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APPLIED ASPECTS

Seasonal Changes in the Content of Lipids and Photosynthetic Pigments in a Brown Alga *Saccharina cichorioides*

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Abstract—Seasonal changes in the contents of lipids and photosynthetic pigments (PSP) were investigated in a brown alga *Saccharina cichorioides* Miyabe (Phaeophyceae, the family Laminariaceae). The content of lipids varied from 0.27 to 0.60% of the algal fresh weight. The content of glyceroglycolipids (GL) was much greater in the time of spore formation (June–July and September–October), phospholipids – in the spring and in September–October, and the content of neutral lipids – in the spring and in November. In the period of spore release (August and October), the level of GL and polyunsaturated fatty acids (PUFA) sharply decreased. A high level of PUFA was observed from March to July and in November. In August and October, the same as in the spring, the proportion of saturated fatty acids (FA) was great. The content of chlorophylls from March to November varied from 20.3 to 26.9%, and the level of carotenoids – from 10.7 to 16.1%. Total content of PSP was relatively high in March and in August–September. Free sterols accounted for 3.4–7.3% of total lipids; their proportion was greater in spring than in summer and autumn.

Keywords: Saccharina cichorioides, brown algae, fatty acids, glyceroglycolipids, phospholipids, photosynthetic pigments, free sterols, gas-liquid chromatography

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INTRODUCTION

Recently, *Saccharina cichorioides* Miyabe (in Algaebase, referred to as *Laminaria cichorioides*) has become a source of biologically active substances. For instance, it was used as a source of fucoidan showing anticoagulant activity [1]. Aqueous alcoholic extract produced from *S. cichorioides* suppressed the growth of cancerous cells in human intestine [2]. Total lipids, particular classes of lipids and their mixtures, fatty acids (FA), and photosynthetic pigments (PSP) showed antimicrobial, hemolytic, cytotoxic, and embryotoxic activity, with the degree of activity depending on the season [3, 4]. Therefore, *S. cichorioides* may be considered as a promising raw material for the production of pharmaceuticals and the investigation of the periods of accumulation of particular compounds of practical importance.

Numerous researchers have shown that the content of soluble carbohydrates, polysaccharides, micro- and macromolecules, lipids, sterols, proteins, and amino acids in the algae vary depending on the season [5–8]. This can be accounted for by such factors as the stage of the life cycle, temperature, light intensity, photoperiod, and water salinity, which influence the algal metabolism [9]. The investigation of changes in their chemical composition is important for understanding the vital functions of algae and their adaptation to changing environmental conditions. The composition of lipids and FA in *S. cichorioides* is studied in the works of Khotimchenko et al. [10, 11]; however, there are no reports about seasonal changes in the content of lipids and their classes as well as FA, free sterols (FSt), and PSP. In this work, we publish the appropriate results.

MATERIALS AND METHODS

The samples of the brown alga *Saccharina cichorioides* (Miyabe) Lane, Mayes, Druehl et Saunders were collected in Trinity Bay, Peter the Great Gulf, the Sea of Japan, at the experimental marine station, Pacific Institute of Bioorganic Chemistry, Far East Branch, Russian Academy of Sciences (Maritime Territory, Khasan region) at a depth from 4 to 8 m. The material was collected from March to November. Each sample

Abbreviations: DGDG—digalactosyldiacylglycerols; FA—fatty acids; FAME—FA methyl esters; FFA—free fatty acids; FSt—free sterols; GL—glyceroglycolipids; MGDG—monogalactosyldiacylglycerols; MGMG—monogalactosylmonoacylglycerols; NAP—N-acyl pyrrolidides of FAs; NL—neutral lipids; PA—phosphatidic acid; PC—phosphatidylcholines; PE—phosphatidylethanolamines; PG—phosphatidylglycerols; PHEG—phosphatidyl-O-(N(2-hydroxyethyl) glycines; PI—phosphatidylinositols; PL—phospholipids; PSP—photosynthetic pigments; SFA—saturated fatty acids; SQDG—sulfoquinovosyldiacylglycerols; TAG—triacylglycerols; TL—total lipids; UI—unsaturation index; USFA—unsaturated fatty acids.

contained 2–3 thallus 1.3–2.0 m in length. From July to October, blades carried sporangia, and in November they showed the signs of destruction and discoloration.

