Hes·so
Haute Ecole Spécialisée de Suisse occidentale
Fachhochschule Westschweiz
University of Applied Sciences and Arts Western Switzerland

Master of Science HES-SO in Life Sciences

Antioxidative pigments of microorganisms for cosmetics : Screening and experimental evaluation of production conditions

Aymerick Bussien

Applied Biosciences

HES-SO Valais

Advisor: Dr. Manfred Zinn // In collaboration with **La Prairie Group AG**

DESCRIPTION

The increase of environmental air pollution and the combination with other factors such UV irradiation is constantly challenging our immune system. Our skin plays an important role in the transport of gases and is therefore also affected by the increased oxidative stress. As a result, the skin ages much quicker and this phenomenon is observable by increased wrinkle formation. The cosmetics industry is therefore interested in developing protective systems that allow a healthier aging.

In nature there are many different types of antioxidants. For the cosmetics industry the large class of pigments are of particular interest due to their known antioxidative activity. For this research project, the focus will be put on the evaluation of phycocyanin and different types of carotenoids that can be extracted from phototrophic, microbial biomass.

Three different cultivation mode namely autotrophy, heterotrophy and mixotrophy were investigated regarding growth parameters and antioxidant content. Three organisms were capable of heterotrophy, namely *Chlorella vulgaris*, *Chlorella minutissima* and *Chlorella zofingiensis* this cultivation mode increased the biomass productivity and the biomass concentration when compared to autotrophy. Moreover it allow the cultivation of these strain in a conventional steel fermenter without the presence of light. However, it has been demonstrated that the antioxidant content of these three organisms were lower when compared to autotrophic biomass. This have been due to a reduction of production in the photosynthetic pigments carotenoids and chlorophylls. Mixotrophy was possible for one more organism, namely *Arthrospiria platensis*. This cultivation mode increased the biomass concentration and the biomass productivity of this organism. Moreover it increased the antioxidant content of the biomass when glucose was used as carbon source. Finally, four extraction techniques namely maceration, high pressure homogenizer, bead milling and sonication were tested regarding antioxidant recovery from microalgal biomass. Sonication and bead milling in water were the most appropriate technique regarding antioxidant recovery from algal biomass.

OBJECTIVES

- Cultivation of the following microalgal and bacterial strains: *Chlorella vulgaris, Chlorella minutissima, Arthrospira platensis,* and *Tetraselmis suecica* and screening them for best growth and antioxidative activity of cell extracts
- Assess the influence of cultivation mode and the extraction method (phycocyanin is hydrophilic whereas carotenoids are hydrophobic) on the final antioxidative performance.
- Optimize the growth in a bioreactor for one selected strain
- Develop the necessary analytics for the characterization and quantification of the pigments of interest.

RESULTS

To truly assess the antioxidant potential of every strain, it was necessary to optimize the extraction protocol. Indeed, some microalgae species produces more lipophilic antioxidants like carotenoids and chlorophylls. Extraction in aqueous solvents would not benefits these species and their antioxidant potential could be underestimated. Four species were grown autotrophically and lysed by French press in ethanol and water. Their antioxidant activity was measured and the affinity for polar or apolar solvents determined.

