

RESEARCH ARTICLE

Fucoxanthin of the brown alga *Cystoseira barbata* (Stackh.) C. Agardh from the Black Sea

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Abstract

Quantitative determination of fucoxanthin was made in the ontogenetic series of 1st order branches of the brown alga *Cystoseira barbata* (Stackh.) C. Agardh growing in upper sublittoral zone of Martyn Bay (Black Sea coast of Crimea). The greatest content (3 mg/g dry mass) was characteristic of the branches 2 – 3 months old. Total amount of this carotenoid which natural *Cystoseira* can hold is evaluated from 508 to 1810 mg per square meter of the sea bed.

Keywords: Brown alga, *Cystoseira*, fucoxanthin, Black Sea

Introduction

Marine algae are the source of natural substances acknowledged as important dietetic food and health improving agents. In this context, a large diversity of biologically active compounds makes edible brown algae most valuable. Multicomponent lipophilic fraction inherent in brown algae includes carotenoids – mainly fucoxanthin (*Fx*), zeaxanthin, violaxanthin, and some minor, such as β -carotene and anthocyanins (Kanazawa *et al.* 2008).

Fx is a common algal pigment which has proved its high antioxidant, antitumor and antimicrobial activity and some other beneficial biological and curative effects (D’Orazio *et al.* 2012). Many recent publications based on the experiments conducted on cell cultures and animals have provided increasing evidence that *Fx* is effective as anticancer agent as well (Mikami *et al.* 2013). Clinical tests have also shown that this pigment effectively reduces visceral fat and insulin in blood of animals and humans (Abidov *et al.* 2010). Noteworthy, easily assimilated xanthophylls are not synthesized by animal tissues, therefore, they should be taken with food (Schoefs 2002).

Earlier, *Fx* was identified and isolated from edible brown algae *Sargassum siliquastrum*, *Hizikia fusiformis*, *Undaria pinnatifida* (Peng *et al.* 2011) and from the Black Sea *Cystoseira* sp. (Nekhoroshev 2000). Nowadays there is a growing need in finding more resources for *Fx* and in designing modern analytical methods for rapid and accurate identification and quantification of carotenoids.

Coastal areas of the Black Sea provide natural habitats for *C. barbata* and *C. crinita*, two structure-forming species of benthic phytocenoses. According to A.A Kalugina-Gutnik (Kalugina-Gutnik 1975) and E.I. Blinova (Blinova 1979), in the 1960-70s the stock of these algae inspected along the integral Soviet shoreline was assessed as 2 million tons, including 687.810 tons in Crimea. Later, the algal stock has decreased to 1.2 million tons (Maksimova and Lucina 2002). Despite general degradation of *Cystoseira* growth, we suppose that with adoption of rational nature exploitation standards these brown algae can be used for industrial production of *Fx*. This paper discusses a possible scenario.

Materials and Methods

The investigation was carried out in September 2013 in Martyn Bay, Sevastopol (44°36'55.8"N, 33°30'12"W) Samples of *C. barbata* were collected from the water of 1 m depth. The freshly collected thalli were disjointed to stem and branches, their age was determined according to Prazukin (1983). The branches were divided into five age groups: to 1.9 month old; from 2 to 3 months old; from 3 to 5 months old; from 5 to 6 months old; and older than 6 months. Further, branches were cleared out of visible epiphytes, washed in distilled water to remove salt, dried with absorbent paper at room temperature and then broken into 8–15 mm pieces. To determine dry mass, the sample was placed into a desiccator at 105 °C for 4 hours.

For analysis, 5 g algal samples were twice extracted with 15 ml ethanol at room temperature for two days. Then the portions of extract were pooled together and subject to preparatory thin-layer chromatography on the glass slides (20×20 cm) covered with 0.5 mm thick silica gel [Kieselgel 60 G, Merck No. 7731 (45 g) and distilled water (90 ml)]. In the chromatographic examination acetone–hexane (3:7) was used. *Fx* fraction was removed and dissolved in ethanol. Spectra of *Fx* were determined by a spectrophotometer SP-2000; its content (*Fx*, mg) in alcoholic extract was calculated by equation (Campbell 1969):

$$Fx = \frac{D \cdot V \cdot 10}{E_{1cm}^{1\%}}$$

where D is the absorbancy at 448 nm wavelength; V is the volume of extract (ml); $E_{1cm}^{1\%}$ = the extinction coefficient equal 1280 (Kanazawa *et al.* 2008). Concentration of Fx (C_{Fx}) was calculated per 1 g of dry algal mass.

