Proc. Estonian Acad. Sci. Chem., 2001, **50**, 2, 95–103

ALGAL BIOMASS FROM *Fucus vesiculosus* (Phaeophyta): INVESTIGATION OF THE MINERAL AND ALGINATE COMPONENTS

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Received 3 November 2000

Abstract. Mineral composition of algal biomass from *Fucus vesiculosus* (the Baltic Sea, Estonia) is rich in light elements (Mg, K, Ca, Na) and halogens (Br, I). Arsenic content is also high (over 300 ppm); the content of other toxic elements (Hg, Sb, Se, etc.) is extremely low. Mineralization of the algal whole biomass does not depend on seasonal varieties. The content of α-L-guluronic acid residues, and therefore the viscosity of alginates from the Baltic *F. vesiculosus*, is relatively low. Viscosic properties depend significantly on the storing and processing conditions of biomass; the polymer chain of alginates from this brown alga species is especially sensitive to high isolation temperatures.

Key words: *Fucus vesiculosus*, mineral composition, alginates, viscosity.

INTRODUCTION

The brown alga *Fucus vesiculosus* is widely distributed in the Baltic Sea. In coastal regions of Estonia, the summary wet biomass of this seaweed exceeds 500 thousand tonnes [1].

In general, the brown algal biomass consists of three major types of components: (1) mineral or inorganic part, (2) alginates, (3) fucans and other carbohydrates. The first two of them will be discussed in this paper.

The mineral composition of brown algae is very rich quantitatively as well as qualitatively, and it has been widely investigated [2]. However, the available data on the content of various elements are not quite exact. Yet the amount of some

elements present, especially of toxic ones, is of interest from the standpoint of bioindication or use as food additives. As to the species *F. vesiculosus* from the Baltic regions, no systematic research on the composition of its microelements has been carried out.

Alginate is a family of polysaccharides composed of (1→4) linked residues of β-D-mannuronic acid and α-L-guluronic acid (Fig. 1) in varying proportions and sequences along the chain [3]. In the Phaeophyceae, alginates are found in cell walls in a crystalline arrangement parallel to the cellulose microfibrils and also in the intercellular matrix [4]. In general lines, the biosynthetic pathway of alginate in algal cells was elucidated about 20 years ago [5].

Many methods exist for analytical determination of uronic acids in alginates [6], but statistical arrangement of uronate residue blocks in the macromolecules is studied chiefly by methods of NMR [7, 8]. An absolutely detailed sequence of the residues along the chain cannot be determined at the present time.

In principle, the isolating process of alginates from brown algal biomass is simple, including stages of pre-extraction with hydrochloric acid (→ alginic acid), followed by washing, filtration, and neutralization with alkali (→ sodium alginate). Sodium alginate is precipitated from the solution by alcohol (iso-propanol or ethanol) and usually re-precipitated (to achieve higher purity) in the same way [9]. However, the real processing scheme for alginate production is quite complicated, including 15 steps [10].

Various types of alginates (sodium, potassium, calcium, ammonium salts, and other derivatives) from different brown algae species have found extensive utilization in numerous branches of technology [11], but mostly they are used as food additives (E 400 … E 405 in Europe). Alginates have been found to have great prospects in biotechnology [12].

The wide use of uronates is based on their rheological behaviour – viscosity and gel-forming properties depending on chain stiffness and extension of the macromolecules [9] according to the Mark–Houwink–Sakurada (MHS) equation:

$$[\eta] = KM^{a},$$

where $[\eta]$ is the intrinsic viscosity; K is a specific constant corresponding to polymer type; M is molecular weight of the polymer, and a is the parameter characterizing the shape and stiffness of the polymer chain.

Generally, the exponent in the equation $a = 0$ for globular molecules and $a = 1.8$ for rodlike ones. Depending on the effectiveness of the solvent, $a = 0.5 – 0.8$ for random coil molecules [9].

Molecular peculiarities of alginates, causing their rheological properties (structure–property relationships), have been studied in correlation with the statistical data on the principal block-structure of the polymers, available at the present time [3, 9, 13, 14]. The alginates from *F. vesiculosus* are usually characterized as low-G alginates having long M blocks of lower extension than high-G alginates (cf. Fig. 1). Accordingly, the exponent *a* for *Fucus* alginates in