Collected algae were bathed in running water, blotted with filter paper, minced, and mixed. From the sample of 0.3 kg, lipids were consecutively extracted with ethanol, mixtures of ethanol with acetone (1 : 1, v/v) and ethanol with chloroform (1 : 1, v/v) as described earlier [12].

Total lipids (TL), phospholipids (PL), glycerolglycolipids (GL), neutral lipids (NL) and their particular classes, as well as free sterols (FSt) and photosynthetic pigments (PSP) were assayed as described earlier [12].

FA methyl esters (FAME) were produced by means of lipid transesterification according to Prevot and Mordret [13]. From 5 to 7 mg of TL in 2 mL of hexane was supplemented with 0.4 mL of 2 M KOH in methanol; the mixture was stirred for 10 s and heated for 30 s at 50°C. Subsequently, the mixture was supplemented with 0.4 mL of 2 M HCl in methanol and shaked for 60 s at room temperature. The mixture was left for 1–2 min to allow phase separation. The upper hexane phase containing FAME was collected. FAME were purified by TLC on silica gel plates (silica gel 60_{F254}, Merk, Germany) using a hexane/benzene (70 : 30, v/v) system of solvents. In order to determine the position of double bonds, we used pyrrolidine derivatives of FA (N-acyl pyrrolidides, NAP). NAP were produced by the direct treatment of FAME with a mixture of pyrrolidine with acetic acid (10 : 1, v/v) at 80°C for 45 min [14] and purified by TLC on silica gel plates in a mixture of hexane/diethyl ether (1 : 2, v/v). FAME were assayed by means of GLC using an Agilent 6890 gas chromatograph equipped with a flame ionization detector. HP-5ms column with 5% phenylmethyl siloxane was used as a stationary phase (30 m × 0.25 mm, Agilent, United States); helium was used as a carrier gas with the flow rate of 1.3 mL/min. In order to determine FAME, we used the following temperature gradients: 190°C for 15 min followed by elevation to 250°C at a rate of 3°C/min and 10 min at 250°C. FAME and NAP were determined by GLC-MS using an identical device equipped with an HP 5973 quadrupole mass selective detector; electron impact type of fragmentation, 70 eV, with the use of column and conditions of chromatography described above.

The unsaturation index (UI) of FA was determined according to the formula: $UI = \Sigma c_i n_i$, where n_i –the number of double bonds in a particular FA, c_i –the relative content of FA in question.

The means and their standard deviations (%) for each analyzed component shown in tables were calculated for 3 replications using Microsoft Excel computer program. Standard deviations (±0.05–0.5% of the mean value) are not shown in tables.

RESULTS AND DISCUSSION

The effect of the season on the content and composition of lipids was investigated only in few brown algae belonging to the order Laminariales growing on the Asiatic shore of the Pacific Ocean, specifically in the perennial alga *S. japonica* [6, 15] and in annual algae *Costaria costata* [16] and *Undaria pinnatifida* [12]. It is worth noting that such investigations were conducted for only a small group of brown algae living in the World ocean [7, 17–19]. Perennial brown alga *S. cichorioides*, we have investigated, extends the list of studied algae.

Some researchers showed that algae are sensitive to the temperature, biogen concentration, light intensity, water salinity, and pH [9, 20]. The content of lipids therein is not permanent and considerably changes during the year under the effect of these factors and depending on the age of algae. The levels of structural and storage lipids vary, with most significant changes occurring in the composition and content of FA [9]. Our investigation revealed marked seasonal changes in the content of total lipids as well as particular classes of lipids and PSP in *S. cichorioides*.

