cycle analysis was carried out, using proper databases and software, in order to evaluate the selected extraction processes' sustainability.

Please cite this article as: Papadaki S. G., Kyriakopoulou K. E., Krokida M. K., 2016, Life cycle analysis of microalgae extraction techniques, Chemical Engineering Transactions, 52, 1039-1044 DOI:10.3303/CET1652174

The comparative analysis between conventional solvent extraction and innovative green extraction methods of bioactive compounds from microalgae constitutes the main scientific innovation aspect of the proposed work.

2. Life cycle assessment methodology

Life cycle assessment (LCA) is a methodology to quantify the potential environmental impacts associated with a product, process or activity throughout its life cycle or lifetime, which is known as a 'from cradle to grave' analysis. According to ISO 14,040 (ISO., 2006), the LCA methodology includes the following stages: the goal and scope definition, the inventory analysis (LCI), the impact assessment (LCIA) and the interpretation of results. The goal definition shows the reason to perform the study and the intended use of the results of the LCA, while the scope states the basic parameters of the study such as the functional unit (FU), the system boundaries, the data quality and assumptions (simplifications).

3. Goal and Scope

3.1 Objectives

The objective of this study was to compare the environmental performance and impacts associated with different extraction methods for the recovery of astaxanthin and to identify the ''hot spots'' such processes as a way to potentially improve their environmental effect. A life cycle assessment from cradle to gate was performed including the cultivation, harvest and extraction treatment of *H.Pluvialis* till the production of astaxanthin rich extracts.

3.2 The product and the system investigated

The product evaluated is an astaxantin rich extract recovered from dried *H. pluvialis* cultivated in a photobioreactor under stressed condition and dried using spray drying (Wan et al., 2014). The dried biomass was extracted using conventional solvent extraction/maceration (CSE), soxhlet extraction (SE), microwave assisted extraction (MAE) and the ultrasound assisted extraction (UAE) using proper solvents (Ruen-ngam et al., 2011).

3.3 Functional unit (FU)

In this case study FU was defined 1 kg equivalent of astaxanthin in the form of oleoresin extract recovered from H. Pluvialis in order to be used as an additive in food, cosmetic or nutraceutical applications.

3.4 System boundaries

The system is modelled for the geographical location of Chios, Greece and is based on fictional microalgae farm. Cradle-to-grave environmental impacts of astaxanthin microalgae and products are considered, broken down into three discrete stages, including: the acquisition of the microalgae rich in astaxanthin (cultivation & harvesting), the treatment or modification of these materials prior to extraction, the extraction processing for the recovery of astaxanthin (see Figure 1). With regards to the extraction stage, just the extraction methods related with the recovery of astaxanthin rich extracts from the different matrices were involved in the analysis, since the rest steps such as the filtration of the samples or the evaporation of the solvents are similar in the all cases. Site works and land occupation for capital infrastructure, such as plumbing, pumps, sheds, processing plant and machinery is not taken into consideration.

