



Original Contribution

**BIOCHEMICAL PROFILE OF GREEN AND RED ALGAE –
A KEY FOR UNDERSTANDING THEIR POTENTIAL APPLICATION
AS FOOD ADDITIVES**

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ABSTRACT

PURPOSE: With the improvements of the microalgal cultivation industry, it became possible to add algal biomass and its metabolites in foods in order to create a balanced and health-food. **METHODS:** By determining the growth and the biochemical composition (lipids, carbohydrates and proteins), two algal strains were evaluated as a potential source of food additives – the newly isolated strain of the green algae *Scenedesmus sp.* and the red algae *Porphyridium cruentum*). **RESULTS:** It turned out that in *Scenedesmus sp.* BGP the most abundant component were the proteins (up to 45 %), which makes this alga an excellent unconventional protein producer. As opposed to it, the biochemical composition of *Porphyridium cruentum* was dominated by carbohydrates (up to 57%), but there was also a high content of some essential polyunsaturated fatty acids: arachidonic (AA, 20:4; 13-29%) and eicosapentaenoic acid (EPA, 20:5, 24-25%). **CONCLUSIONS:** Microalgae are a really remarkable source of biomass and a wide range of substances, but this area is poorly explored. Both of the microalgal strains proved to be important sources of functional ingredients that could be successfully used as food additives together or separately.

Key words: *Scenedesmus*, *Porphyridium*, protein, carbohydrate, arachidonic acid, eicosapentaenoic acid

INTRODUCTION

Microalgae have been used for a long time as food for humans and for animals in aquaculture. With the increasing of world's population and predictions of an insufficient protein supply, in the early 1950's started a search for new alternative and unconventional protein sources. Algal biomass proved to be a good candidate for this purpose. Due to their relatively simple morphological organization, fast growth, high productivity and enormous and diverse biosynthetic potential, microalgae became desirable research objects. Improvements in the microalgal biotechnology give opportunity to add microalgal biomass and its metabolites in foods in order to create balanced and health-food (1). Microalgae for

human nutritional needs are currently being manufactured in different forms such as tablets, capsules, pastilles, liquids and nutritional supplements and are also incorporated into snacks, pastas, candy bars or chewing gum and in beverages (1, 2).

Microalgal biomass consists of different nutritional components of which the main three are proteins, carbohydrates and lipids. Some of the reasons why microalgae came to be of a such commercial importance in relation to its nutritional composition are presented in this article. The presence of high protein content in microalgae is the main reason why it should be kept in mind as an unconventional source of protein. Their amino acid pattern compares favorably with other foods. Carbohydrates are obtained in various forms such as starch, glucose, sugars, other polysaccharides. This fact explains why there are no limitations to its use in food and animal feed (3, 4).

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Scenedesmus sp. is particularly found to contain all essential amino acids and a good amount of protein, lipid and essential minerals (5). According to (3) and (6), *Scenedesmus* contains lipids, proteins and carbohydrates that can compare favorably with the traditional sources (**Table 3**).

Scenedesmus is among the most used microalgae (without any toxic impacts or abnormalities in experiments with test animals) that has attracted the attention of manufacturers in the food and health-food market (7). Another promising microalga for commercial purposes is the unicellular red alga *Porphyridium cruentum* (Rhodophyta). This alga has spherical cells that lack a cell wall and instead it has a polysaccharide capsule (8). This feature helps its assimilation by the animals and humans. The red microalgal polysaccharides exhibit various bioactivities that have nutritional, medical, and cosmetic significance. Animal feeding experiments have shown that rodents whose diets are supplemented with low concentrations of red microalgal polysaccharides have considerably lowered levels of serum cholesterol, triglycerides, and low-density lipoprotein levels (9, 10) with no evidence of toxic side effects. This strain is a potential source for several products, such as lipids (11), pigments (7) and carbohydrates (12). Only plants and microalgae are able to synthesize essential ω -

3 and ω -6 essential PUFA. Therefore, microalgae supply entire food chains with these vital components. One of the innovative approaches is the preparation of eicosapentaenoic acid (EPA) and arachidonic acid (AA) from microalgae (like *Porphyridium sp.*) for baby food and the health food market (13).

The aim of the study was to prove the suitability of the two algal strains as sources of functional ingredients by determining their biochemical composition. The high protein content of *Scenedesmus sp.* BGP and the production of polyunsaturated fatty acids by *Porphyridium cruentum* are the characteristics that reveal the potential of the strains as food additives.

