ABSTRACT

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Title of Doctoral Thesis **Phytochemical analysis and biological activity of the alga Haematococcus pluvialis and Chlorella sp.**

Nowadays, microalgae represent a novel and promising source of various bio-active compounds. This research work focuses on two carotenoids – astaxanthin and lutein, which are attracting interest from various industrial sectors. However, the production methods so far developed for obtaining these two valuable carotenoid pigments from microalgae imply time- and solvent-consuming operations.

This work deals with two aspects. Firstly, the investigation and development of efficient and scalable isolation methods for producing the target carotenoids from microalgae biomass using high-performance countercurrent chromatography (HPCCC). Secondly, the investigation of the biological activity of astaxanthin and its esters, which have been little studied.
In our study, lutein was isolated from the green microalgae Chlorella vulgaris using the lower phase of the biphasic solvent system composed of n-heptane–ethanol–water (5:4:1.5, v/v/v) (LP4), which served both as a solvent for microalgae biomass extraction and as a mobile phase for lutein isolation by HPCCC. The ultrasound-assisted extraction of biomass with LP4 for 30 min led to an extract enriched in lutein (3.20 mg/g dried biomass). In total, an amount of 2 g of Chlorella vulgaris extract was processed through HPCCC affording 60 mg of lutein (92% purity), which was further cleaned up by gel permeation chromatography yielding 50 mg of lutein (97% purity). The same method was also applied for lutein isolation from a chlorophyll-deficient strain of the microalgae Parachlorella kessleri HY1, yielding 150 mg of lutein (95% purity, 97% recovery). Next, five astaxanthin monoesters were isolated from Haematococcus pluvialis by HPCCC, where the lower phase (LP) of a biphasic solvent system (n-heptane:acetonitrile, ratio 5:5, v/v) was used as a mobile phase. The isolated astaxanthin monoesters were finally cleaned up using high performance liquid chromatography (HPLC) affording five astaxanthin derivatives esterified with α-linolenic acid (4 mg), linoleic acid (8 mg), palmitic acid (8 mg), oleic acid (12 mg) and stearic acid (1 mg) (98% purity). To further increase the processes productivity, a multi-injection HPCCC method was developed to obtain higher amounts of astaxanthin monoesters by combining two elution modes (reverse phase and co-current). In co-current elution mode, both the mobile and stationary phases were pumped simultaneously so that the stationary phase that gets lost during each separation cycle was replenished.

Astaxanthin is a potent natural antioxidant with beneficial bioactivities, which has been demonstrated primarily for its free (non-esterified) form. However, its natural producer, the microalgae Haematococcus pluvialis synthesizes astaxanthin mostly in ester forms which have been little valorized so far. To contribute to the possible commercial use of these compounds, several biological activities were tested. The Haematococcus pluvialis extract together with the HPCCC isolated fractions enriched in astaxanthin monoesters and diesters were tested for their antioxidant, antiparasitic, cytotoxic, anti-allergic, immunomodulatory, antiaggregant and vasodilatory activity as well as their capacity to inhibit the tyrosinase activity and melanin production. For the allocation of antioxidant and cytotoxic effects, five previously isolated astaxanthin derivatives were also used. A significant antioxidant, tyrosinase-inhibitory and cytotoxic activities were observed in the Haematococcus pluvialis extract together with the isolated astaxanthin monoesters. The weak antioxidant activity was noticed when examining the astaxanthin monoesters bonded with the α-linolenic (C18:3), palmitic (C16:0) and stearic (C18:0) acids. The astaxanthin esterified with oleic acid (C18:1) exerted a cytotoxic effect against the AGS human gastric cancer cells. In addition, the potential use

of astaxanthin esters as antiparasitic agents as partially demonstrated by their negative effect on the larval antioxidant systems was observed. The preliminary evaluation of the tested substances did not show significant alterations of larval motility, however some negative impact on their morphology was shown. The capacity of astaxanthin esters to act as anti-allergic agents was also tested by demonstrating the inhibition of antigen-induced β-hexosaminidase release at a concentration of 5 μM. Finally, astaxanthin esters and their parent extract showed no immunomodulatory, antiaggregant, and vasodilatory activity; besides, they did not inhibit melanin production on cells.