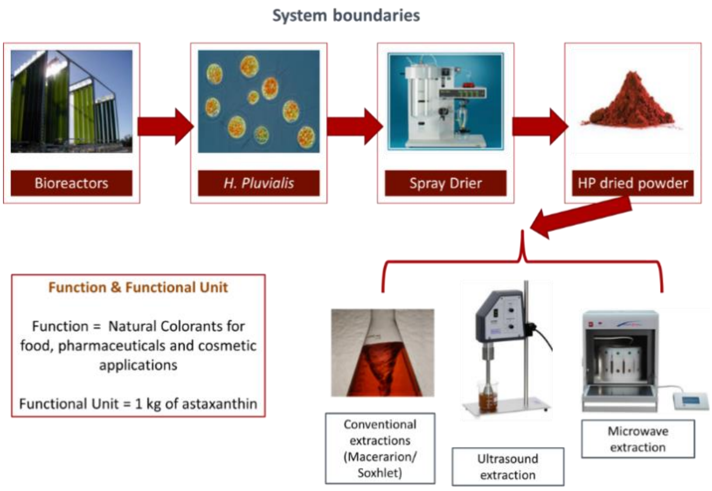

Figure 1: System boundaries for astaxanthin production

4. Life Cycle Inventory

Life cycle inventory includes input data for the production and processing stage of each principal process for the recovery of astaxanthin. For electricity from the grid, average Greek electricity mix production was estimated as presented from Ecoinvent 2.0 references, while typical raw material extractions and manufacturing processes were inventoried based on readily available data from the Ecoinvent 2.0 database. In this study, no allocation procedure was required since algae cultivation was only focused on astaxanthin production. Therefore, all the environmental burdens were allocated to the amount of astaxanthin produced. Moreover, since the main focus of the study is on the recovery treatments suggested, information regarding the cultivation, harvesting and drying chain were considered the same for all examined procedures. For the cultivation and harvesting step information was acquired from the study of Pérez-López et al. (2014). Drying is an essential step for the recovery of astaxanthin, especially since the initial moisture content of H. Pluvialis biomass can reach up to 92 % with a concentration of TSS around 25 %. After drying treatment, the moisture content is less than 5 % w.b., leading to powder with 96 % TSS. In order to succeed that 6.17 MJ/kg H. pluvialis biomass is requested for the drying procedure (Guohua Chen and Arun S . Mujumdar, 2014). For the recovery of astaxanthin from the dried biomass acetone is suggested as the most common solvent. However, since the astaxanthin rich extracts produced are considered for food and nutraceutical applications alternative food grade solvents such as vegetable oils and essential oils were considered. Moreover, the selected solvents were assumed to be recycled and reused in a rate of 80 %, while vegetable oils were considered to be filtered and reused in a ratio of 50 % regarding scale up and industrial application. The extraction conditions, solvent system, energy consumption, yielding and recovery are presented in Table 1, as they were retrieved from the literature.

Table 1: Extraction conditions, solvent system, energy consumption, yielding and recovery of Astaxanthin

	Maceration 1	**Maceration 2**	**Soxhlet**	**Microwave Assisted Extraction**	**Ultrasound Assisted Extraction 1**	**Ultrasound Assisted Extraction 2**
Solvent (L)	10 L Acetone	10 L Vegetable Oil	30 L Acetone	10 L Acetone	10 L Acetone	10 L Essential Oil
Conditions	40 ºC, 24 h	40 ºC, 24 h	56 ºC, 4 h	75 ºC, 5 min	45 ºC, 20 min	45 ºC, 20 min
Electricity usage (Wh)	20	20	360	750	650	650
Yield (mg/g d.b.)	**28.5**	**43.8**	**27.7**	**37.0**	**36.5**	**35.0**
Yield %	57	87.5	70	74	73	70
References	(Ruen-ngam et al., 2011)	(Kang and Sim, 2008)	(Ruen-ngam et al., 2011)	(Ruen-ngam et al., 2011)	(Ruen-ngam et al., 2011)	(Ruen-ngam et al., 2011; Kang and Sim 2008)

5. Life cycle impact assessment

Among the different LCA impact assessment methods available with Simapro 7.1 software, the CML2 baseline 2000 method was chosen in order to provide a measure for the potential environmental damage of airborne, liquid and solid emissions by means of appropriate equivalence factors to selected reference compounds for several impact categories such as Abiotic Depletion Potential (ADP), Global Warming Potential (GWP), Ozone Depletion Potential (ODP), Human Toxicity Potential (HTP), Acidification Potential (ACP) and Eutrophication Potential (EP), Photochemical Oxidation Potential (POP), Terrestrial Ecotoxicity Potential (TEP), Marine Aquatic Ecotoxicity Potential (MAEP), and Fresh Water Aquatic Ecotoxicity Potential (FWAEP). More specifically, the environmental impacts of cultivation and harvesting, drying and extraction procedure were evaluated.