MATERIALS AND METHODS

Algal material and cultivation conditions

Scenedesmus sp. strain BGP (Chlorophyta) was isolated from a rainwater puddle (Sofia, Bulgaria). Monoalgal, non-axenic cultures of *Scenedesmus sp.* BGP were grown autotrophically on $\frac{1}{4}$ medium of (14), modified by (15) (**Table 1**). The red microalga *Porphyridium cruentum* (Rhodophyta), strain VISCHER 1935/107 was acquired from the culture collection of the Institute of Botany, Trebon, Czech Republic and was grown on the modified culture medium of (16) (**Table 2**).

Table 1. Composition of the modified nutritive medium, used for cultivation of *Scenedesmus sp.* BGP.

Elements	[mg/l]
NH ₄ NO ₃	400
CO(NH ₂) ₂	300
KH ₂ PO ₄	170
MgSO ₄ ·7H ₂ O	494
CaCl ₂ ·2H ₂ O	5.478
H ₃ BO ₃	1.545
CuSO ₄ ·5H ₂ O	0.623
Fe ₂ (SO ₄) ₃ ·9H ₂ O	7.025
MnSO ₄ ·4H ₂ O	0.558
ZnSO ₄ ·7H ₂ O	0.718
CoSO ₄ ·7H ₂ O	0.703
(NH ₄) ₂ MoO ₄ ·7H ₂ O	0.605
NaHCO ₃	2000

Table 2. Composition of the modified nutritive medium, used for cultivation of *Porphyridium cruentum*

Macroelements	[g/l]
KH ₂ PO ₄	0.8
KNO ₃	1
NaCl	27
MgSO ₄ .7H ₂ O	6.6
MgCl ₂ .6H ₂ O	5.6
CaCl ₂ .2H ₂ O	1.5
NaHCO ₃	0.04
Microelements	[mg/100ml]
ZnCl ₂ *	4
H ₃ BO ₃ *	60
CoCl. 6H ₂ O*	1.5
CuCl. 2H ₂ O*	4
MnCl ₂ . 4H ₂ O*	40
(NH ₄) ₆ Mo ₇ O ₂₄ . 4H ₂ O*	37
FeCl ₃ . 4H ₂ O**	240

*All the components are dissolved in 100 ml dH₂O. 1 ml of the solution is added to 1L medium.

** Dissolved in 100 ml of 0.05 M Na₂EDTA. 1 ml of the solution is added to 1L medium.

The experiments were carried out on a specially equipped block. The cultures, in 200 ml flasks, were continuously supplied with carbon dioxide (2% in air) at a constant temperature (about 24°C) and illumination - 8000 lux. Initial culture densities of ~ 0.6 g/l and ~ 0.8 g/l was used for all the treatments of *Scenedesmus sp.* BGP and *Porphyridium cruentum*, respectively. To analyze the biochemical composition, experimental cultures were harvested in both exponential and stationary growth phase. Cells were collected by centrifugation (5000×g, 20 min), rinsed three times with water, frozen, and stored at -70°C. The concentration of the dried algal biomass (dry weight, DW) was determined gravimetrically. The growth of *Scenedesmus* and *Porphyridium* was evaluated by the increase in the DW. The protein content was measured according to the method of (17). The lipid content was determined after extraction with a mixture of methanol and chlorophorm (18). The carbohydrates were quantified using the phenol-sulfuric acid method of (19). The viscosity of the culture's supernatant indicated the amount of extracellular polysaccharide synthesized. It was measured by a viscosimeter B3 (VEB MLW, DDR). The algal culture was centrifuged for 30 min. at 8000 g and the cells were removed. The supernatant's viscosity was determined by the following formula:

$$\eta = t(Q_1 - Q_2) K \quad (1)$$

where η is the dynamic viscosity of the liquid (mPa.s); t – the time for the ball to fall in the viscosimeter; Q_1 - ball density; Q_2 - density of the studied liquid, K - ball constant (m Pa.cm³/g).

All experiments were conducted in three repetitions.