Earlier we isolated crystalline Fx from *C. barbata* and to identify it by means of high-performance liquid chromatography (HPLC) and nuclear magnetic resonance (HNMR) at the Research Institute for Production Development (Kyoto, Japan).

Results and Discussion

Macro-morphologic architecture of *C. barbata* is composed of an old trunk and branches of different age. Branches are the structural elements of special importance because they form photosynthetic surface and contribute 40–80 % to the total mass of the plant (Blinova 1979). Moreover, as our previous investigations, branches, compared to stem, are richer with Fx – 1.2–3.0 mg/g of dry mass vs. 0.466 mg/g of dry mass.

Fx measured in the ontogenetic series of *C. barbata* branches of the 1st order shows a single-peak distribution; the maximum (3 mg/g of dry mass) was registered in the age group of 2–3 months (Figure 1). Branches of this age group are equipped with a complete set of axial structures swiftly increasing in number, by total mass and total surface area during this ontogenetic interval (Prazukin 1983) and showing high photosynthetic rate (3 mcg C·mg⁻¹·h⁻¹).

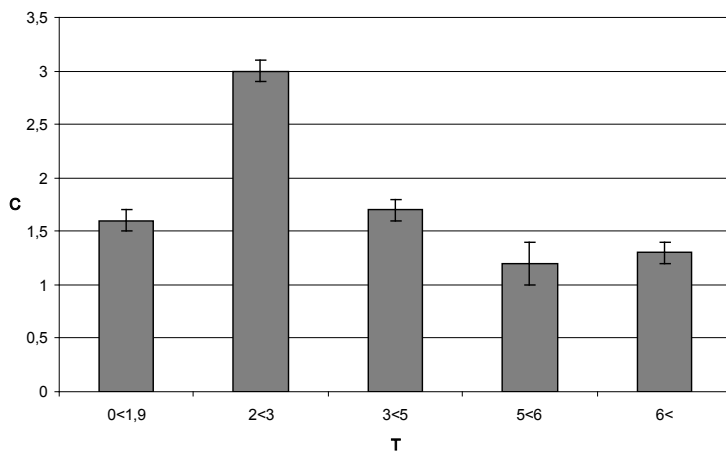


Figure 1. Fucoxanthin content in 1st order branches of *Cystoseira barbata* depending on age group; T – age (months), C – fucoxanthin (mg/g)

In the age groups of $0 < 1.9$ months and older than 3 months, Fx estimates were notably less than the C_{Fx} maximum. In the younger branches it was because their axial apparatus was not fully formed, therefore functionally active axial structures of higher branching orders at that time were either absent or, being still growing, could only inconsiderably contribute to total mass of the branch. In branches of the older group low estimates of C_{Fx} were due to natural decay with age (Prazukin 1983). Noteworthy, in both age groups photosynthetic rate was relatively low ($0.5-0.8 \text{ mcg C}\cdot\text{mg}^{-1}\cdot\text{h}^{-1}$).

Assessing Fx in thalli of each age group, we took into consideration ontogenetic changes in their morphological structure, primarily the ratio between trunk mass and integral branch mass, and used the averages of Fx content determined for the stems and branches (0.466 and $1.633 \text{ mg}^{-1}\cdot\text{g}^{-1}$ of dry mass, correspondingly) (Figure 2).

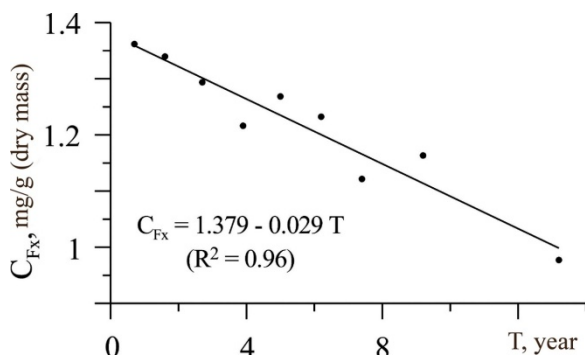


Figure 2. Fucoxanthin content (C_{Fx} , mg/g dry mass) in relation to the ontogeny of thalli (T) of *Cystoseira barbata*

The earlier explored size-age structure of *C. barbata* populations growing at 1 m depth near the shores of Sevastopol suggests that the total amount of Fx there can range from 508 to 1810 mg per m^2 of the sea floor. Hence, rational exploitation of the natural *Cystoseira* growth (Khailov *et al.* 1992) allows harvesting the pigment of 254-905 mg per m^2 every four years, i.e., the sea bed 1 km long and 10 m wide would supply 2.5-9 kg of Fx .

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