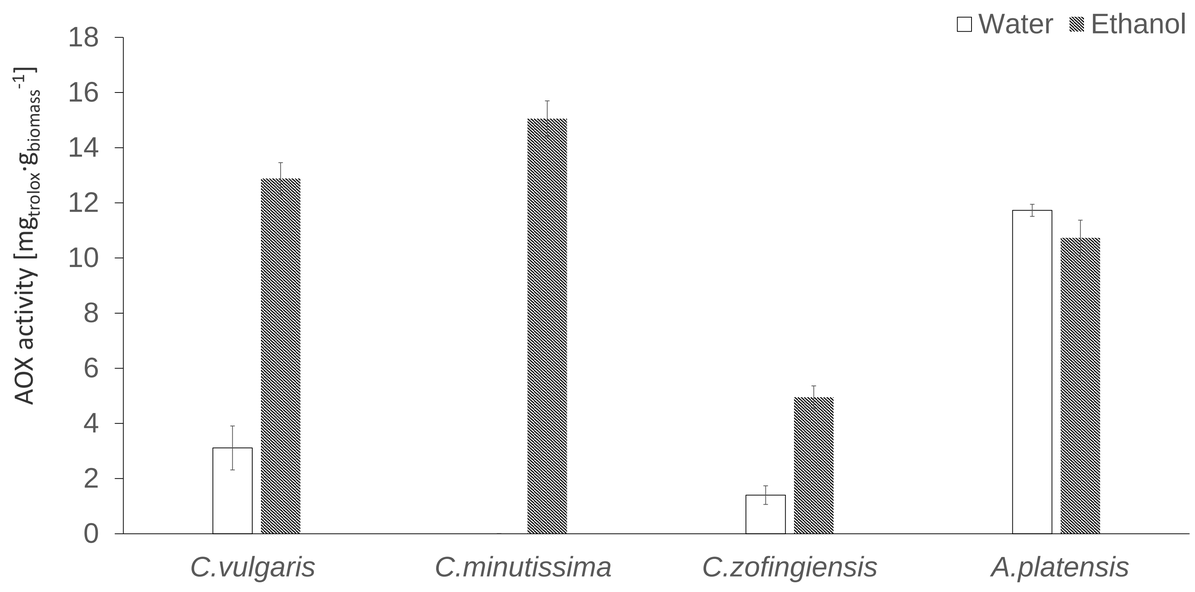

Figure 1 : Antioxidant capacity normalized by the biomass of four organisms; *Chlorella vulgaris*, *Chlorella minutissima*, *Chlorella zofingiensis* and *Arthrospira platensis* cultivated autotrophically in 125mL shake flasks lysed by French press in water (white bars) and in ethanol 95% (striped bars)

The three *Chlorella* species *(vulgaris, minutissima, zofingiensis*) showed extremely low antioxidants activity of 3,11; 0 and 1,4 mg_{trolox} g^{-1} when extracted with water. On the other hand, ethanolic extraction showed much higher results for these three organisms, indeed, antioxidant activity of 12,88; 15,05 and 4,95 mg_{trolox} g^{-1} were obtained, thus showing the higher content in apolar antioxidants of these species such as carotenoids and chlorophylls. *A.platensis* extracts showed slightly more activity in aqueous extract than in ethanolic extracts. Reflecting the high content in polar antioxidants of this specie. Indeed*, A.platensis* produces a photosynthetic blue protein that have antioxidant properties, phycocyanin-C. It has been demonstrated in this thesis that autotrophy enhance the production of phycocyanin-C. However, mixotrophy showed higher antioxidant content for this organism. Several mixtures were investigated regarding the recovery of phycocyanin-C from *A.platensis*. Water with 1% $CaCl_2$ allowed to collect higher phycocyanin-C content from *A.platensis* biomass. Finally, bead milling in $CaCl_2$ was selected has the method of choice for phycocyanin-C recovery.

CONCLUSION

While the heterotrophic growth presented some advantages such as higher biomass productivities and concentrations, it also showed some downsides. Indeed, this cultivation without light induced a reduction in the antioxidant activity of each strain probably due to a lower photosynthetic pigment content. Knowing that these three strains are totally capable of heterotrophy in shake flask it would be judicious to scale up into a 3,7L fermenter. Indeed, this would allow more tuning and consistency of their bioprocess, pH could be controlled and fed-batch with glucose could be implemented to attain higher biomass concentration without undergoing substrate inhibition. Further investigation of mixotrophy with *A.platensis* was implemented by trying three organic carbon sources namely, acetate, glucose and glycerol. It resulted that only glucose was consumed and promoted higher specific growth rate and higher productivity. Moreover, this organic carbon source increased the extract's content of protein, total nitrogen and antioxidant activity. Since this culture method does not rely solely on the photosynthesis and light supply it could be considered a scaling up into a 3,7 L glass fermenter equipped with light panels. This scale-up would allow to work in fed-batch and produce more biomass for antioxidants and phycocyanin-C recovery. Regarding antioxidant recovery from the different strain, several solvents and techniques were tested. It was observed that ethanol extracted more antioxidant from *C.vulgaris*, *C.minutissima* and *C.zofingiensis* biomass, reflecting to their higher content in lipophilic antioxidants such as carotenoids and chlorophylls. While aqueous mixture such as $CaCl_2$ collected more activity from *A.platensis* biomass, highlighting the higher content in antioxidant protein from this organism. These aqueous extracts offer several advantages in the case of a cosmetic formulation. First, they do not involve any toxic solvents that could cause more adverse effect than benefits on the skin. Then they are mainly composed of phycocyanin-C, a deep blue protein that is more attractive in term of color than a green-brown chlorophyll rich extract. Moreover, this protein rich extracts shows high antioxidant activity when tested by colorimetry with the ABTS assay. However additional research is necessary to truly assess the efficacity of these extract in actively mitigating ROS in vivo. First skin permeation needs to be assessed. Proteins are relatively large molecules, 232 kDa for the phycocyanin-C, that are hydrophilic, which can present difficulties for epidermis permeation. Moreover, effect on keratinocytes could be assessed in vitro in order to better characterize the antioxidant activity of phycocyanin-C extracts. In vitro assays involving keratinocytes cells stressed by the addition of a ROS generator such as AAPH could be a great model to assess the efficacy of C-PC extract to mitigate ROS damage.

SWISS UNIVERSITIES APPLIED SCIENCES AND ARTS

MLS | MASTER OF SCIENCE IN LIFE SCIENCES