RESULTS

Growth *Porphyridium cruentum* is easily cultivated in an artificial seawater medium. The green algae of genus *Scenedesmus* are commonly found in fresh waters. Both strains are very suitable producers of biomass as they have a high tolerance towards variation of the most important environmental factors such as light intensity, temperature, pH, as well as the content of the nutrient medium (20). The cultivation time is an important factor for increasing the biomass concentration (11). The growth curves of *Porphyridium cruentum* displayed the expected characteristics with a lag phase during the first one or two days, an exponential growth phase till the 120th h., and stationary phase when growth reached it maximal value (**Figure 1**). The peak of the cell concentration of the *Porphyridium cruentum* culture was reached on the 144h. The microalgal biomass increased from 0.8 to 5.15 g/l.

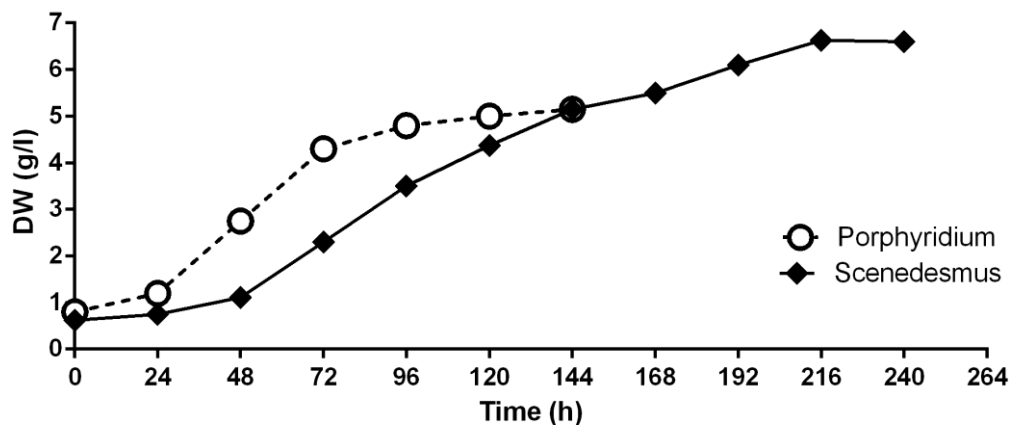


Figure 1. Growth of *Porphyridium cruentum* and *Scenedesmus sp.* BGP at intensive conditions for 144 h.

The growth of *Scenedesmus sp.* BGP started with a short lag phase (Figure 1). After that it continued with a rapid exponential phase between the 48th and 144th hour when it slowed down but reached the maximal yield of 6.6 g/l on the 216th hour after which the culture entered stationary phase.

Extracellular polysaccharide production of *Porphyridium cruentum* Our results for polysaccharide production of *Porphyridium cruentum*, presented on Figure 2, showed that the viscosity grew from 1.8 mPa.s (0h) to 5 mPa.s at the end of the experiment (144h).

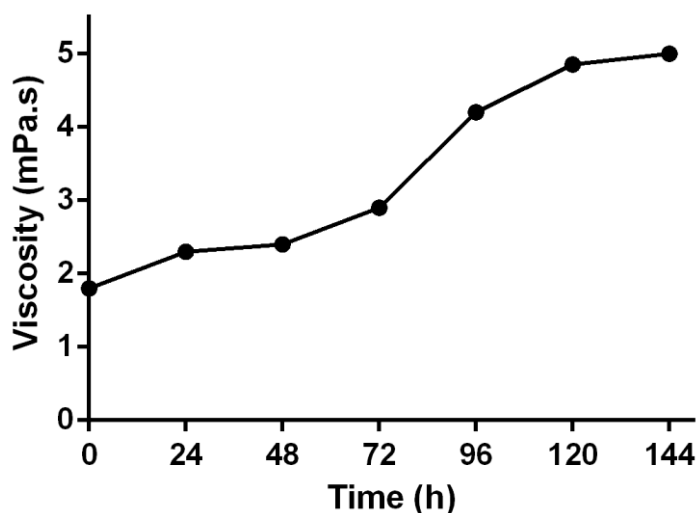


Figure 2. Extracellular polysaccharide production of *Porphyridium cruentum* at intensive conditions.

Biochemical characteristics of *Porphyridium cruentum* and *Scenedesmus sp.* BGP

Table 3. Biochemical characteristics of two species of microalgae depending on the growth conditions.

Biochemical components	Percent from dry weight	
	<i>Porphyridium cruentum</i>	<i>Scenedesmus sp.</i>
Proteins	27-38 %	24 – 45%
Lipids	9-12%	23%
Carbohydrates	40-57 %	25 - 28 %

The results revealed that the protein content of *Porphyridium* reached up to 27-38% on the 120h (**Table 3**). *Scenedesmus* sp. BGP revealed high values in the range of 24 – 42% during the different growth phases (**Table 3**).