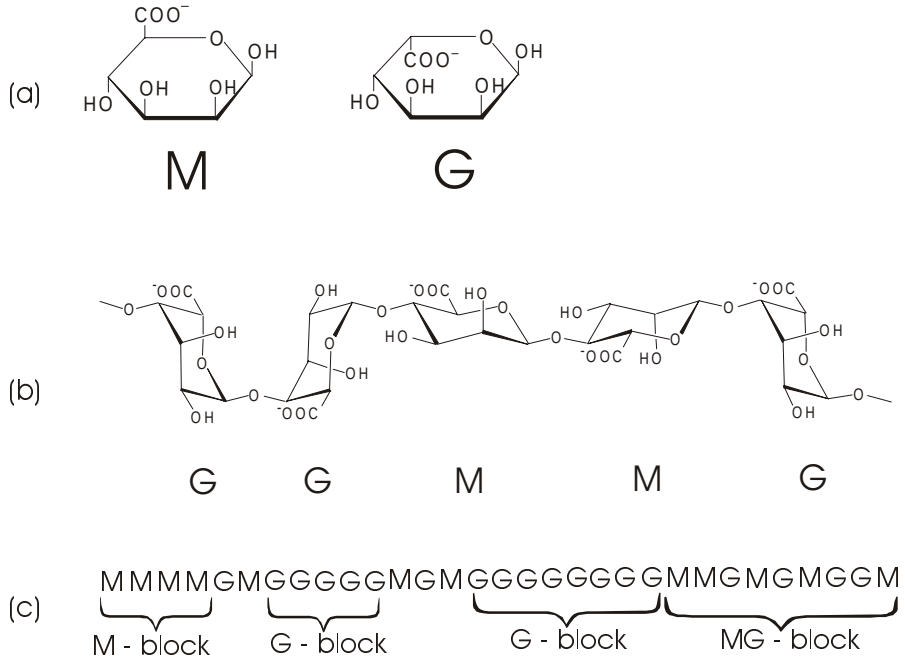

Fig. 1. Principal structure of alginates. (a) The structure units (monomers), Haworth conformation. M: β-D-mannuronate; G: α-L-guluronate. (b) An example of alginate chain fragment, chair conformation. (c) A symbolic cut from alginate chain: a sequence of monomers arranged in groups.

the MHS equation is to some extent lower than for other brown algal alginates [9, 15]. Structural characteristics of alginates depend not only on algal species or geographical, seasonal, and technological (processing) variations but also on location along the algal thallus, i.e. on the tissue age.

In general the alginate component of brown algal species has been widely investigated and used. But alginates from the Baltic *F. vesiculosus* have attracted less interest, especially concerning the dependence of their rheological properties upon processing conditions. The elementary composition of the algal biomass has been also insufficiently researched. Therefore, the purpose of the present introductory study was to characterize the mineral (multielementary) composition of the algal biomass and rheological peculiarities of alginates from the Baltic *F. vesiculosus*, extracted from the seaweeds under various conditions.

MATERIAL AND METHODS

Algae samples. Seaweeds *Fucus vesiculosus* were collected from coastal regions of Kakumäe Bay (the Baltic Sea, North Estonia) near the tip of the peninsula in the autumn of 1998. The samples for ash content determinations

were collected in various seasons from July 1998 to February 2000. The plants were washed in tap water and dried in air.

The ash content of the seaweeds was determined by heating the dried (at 95 °C) samples in a muffle (at 620 °C, 4 h).

Extraction and precipitation of alginates. In all cases, alginates were isolated from the seaweeds in sodium form. Algae were processed at three principal regimes: (a) "cold" method, (b) "hot" method, and (c) modified method including extraction in boiling state.

(a) *Cold method*. 20 g of air-dry algae was stored (18 h) in 300 mL of 1% $CaCl_2$ solution at room temperature. After that, the seaweeds were washed with water (3×300 mL), stored (1 h) in 5% solution of hydrochloric acid, and washed again with water (3×300 mL). Then algae were treated with 3% Na_2CO_3 solution (100 mL, 1 h) and water (300 mL) was added to stand overnight. The viscous mixture was filtered and the sodium alginate formed was precipitated from the solution by adding isopropanol (1 : 4 v/v). The precipitate was filtered, washed by isopropanol, and dried in air.

(b) *Hot method*. The seaweeds were treated as described above (method a) with the only differences that the storing time was 3 h and the processing temperature was 50 °C.

(c) *Modified method*. 20 g of air-dry algae was treated with boiling water (300 mL, 30 min) and with 0.5% $CaCl_2$ solution (300 mL, 100 °C, 30 min). The extract was separated and the algal residues were treated with 0.5% NaCl solution (300 mL, 1 h) at 100 °C. The dark-coloured extract was removed and 3% Na_2CO_3 solution (100 mL, 100 °C) was added to the seaweeds by intensive stirring (30 min, a boiling water bath). Then, additional 100 mL of water was added and the mixture was filtered. Sodium alginate was precipitated from the solution as described above.

Viscosity measurements were performed for 0.5% alginate solutions at 25 °C (if not specified otherwise). The dependence of viscosity on ion strength was investigated with a glass viscometer having a capillary diameter of 0.56 mm. For comparison of viscosity parameters at different treatment temperatures, a capillary with a diameter of 1.63 mm was used. Viscosity was measured relative to water (and therefore, has no unit).

Mineral (multielementary) composition was determined by neutron activation analysis (NAA). The samples were irradiated in a nuclear research reactor [16] of the Latvian Centre for Nuclear Research. A pneumatic tube conveyer was used to transport samples to and from the reactor.

