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Nutritional characterization of the microalga *Ruttnera lamellosa* compared to *Porphyridium purpureum*

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ABSTRACT

Sustainable food and human health are the major concerns of the society in the last decades. Functional foods and nutraceuticals from natural sources such as microalgae are regarded as a solution. In this study the nutritional composition of *Ruttnera lamellosa* ACOI 339 has been evaluated and compared to the widely studied *Porphyridium purpureum*. *R. lamellosa* showed a proximate composition with 8.81% of protein and 43.88% of carbohydrate and also a convenient lack of fiber (0.94%). The strain revealed a lipid content of 2.68% with substantial amount of long chain polyunsaturated fatty acids, especially docosahexaenoic fatty acid (C22:6ω3 – DHA) representing 6.36% of total fatty acids. The intracellular polysaccharide is rich in xylose, and also a promising antioxidant capacity of 12.02 mg/L equivalent to ascorbic acid was detected as an additional feature of the valuable biomass.

1. Introduction

Innovation in functional food design and nutritional coaching programs aiming to promote health are under development [1] Concepts such as functional foods and nutraceuticals reshaped the entrepreneurial critical mass of the food industry [2]. There is no universally accepted definition for functional food, however the European Commission Concerted Action on Functional Food Science in Europe defined functional foods as “the food that besides its nutritious effects, has a demonstrated benefit for one or more functions of the human organism, improving the state of health or well-being or reducing the risk of disease” [3]. With this new perspective of advanced human nutrition towards health and longevity, there is growing demand for functional food and nutraceuticals from natural sources. So, the quest for organisms that are natural producers of nutritionally interesting compounds emerged to face the possibility of new naturally-derived products for human nutrition.

Among all natural sources, microalgae are resurfacing as a source of nutritional material [1,4]. These microorganisms are able to synthesize metabolites with enormous interest in the modern food industry such as fatty acids, carbohydrates, proteins, vitamins, amino acids and carotenoids. They also have the ability to grow rapidly, efficiently use natural and artificial light, and can be stimulated to synthetize and accumulate the compound of interest by manipulation of the culture conditions [5].

Porphyridium purpureum (Bory de Saint-Vincent) Drew & Ross is a spherical microalga with phycoerythrin as an accessory pigment, which provides the characteristic red color to the cells [6]. This species has reknown biotechnological interest due to the production of sulphated polysaccharides, phycoerythrin, protein and polyunsaturated fatty acids, mainly arachidonic (C20:4ω6 – AA) and eicosapentaenoic (C20:5ω3 - EPA) acids [7]. The biomass nutrient profile of this species has been characterized and it has the potential to be used for nutritional purposes due the diversity and amount of nutrients it contains [8].

Ruttnera lamellosa (Anand) Andersen, Kim, Tittley & Yoon is an understudied spherical golden microalga, surrounded by lamellate layers of mucilage [9]. It belongs to the order Isochrysidales, known for the importance of the genus *Isochrysis galbana* in aquaculture. *I. galbana* is widely used as feed for commercial rearing of many aquatic animals, particularly larval and juvenile mollusks, crustaceans and fish species due to its high ω3 polyunsaturated fatty acids content [10]. Also this organism accumulates EPA and docosahexaenoic (C22:6ω3 - DHA) ω3 polyunsaturated fatty acids, sterols, tocopherols and coloring pigments. Therefore, it is regarded as a promising source for the food industry. Other species of Isochrysidales, such as *R. lamellosa*, may be found equally interesting for that purpose.

In order to progress with microalgal-derived compounds as natural sources of functional foods and nutraceuticals, the nutrient composition of new microalgae strains is required. The aim of the present study was to evaluate the nutritional composition of *Ruttnera lamellosa* ACOI 339

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by studying its growth, biomass production, the proximate composition of biomass, fatty acid profile, polysaccharide production and corresponding monosaccharide composition, and also its antioxidant potential. Biomass nutrient profiles are already available for *P. purpureum*, enabling a direct comparison with published values. Nevertheless, this could lead to erroneous assumptions, since different methods used for biochemical profiling are known to yield variable results [11]. Therefore we included *P. purpureum* ACOI/SAG 13.80 strain in the study to reassess its composition in parallel with *R. lamellosa*, in order to provide an internal comparison model.

2. Material and methods

2.1. Establishment and culture conditions of microalgae

Porphyridium purpureum ACOI/SAG 13.80 and *Ruttnera lamellosa* ACOI 339 were obtained from the Coimbra Collection of Algae (ACOI) (www.acoi.ci.uc.pt). The strains were cultivated in a 2-step batch system, under defined conditions of 23 °C room temperature, 21.62 $\mu mol \cdot m^{-2} \cdot s^{-1}$ cool white light intensity and 16:8 light:dark photoperiod. The culture media used were M6 (brackish) and M5 (marine) at pH 8.5 (www.acoi.ci.uc.pt) for *P. purpureum* and *R. lamellosa*, respectively. The cultivation started with a mother-culture, established by diluting a dense culture of the strain 1:1 (v/v) with fresh culture medium. After 5 days, 100 mL of this mother-culture was used to inoculate the culture, and fresh medium 1:1 (v/v) was added. The strain was then cultivated for 15 days with air supply provided by air bubbling at a controlled rate of 0.5–1 L/min, in triplicate (3 batches). For total biomass determination, both strains were cultivated in a vertical column photobioreactor with air supply, starting by diluting 5 L of mother-culture with fresh medium, 1:1 (v/v), then cultivated in standard conditions during 15 days. All analysis were performed on both strains.