Under natural conditions, *S. cichorioides* actively grows in April–May. In the beginning of summer, the rate of growth decreases; sporogenous tissue develops at water temperature above 10–12°C. By July, the greater part of algae form developed sori. In natural population of *S. cichorioides*, zoospores are released from the end of summer to the autumn [21]. We found that the content of lipids in the tissues of mature algae varied during the year from 0.27 to 0.60% of algal fresh weight (Table 1). When water temperature was below zero (March) or in the range from 8 to 10°C (October), the content of lipids in *S. cichorioides* was the greatest. When vital functions were most active (April–May), the content of TL was lower, whereas in the periods of a relative rest (March, October) and sporogenesis (June–July), lipids accumulated. When the release of spores started (August), the content of lipids in the algae sharply decreased. In November when blades were distinctly destroyed and discolorated, the content of TL was also low. Dynamics of changes in the content of TL in *S. cichorioides* was identical to their time-course in the alga *S. japonica* investigated earlier by Honya et al. [6]. These researchers investigated a wider time span including winter months when algal population consisted of juvenile individuals where the content of TL was greater in autumn and winter with the peak in December. Thallus of *S. japonica* (the same as in *S. cichorioides*) actively grew from April to May; in this period, the researchers noted a decrease in the lipid content. Subsequently, changes in the content of TL were identical in both algae. In annual brown alga *Costaria costata* investigated earlier [16], we observed similar dynamics of the content of TL: the level of TL decreased in the period of active growth, and the

Table 1. Content of TL, particular classes of lipids, and PSP in *S. cichorioides* at different seasons

Substance	Time of collection								
	march	april	may	june	july	august	september	october	november
TL*	0.49	0.30	0.38	0.43	0.46	0.27	0.38	0.60	0.32
Total GL**	12.9	22.9	28.1	33.5	37.7	27.2	32.6	31.2	29.3
MGDG	6.1	10.2	11.9	16.3	18.5	8.9	13.8	13.1	8.7***
DGDG	2.6	5.9	7.0	8.0	9.0	10.3	8.2	8.0	8.0
SQDG	4.2	6.8	8.2	9.2	10.2	8.0	10.6	10.1	9.1
Total PL**	11.9	18.0	13.1	11.9	9.2	9.2	10.3	12.6	8.2
PC	7.8	5.8	6.1	6.1	5.8	3.5	6.2	5.8	5.3
PE	1.7	3.2	1.8	1.8	1.2	1.4	1.0	1.9	0.9
PG	2.2	7.0	3.1	3.7	1.9	3.0	2.8	3.0	1.2
PI + PA + PHEG (total)	0.2	1.5	0.8	0.3	0.3	1.3	0.3	1.1	0.8
Total NL**	29.6	24.6	22.3	16.5	15.3	17.9	18.0	18.8	22.7
TAG	21.2	16.4	13.4	11.5	10.6	11.5	11.7	11.6	18.4
FFA	1.1	1.3	2.1	1.0	1.0	0.8	0.6	1.4	0.9
FSt	7.3	6.9	6.8	4.0	3.7	5.6	5.7	5.8	3.4
PSP**	40.8	34.5	35.0	34.3	34.8	43.0	37.9	36.7	32.1
Chlorophylls	26.8	21.1	24.3	22.2	20.3	26.9	24.4	24.4	20.8
Carotenoids	14.0	13.4	10.7	12.1	14.5	16.1	13.5	12.3	11.3
Unidentified substances	4.8	–	1.5	3.8	3.0	2.7	1.2	0.7	7.7

* % of algal fresh weight.

** % of TL level.

*** The sample contains MGMG (3.5%).

accumulation of lipids occurred in the time of sporogenesis. The level of TL remained high by the end of sporogenesis. It is worth noting that in contrast to algae growing in moderately cold waters on the Asiatic shore of the Northern Pacific, in brown algae of Australian tropic waters there were no distinct species and seasonal variations in lipid content, although in summer the content of TL was somewhat greater [7].