5.1 Impact of the production of dry H.pluvialis

According to recent studies, the cultivation systems (e.g. tubular bioreactor, open ponds) influences drastically the environmental impacts associated to microalgal production, specifically in terms of energy requirements and therefore global warming potential (Grierson et al., 2013). Cultivation represents the most energy demanding stage over the life cycle of microalgal production since artificial lightning is needed not only for the growth stage but also for the stress induced conditions that are need to convert the green *H. Pluvialis* microalga to a rich in astaxanthin biomass (Ugwu et al., 2008). Moreover, the harvest and drying stage that follows consumes also high amounts of energy as long as vigorous centrifugation is needed to achieve the optimal TSS percentage leading to an efficient drying process. Specifically, spray drying technique is applied at high temperatures up to 220 °C in order to retrieve 1 kg of dry biomass from almost 10 kg of wet mass. The environmental impact for the production of 1 kg of dry *H. Pluvialis* is presented in Figure 2. Apart from the GWP, the eutrophication, abiotic depletion and acidification potential are also high mainly due to the use of nutrients and fertilizers during the growth stage.

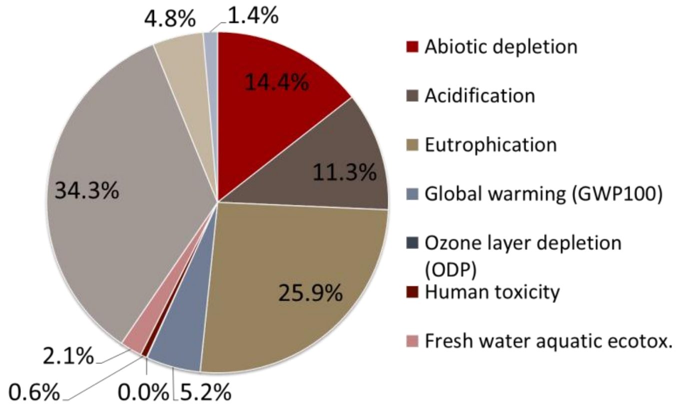

Figure 2: Environmental impact for the production of 1 kg of dry H. Pluvialis

5.2 Impact of the recovery of 1kg Astaxanthin from dry H.pluvialis

Astaxanthin production is optimized when *H. Pluvialis* is subjected to stress cultivation conditions and dried in order to remove the moisture content that acts as a barrier for the extraction process. The solvents selected in this study were mainly non-polar in order to recover the lipophilic fraction containing astaxanthin. However, major differences on the recovery of astaxanthin are presented among the different solvents and extraction procedures applied. The use of acetone as solvent is suggested in literature as the optimum system for the recovery of astaxanthin, therefore it is applied in all extraction processes. According to Table 1, the yielding of astaxanthin varies from method to method affecting the environmental footprint of the production of 1 kg of astaxanthin. Therefore, techniques that present high recovery are environmental friendly even though sometimes present high energy demands. A comparison among conventional and short processing green methods for the recovery of 1 kg of astaxanthin is presented in Figure 3. Specifically, although MAE and UAE have high electricity demands the processing time is significantly lower and the yielding is higher leading to low impacts. The solvent usage in such processes is also significantly lower, whilst also recycling of the solvent can be achieved, leading to lower impacts on the abiotic depletion potential. Furthermore, the replacement of organic solvents with food grade alternatives (vegetable or essential oil) leads to a slight reduction of the overall impact even though the yielding is lower (see Table 1, Figure 4). The advantages of using such solvents are counterfeited by the effect they have on Eutrophication and Marine aquatic ecotoxicity. The nature of the solvent makes separation of astaxanthin difficult, but with propriate handling these products can be incorporated to food and cosmetics high added value products since they consist of food grade oils avoiding potential hazards from mishandling.

6. Interpretation of results and discussion

6.1 General

The weather conditions of cultivation areas could affect the selection of proper cultivation systems, although closed controlled indoor photobioreactors illuminated with artificial light are being currently applied for high-value products including astaxanthin (Lorenz and Cysewski, 2000). Moreover, even though sunlight is eco-friendlier the number of days required to obtain the same amount of microalgae cell paste under solar light conditions are considerably higher than under artificial illumination since the growth of microalgae and the composition of biomass are strongly dependent on the light supply (light source and light intensity) (Yeh et al., 2010). Therefore, a more focused research on the optimum cultivation conditions is suggested. Harvesting and drying of microalgae as shown in our previous research plays an important role on the environmental footprint of the production of functional extracts (Kyriakopoulou et al., 2015). Selecting the right combination of centrifugation and drying systems can lead to highly efficient dry mass production. However, all the affirmation stages can significantly affect the bioactive content of the dried biomass. The selection of a suitable extraction procedure should be done not only based on the yielding of the process but also the overall environmental performance, since otherwise green processes are not so environmental friendly as they are expected.