The accumulated quantity of carbohydrates under intensive cultivation of *P. cruentum* was between 40-57% of DW (**Table 3**). The other microalgae – *Scenedesmus* sp. BGP also produced this biochemical component in the range of 38-45% (**Table 3**).

Our results revealed that lipid content of *P. cruentum* is between 9-12 % (**Table 3**). *Scenedesmus* sp. BGP showed ability to produce a better amount of lipids – around 23% that changed with less than 1% during the cultivation (**Table 3**).

The alga *P. cruentum* accumulated large amounts of fatty acids (**Table 4**), especially AA (20:4 ω 6; accumulated to 36% of total fatty acids) and noticeable amounts of EPA (20:5 ω 3; (21). *Scenedesmus* sp. is rich in oleic and linoleic acids (**Table 4**, 22).

Table 4. Fatty acids composition of *Porphyridium cruentum* and *Scenedesmus* sp. (22).

Fatty acids% of Lipids	<i>Porphyridium cruentum</i>	<i>Scenedesmus</i> sp.
16:0 palmitic acid	27-32	30.3
16:1 palmitoleic acid	0.3-1.8	6.5
18:1 oleic acid	0.6-1	17.5
18:2 linoleic acid	4-8.3	21.1
20:2 eicosadienoic acid	0.6-1.2	0.3
20:4 arachidonic acid	13-29	-
20:5 eicosapentaenoic acid	24-25	0.8

DISCUSSION

The growth rate of both microalgae was almost the same ($[\mu] = 0.27$ and $[\mu] = 0.31$). They showed fast adaptation to the growth conditions and sufficient biomass accumulation was observed. In general, the data for the biochemical composition of *Porphyridium cruentum* were similar to those obtained by (23). The carbohydrate content can be increased after different modifications (24). Our results showed that the cultivation of both microalgae as perspective producers of carbohydrates is in order. Some authors obtained larger amounts of lipids up to 19% (**Table 3**) (11). Although the percent of lipids in the *Porphyridium* cells is not very high, the qualitative composition (polyunsaturated fatty acid) is what makes the lipid content of this red alga extremely valuable for the food industry. When it comes to *Scenedesmus* sp. BGP, the strain showed to be a better producer of protein than *S. obliquus* and *S. dimorphus* (6-18 %) (25). The produced amount of protein from our strain in up to 45%, which creates a field for perspective potential application of this alga as an unconventional source of protein. From other microalgae from *Scenedesmus* sp. (*S. obliquus* and *S. dimorphus*) were extracted 6-14% lipids (23,6), which is less than what *Scenedesmus* sp. BGP is able to produce. This stable biochemical composition combined with

higher amount of produced proteins than in other microalgae from the same genus, made the strain desirable for biotechnological studies.

The principal use of oleic acid as a component in many foods, is in the form of its triglycerides. It is a component of the normal human diet as a part of animal fats and vegetable oils. Linoleic acid is an essential fatty acid that must be consumed for proper health. A diet only deficient in linoleate (the salt form of the acid) causes mild skin scaling, hair loss and poor wound healing in rats (26). It is of a paramount importance to optimize the growth conditions of microalgae to maximize EPA and AA production (27).

AA and EPA were the dominating polyunsaturated fatty acids (PUFAs) in *P. cruentum*. Nutritionally, EPA (20:5) is one of the most important fatty acids belonging to this group. These long chain omega-3 fatty acids provide significant health benefits to the human population, particularly in reducing cardiac diseases, stroke and high blood pressure, depression, rheumatoid arthritis, and asthma. They have also been reported to inhibit tumor growth (28).

Currently, the sulphurized polysaccharides (EPS) have received much attention due to

their antibacterial, anti-oxidative, and anticancer properties (29). During cultivation, the cells of *Porphyridium cruentum* excreted EPS, which caused the cultures to become viscous, which would have been more visible under limiting conditions. Since EPS are released into the culture medium, they can easily be recovered and purified (30). The results were confirmed by (31), who established that viscosity increase at the end of the exponential phase. They also found out that the amount of polysaccharide depends on the intensity of the light.

CONCLUSION

Microalgae provide numerous opportunities for developing healthier food products.

As the research showed, both of the strains have balanced biochemical content which proves them to be important sources of functional ingredients that could be successfully used as food additives together or separately.

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