The determination of lipid content at different seasons showed that in *S. cichorioides* TL contained the same groups of lipids as in *C. costata* [16] and *U. pinnatifida* [12]: GL comprised monogalactosyldiacylglycerols (MGDG), digalactosyldiacylglycerols (DGDG), and sulfoquinovosyldiacylglycerols (SQDG); PL—with phosphatidylcholines (PC), phosphatidylethanolamines (PE), phosphatidylglycerols (PG), phosphatidyl-O-(N(2-hydroxyethel) glycines (PHEG), phosphatidylinositols (PI), and phosphatidicacid (PA); NL—with triacylglycerols (TAG), free sterols (FSt), and free FA (FFA) (Table 1). In November when the thallus of *S. cichorioides* were destroyed, among GL we detected a lysoform of MGDG.

According to Khotimchenko et al. [11], in June–August the lipids of *S. cichorioides* mainly contained GL that accounted for 71% of TL; NL and PL accounted for 15.1 and 13.9%, respectively. At the same time, the researchers did not take into account the presence of PSP among TL, which distinctly overestimated the content of lipid components. The researchers did not specify the month when lipids were assayed. However, below we will show that, in certain summer months, the same as during the year, appreciable changes in the content of particular classes of lipids occurred (Table 1). The content of GL was the lowest in March. From March to July, it rose and reached peak values in June–July. In August, the content of GL significantly decreased; in September and October, it rose again; in November, it slightly decreased. The analysis of the content of particular GL classes showed that irrespective of the season MGDG predominated among total GL, which is characteristic of the majority of brown algae [22]. The content of GL of this class considerably changed depending on the season. In March, the content of MGDG was the lowest, the same as the level of DGDG and SQDG. In April and May, when vital functions of the algae were more active, the content of GL of all classes considerably increased. The peak content of MGDG was observed in the time of spore formation in June–July. In August when the spores were released, the content of MGDG decreased almost two times as compared with July, and in Sep-

tember and October it rose. In November when thallus destruction started, the content of MGDG decreased again but a lysoform of MGDG (MGMG, monogalactosyl monoacylglycerols) appeared. In the content of DGDG the same as SQDG, variations were also evident. The share of DGDG in GL rose from March to August, and share of SQDG – from March to July. The highest level of DGDG was observed in July–August. The share of SQDG in August considerably decreased and in September–October it rose again (Table 1).

Dynamics of PL content differed from the time-course of GL (Table 1). The content of PL was greater in spring and in October. In summer and especially in November, the level of PL decreased. Irrespective of the season, PC, PG, and PE predominated among PL. The peak of PC content was only in March. In April, its content decreased and then to July the level was essentially the same. In August, the content of PC decreased almost 1.5 times, and in September and October its content became the same as in April–July. In November, the content of PC in collapsing alga decreased. Variations in the content of PE were insignificant except for April when the level of PE was peaked. The content of PG was the greatest from April to June and from August to October, whereas in March, July, and November it was the lowest.

The level of NL was maximal in spring, very low in the period of spore formation in June–July, and starting from August their content rose again (Table 1). NL were predominantly accounted for by TAG. In spring, their content was peaked. The greatest level of TAG was found in March. When algal growth was the most active (April–May), their content decreased. The lowest level of TAG was observed in summer when the formation of spores started and remained low up to October. In November, the level of TAG rose again. The accumulation of TAG in spring was earlier observed by Nelson et al. [18] in a brown alga *Egregia menziesii*. At the same time, there was no general trend in TAG accumulation. For instance, in a brown alga *Fucus serratus* [17] TAG accumulated in summer and autumn, whereas in spring and winter their content decreased 4–5 times. In annual brown alga *C. costata*, we also found the higher level of TAG in July [16]. Different terms of accumulation of storage lipids are most likely associated with developmental biology of each algal species.