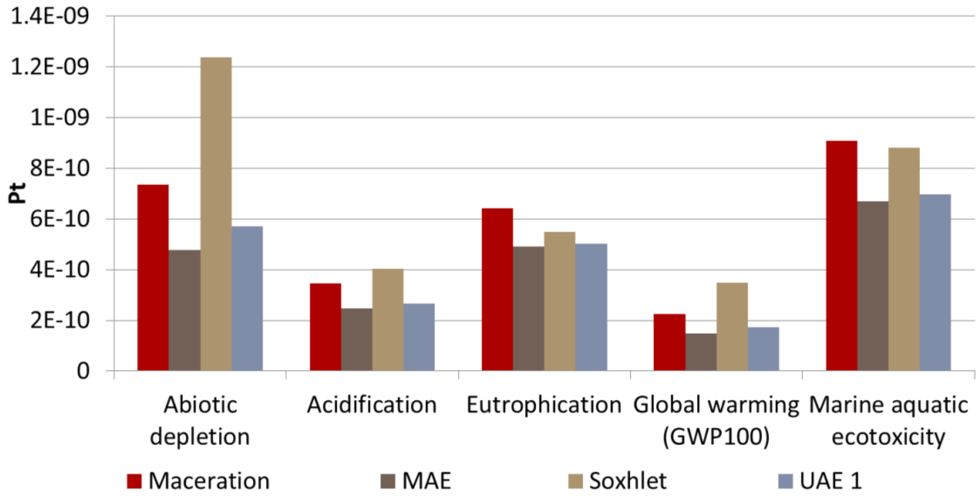

Figure 3: Environmental impact of conventional and short processing green methods for the recovery of 1 kg of astaxanthin

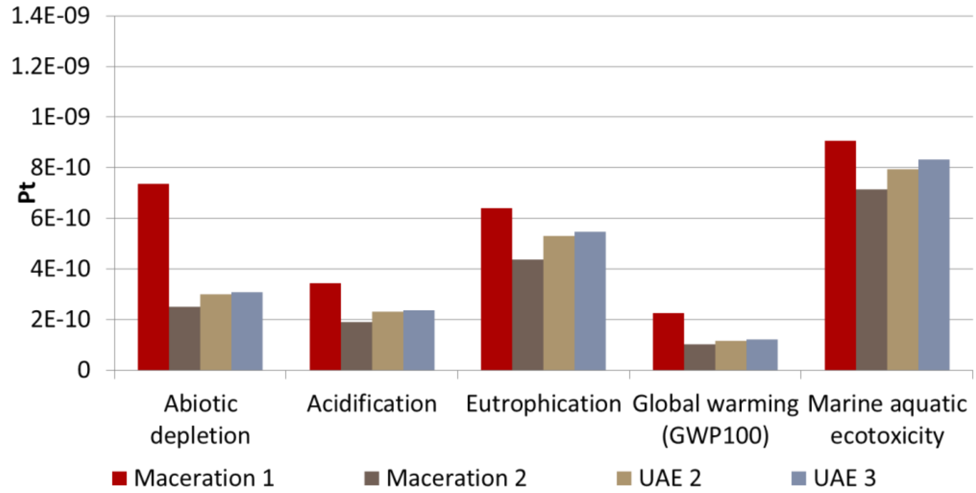

Figure 4: Environmental impact of selected extraction methods using vegetable oils as solvent for the recovery of 1 kg of astaxanthin