2.2. Microalgal growth analysis

The growth curve was determined for both strains during 21 days by the establishment of triplicate cultures. At days 0, 3, 6, 9, 13, 17, 21, dry weight (g/L) was determined by filtering three samples of 10 mL of each replicate culture (Wathman GF/C), rinsing with 20 mL of distilled water and drying at 60 °C. Filters were kept in a desiccator for moisture stabilization until the weight was determined. The initial and final cell density was determined by cell counting (cel/mL) using a Neubauer haemocytometer. The specific growth rate of both strains was determined according to Mohsenpour and Willoughby [12] using the equation $\mu = \ln (X_t / X_0) / (t - t_0)$, where μ is the specific growth rate (day), X is the biomass concentration (g/L) and t is the number of days respectively. At the time of analysis the strains were at the stationary phase of development.

2.3. Biomass production

The biomass production was performed in triplicate cultures. Determination of dry weight was performed at the beginning (day 0) and at the end (day 15) of cultivation in triplicate samples from each replicate culture. A volume of 10 mL of culture was centrifuged at 4500 rpm for 15 min and the pellet was washed with distilled water, dried at 60 °C and kept in a desiccator until the weight was determined. The biomass production after 15 days was calculated as the difference between dry weight at day 15 and day 0 and expressed in g/L.

2.4. Proximate composition analysis

For proximate composition analysis the strains were cultivated in a 10 L volume (15 L photobioreactor), as described previously. The biomass was harvested by centrifugation at 4500 rpm for 15 min and the pellet washed with distilled water and dried in an oven at 60 °C. The biomass was characterized based on Isaac [13] to quantify moisture, ash, lipid, protein, crude fiber and nitrogen free extract (mainly carbohydrates). Each parameter was determined in triplicate. Moisture was determined by drying 2 g of biomass in an oven with air circulation at 100–105 °C until the weight was constant. Total ash was determined by incineration of 0.5 g biomass in an oven at 550 °C. Lipids were determined by Soxhlet with hexane as the extraction solvent. The crude protein was determined by total organic nitrogen (N) using a Kjeldahl apparatus. The digestion was performed with sulfuric acid. Estimation of the total protein was calculated multiplying the total N by 4.78 [14]. The dietary fiber was determined by hydrolysis of carbohydrates and proteins by a boiling treatment with sulfuric acid and filtered using a filter crucible (G2), where the residue was retained. Nitrogen-free extract (NFE) was determined by calculation of the difference of the previous values according to the following expression: NFE% = 100 − (% ash + % lipid + % crude fiber + % crude protein).

2.5. Fatty acid profile

The fatty acid content was determined according to Lim et al. [15] with some modifications, in triplicate cultures. After 15 days of cultivation, the biomass of each replicate culture was harvested by centrifugation at 4500 rpm for 15 min and total lipids were extracted from the pellet by adding 1 mL of hexane and 0.4 mL of methanol assisted by ultrasound (35 kHz water bath). The combined fatty acids extracted were converted to methyl esters of fatty acids (FAMEs) by transesterification with addition of 100 μL of sodium methoxide (2 M) and analyzed by gas chromatography (GC) performing two injections for each replicate culture. The gas chromatography was performed in a Chrompack CP 9001 chromatograph equipped with a flame ionization detector and a TR_CN 100 capillary column (60 m × 0.25 mm × 0.20 μm). Helium was used as carrier gas at a pressure of 150 kPa at the top of the column and the temperature of the injector and detector was 260 °C. The temperature of the column oven program was as follows: the initial temperature was maintained at 90 °C for 7 min after the injection, and then increased by 5 °C/min to 220 °C and held for more 15 min. The sample (1 μL) was injected using a Hamilton syringe in splitless injection mode. Peaks of fatty acids (FA) were identified by comparison of the relative retention times (RRT) with an external authentic standard, 37 Component FAME Mix (SupelcoTM). Quantification of FA was based on the internal standard method described by Lim et al. [15]. Internal standards used were a mixture of n-hexadecane and methyl pentadecanoate with final concentration of 0.03 mg/mL. The results were expressed in percentage of total FAME (%).

2.6. Polysaccharide extraction and identification of monosaccharides

Extraction of polysaccharides was performed for each replicate of the culture. Each 200 mL replicate was centrifuged at 4500 rpm for 15 min, the supernatant was recovered and the biomass pellet was washed with distilled water, dried at 60 °C and stored.

2.6.1. Exopolysaccharide extraction

The exopolysaccharides were directly precipitated by adding absolute ethanol to the supernatant. The mixture was passed through a 0.10 mm sieve, the exopolysaccharides were collected, dried at 60 °C and stored until used.

2.6.2. Intracellular polysaccharide extraction

The dried biomass pellet was pre-treated with 40 mL of a mixture of methanol: acetone, 1:1 (v/v) in an ultrasonic bath for 2 h to eliminate the organic-soluble fraction, and dried at 60 °C until complete evaporation of the solvents [16]. An alkaline treatment of the biomass was done by adding 150 mL NaOH (0.1 M), and then inserted in a water