At different seasons, the content of FSt in *S. cichorioides* varied from 3.4 to 7.3% of TL (Table 1). Free sterols are known to act as regulators of liquid crystalline state of membranes affecting the flexibility of FA chains of membrane lipids. Data about seasonal changes in FSt content in brown algae are sparse except for the works of Japanese researchers. For instance, Terasaki et al. [19] noted the peak content of one of the major sterols (fucosterol) in brown algae from January to March in six species of brown algae in the Sea of Japan. In late spring as well as in summer and autumn months, the content of fucosterol considerably decreased depending on algal species. Similar changes in the content of sterols were earlier detected in *S. japonica* [6]. The same as in *S. japonica*, in *S. cichorioides* the proportion of FSt in spring was greater than in warmer summer and autumn months.

The level of PSP in *S. cichorioides* was high, and depending on the season it varied from 32.1 to 43.0% of TL (Table 1). As it was shown earlier, the content of PSP depended on light intensity: they accumulated in low light, and in high light the concentration of PSP decreased [9]. In Peter the Great Bay, the alga lives in locations ranging from the littoral zone to the depth of 20 m with strong current or surf, which ensures delivery of nutrients to the thallus and in the sites with sludgy bottom. Such places are known for reduced light intensity that apparently affects the activity of the photosynthetic machinery. The level of PSP was relatively high in March and August. They were predominantly accounted for by chlorophylls: 20.3–26.9% of TL (Table 1). In April, when the most active growth of algae started, the level of chlorophylls dropped to 21%, whereas in May and August–October it rose. A high content of chlorophylls in *S. cichorioides* at all seasons points to the metabolic activity of the alga during the year. The proportion of carotenoids varied from 10.7 to 16.1%: their content in the tissues of *S. cichorioides* was relatively high in March and in the months rich in sunshine (July and August).

During the tested months, the algae had identical composition of FA but differed in the content of main components and first of all of 14:0, 16:0, 16:1 n-7, 18:1 n-9 (together with 18:3 n-3), 18:2 n-6, 18:4 n-3, 20:4 n-6, and 20:5 n-3 acids (Table 2). The content of saturated FA (SFA) was 22.0–38.2% and that of unsaturated FA (USFA) – 61.8–78.0% of total FA. The UI slightly varied from March to August from 196 to 206. Only in September–November, its values fluctuated considerably: in September, the UI was 219, in October it decreased to 155, and in November it rose to 245. Among SFA, FA 16:0 predominated (up to 27.7%), the proportion of FA 14:0 was much lower (up to 10.0%), and the level of FA 18:0 and 20:0 was low. On the whole, the proportion of SFA was somewhat lower in summer and autumn as compared with spring.

Among USFA, the total concentration of 18:1 n-9 and 18:3 n-3 acids was high (Table 2); in August and October, it was much greater (24.5 and 30.2%, respectively) than in other months. Honya et al. [6] also showed that in closely related *S. japonica* the total content of 18:1 n-9 and 18:3 n-3 acids in August–October reached peak values (23.8–28.7%). A rise in the content of such acids as 18:1 n-9 was accounted for by the formation of sori. One should also note a considerable decrease in the content of 18:3 n-3 acid in fertile samples of *S. japonica*. The content of another monounsaturated acid 16:1 n-7 in *S. cichorioides* was low. Only in June, July, and September, the level of this

Table 2. Composition of fatty acids (% of total FA) in the lipids of brown alga *S. cichorioides* at different seasons