7. Conclusions

H. Pluvialis is a high rich source of astaxanthin which can be recovered sufficiently using not only organic solvent but also edible vegetable and essential oils. Among the extraction procedures applied, ultrasound assisted extraction was found the most sustainable method so far for astaxanthin extraction, regarding its high yielding, low cost, short time and medium environmental impact. Microwave assisted extraction is considered also a rapid and overall eco-friendly technique suffering though from low yielding due to thermal degradation of carotenoids in long processing times. The maceration and the soxhlet extraction techniques are highly time consuming, expensive and potentially hazardous due to the large amount of solvents used, soxhlet also exhibits low yielding due to thermal degradation and high energy demand. The extraction techniques examined showed their potential for the recovery of astaxanthin rich extracts which can be used in nutraceutical, cosmetic and food industries. The overall environmental impacts of the processes showed that extraction of astaxanthin affects mainly the eutrophication, marine aquatic ecotoxicity and global warming, while abiotic depletion impact is minimized through the recycling of solvents and other materials though the cradle to gate process.

Ackonowledgements

This research was founded by the IKY FELLOWSHIPS OF EXCELLENCE FOR POSTGRADUATE STUDIES IN GREECE – SIEMENS PROGRAM.

References

Chen G., Mujumdar A.S., 2014. Drying of Herbal Medicines and Tea. In: Mujumdar, A. (Ed.), Handbook of Industrial Drying. CRC Press, Boca Raton, FL, United States, 637–646.

Galvão R., Santana T., Fontes C., Sales E., 2013. Modeling of biomass production of Haematococcus pluvialis. Appl. Math. 4, 50–56.

Grierson S., Strezov V., Bengtsson J., 2013. Life cycle assessment of a microalgae biomass cultivation, bio-oil extraction and pyrolysis processing regime. Algal Res. 2, 299–311.

Guerin M., Huntley M.E., Olaizola M., 2003. Haematococcus astaxanthin: applications for human health and nutrition. Trends Biotechnol. 21, 210–216.

ISO., 2006. ISO 14044: Environmental Management — Life Cycle Assessment — Requirements and Guidelines. Environ. Manage. 3, 54.

Kang C.D., Sim S.J., 2008. Direct extraction of astaxanthin from Haematococcus culture using vegetable oils. Biotechnol. Lett. 30, 441–444.

Kyriakopoulou K., Papadaki S., Krokida M., 2013. Natural aspect of sustainable food and cosmetics manufacturing. In: Green Design, Materials and Manufacturing Processes - Proceedings of the 2nd International Conference on Sustainable Intelligent Manufacturing, SIM 2013, 197–201.

Kyriakopoulou K., Papadaki S., Krokida M., 2015. Life cycle analysis of β-carotene extraction techniques. J. Food Eng. 167, 51–58.

Lorenz R.T., Cysewski G.R., 2000. Commercial potential for Haematococcus microalgae as a natural source of astaxanthin. Trends Biotechnol. 18, 160–167.

Miao F., Lu D., Li Y., Zeng M., 2006. Characterization of astaxanthin esters in Haematococcus pluvialis by liquid chromatography-atmospheric pressure chemical ionization mass spectrometry. Anal. Biochem. 352, 176–81.

Pérez-López P., González-García S., Jeffryes C., Agathos S.N., McHugh E., Walsh D., Murray P., Moane S., Feijoo G., Moreira M.T., 2014. Life cycle assessment of the production of the red antioxidant carotenoid astaxanthin by microalgae: from lab to pilot scale. J. Clean. Prod. 64, 332–344.

Ruen-ngam D., Shotipruk A., Pavasant P., 2011. Comparison of Extraction Methods for Recovery of Astaxanthin from Haematococcus pluvialis. Sep. Sci. Technol. 46, 64–70.

Ugwu C.U., Aoyagi H., Uchiyama H., 2008. Photobioreactors for mass cultivation of algae. Bioresour. Technol., 99(10), 4021–4028.

Wan M., Zhang J., Hou D., Fan J., Li Y., Huang J., Wang J., 2014. The effect of temperature on cell growth and astaxanthin accumulation of Haematococcus pluvialis during a light-dark cyclic cultivation. Bioresour. Technol., 167, 276–283.

Yeh K.L., Chang J.S., Chen W.M., 2010. Effect of light supply and carbon source on cell growth and cellular composition of a newly isolated microalga Chlorella vulgaris ESP-31. Eng. Life Sci. 10, 201–208.