FA	Time of collection								
	march	april	may	june	july	august	september	october	november*
14:0	8.0	8.2	9.5	6.0	6.7	8.5	10.0	4.9	3.0
15:0	0.4	1.9	0.4	0.3	1.4	–	0.4	0.2	0.2
16:0	23.0	23.4	18.8	20.1	19.2	23.1	17.6	27.7	15.6
16:1 n-9	0.6	–	0.2	–	–	–	–	–	–
16:1 n-7	3.8	4.0	7.2	14.6	9.4	6.6	9.2	3.7	4.0
16:2 n-6	0.7	0.7	0.9	–	1.9	–	0.6	0.4	0.5
18:0	1.2	2.2	1.4	1.4	0.9	0.8	0.5	3.2	2.0
18:1 n-9**	17.9	16.8	16.1	13.6	15.3	24.5	11.1	30.2	19.6
18:1 n-7	1.1	–	0.9	1.4	1.1	–	0.5	0.5	1.3
18:2 n-6	9.4	9.3	8.8	10.1	8.5	6.7	8.6	9.4	8.6
18:3 n-6	2.0	3.4	5.5	3.7	8.3	0.4	6.8	2.4	5.3
18:4 n-3	8.0	9.3	7.8	6.2	5.5	5.7	6.3	2.0	5.5
20:0	0.4	0.4	0.4	0.3	0.3	0.2	0.4	0.9	1.2
20:2 n-9	0.5	–	0.1	0.2	–	–	0.4	0.7	1.5
20:1 n-9	–	–	–	–	–	–	0.2	0.2	–
20:4 n-6	13.0	11.3	11.1	8.5	9.8	11.1	13.1	7.0	15.5
20:3 n-6	0.4	–	0.4	–	–	–	0.5	0.2	–
20:4 n-3	0.5	–	0.5	–	–	–	–	–	0.8
20:5 n-3	9.1	9.1	10.0	13.6	11.7	12.4	13.7	5.1	15.2
22:0	–	–	–	–	–	–	0.1	1.3	–
ΣSFA	33.0	36.1	30.5	28.1	28.5	32.6	29.0	38.2	22.0
ΣUSFA	67.0	63.9	69.5	71.9	71.5	67.4	71.0	61.8	78.0
ΣPUFA***	42.5	42.4	44.2	42.3	43.8	36.3	50.0	27.2	52.4
UI	200	196	205	202	206	199	219	155	245

Dash—FA not detected.

* Contains FA 17:0 (0.2%);

** together with FA 18:3 n-3;

*** without FA 18:3 n-3.

acid reached 9.2–14.6%. The proportion of PUFA from March to July was considerable (Table 2). In August and October, when the algae released spores, the level of PUFA went down, and in September and November it increased. Honya et al. [6] also detected a high level of PUFA in *S. japonica* in March–July and in juvenile algae – in December–January. In spring and summer, the main PUFA in *S. cichorioides* were 18:2 n-6, 18:4 n-3, 20:4 n-6, and 20:5 n-3 acids. The relative content of 18:4 n-3, 20:4 n-6, and 20:5 n-3 acids considerably decreased in October, and in November it rose again. On the whole, *S. cichorioides* was notable for a high concentration of PUFA of (n-3) and (n-6) series. As Honya et al. [6] noted, seasonal changes in the content of FA in *S. japonica* differed from the changes in certain algal species: often the levels of SFA and MUFA therein were high in warm months while the level of PUFA was low. In *S. cichorioides* the same as in *S. japonica*, the levels of SFA and PUFA were high all the year round, and the proportion of PUFA was reduced at the time of reproduction.

S. cichorioides is rich not only in amino acids and fucoidans [23]. It is an important source of indispensable FA, for instance, linoleic acid that is not produced by human cells [24] and accumulates a relatively high content of 20:5 n-3 acid.

Thus, in *S. cichorioides*, we found significant differences in the content of total lipids and their particular classes, as well as in the content of PSP depending on the season and the stage of the algal life cycle. The extent of lipid unsaturation also depended on algal physiological state and surrounding temperature. The obtained data about the periods of the accumulation in *S. cichorioides* of particular classes of lipids, FA, and carotenoids are of applied interest. As we have shown earlier, algal TL, certain fractions of lipids and PSP

have antimicrobial activity and may be used in medicine and agriculture [3, 4].

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