For the determination of Mg, Al, Cl, Ti, V, Mn, Cu, and I short-lived radionuclides were used. The samples were irradiated in horizontal channels at a flux of 1.6×10^{13} n $cm^{-2}s^{-1}$ for 30 s. The gamma spectra from the irradiated samples were measured using a Ge (Li) detector connected to a 4000-channel analyser (NTA-1024, Hungary).

When long-lived nuclides were used for analysis, the irradiation of samples was performed in vertical channels at two regimes: with reactor neutrons at a flux

of 1.4×10^{13} n $cm^{-2}s^{-1}$ for 24 h, and with resonance neutrons at a flux of 1.3×10^{11} n $cm^{-2}s^{-1}$ for 72 h. In both cases, the irradiated samples were measured twice: 4 days and 3 weeks after irradiation. The gamma activity initiated was measured using a Ge (Li) detector connected to a 4000-channel analyser (LP-4900, Nokia, Finland). A more detailed description of the method is given in our earlier paper [17].

RESULTS AND DISCUSSION

Mineralization of algal biomass from the Baltic *Fucus vesiculosus* proved to be comparable to this characteristic of red algae from the Baltic Sea [18]. The ash content of *F. vesiculosus* changes from 14.2 to 21.4% and has no clear dependence on seasonal varieties. Storm-castings have also a medium mineral content (16–17%).

The ash content (mineral composition) of algal biomass is distributed between various elements as shown in Table 1. In general lines, the distribution of light elements in algae is comparable to that in seawater though the sodium and chlorine contents are relatively low. As it is typical of brown algae, the Baltic *F. vesiculosus* contains much halogens (Br and I); also arsenic content is high. The contents of other toxic elements (Hg, Sb, Se, etc.) are extremely low.

As to the method of NAA for such a purpose in general, previous ashing of the samples would be expedient to further detailed determinations of multielementary composition. In the case of such elements as K, Ca, Ti, Cu, and Mo, where NAA has typically low sensitivity, any other method of analysis would be preferable.

The viscosity of alginates from the Baltic *F. vesiculosus* is relatively low and depends strongly on the collection time and drying conditions of the seaweeds. To avoid destruction of alginates, the algae should be collected, transported, and dried as quickly as possible. Besides, the drying process of the seaweeds must proceed without any warming. Even close lots of algae samples gave alginate solutions of variable viscosity (Table 2).

Rheological properties of the alginates are also greatly dependent on processing temperature (Fig. 2). In general, even a slight rise in the treatment temperature of algal biomass leads to a drastic fall in viscosity and a rise in the extracting yield. The actual yield of alginates from the *Fucus* biomass is 7–8% for methods a and b and up to 15% for method c, although their content in algal tissue is considerably higher. So, the three different extraction methods used have quite different effect on the rheological behaviour of the isolated alginates. At low salt concentrations (0.01–0.1 M NaCl), the ionic strength increase causes a reduction of viscosity.

Table 1. Composition of the inorganic part of *Fucus vesiculosus*

Atomic number	Element	Element content in algae, ppm	Detection limit, ppm	Statistical error, %
11	Sodium	460–510	30	9
12	Magnesium	4300–6000	800	13
13	Aluminium	120–140	12	10
17	Chlorine	–	100	
19	Potassium	≤2000–7000	2000	42–66
20	Calcium	≤1200–5400	1200	18
21	Scandium	0.056–0.058	0.004	4
22	Titanium	–	120	
23	Vanadium	0.6–0.8	0.5	36–42
24	Chromium	1.8	0.6	16–17
25	Manganese	410–420	1–10	1–2
26	Iron	280–320	30–40	6–8
27	Cobalt	–	2–4	
28	Nickel	9.2–13.2	7	27–37
29	Copper	≤10–20	20	45–57
30	Zinc	250–310	4	1
33	Arsenic	327	2	2
34	Selenium	–	0.2	
35	Bromine	140–160	1	1–2
37	Rubidium	–	4	
38	Strontium	2180–2210	40	1
40	Zirconium	10.7	10	59
42	Molybdenum	≤0.27	0.19–0.3	13
51	Antimony	0.068	0.03	22
53	Iodine	130–160	10	6–7
55	Caesium	0.047–1.08	0.04	22–42
56	Barium	372–381	70	9
57	Lanthanum	–	0.2–0.9	
62	Samarium	0.09–0.12	0.04	10
63	Europium	0.017–0.032	0.003–0.006	14–16
65	Terbium	0.046	0.03	33
70	Ytterbium	–	0.05	
71	Lutetium	≤0.05	0.08	19–22
72	Hafnium	–	0.03	
79	Gold	0.011–0.012	0.01	19
80	Mercury	0.0063–0.0074	0.001	5–6
90	Thorium	–	0.05–0.06	
92	Uranium	1.5–1.8	0.5	9–10

– below detection limit.

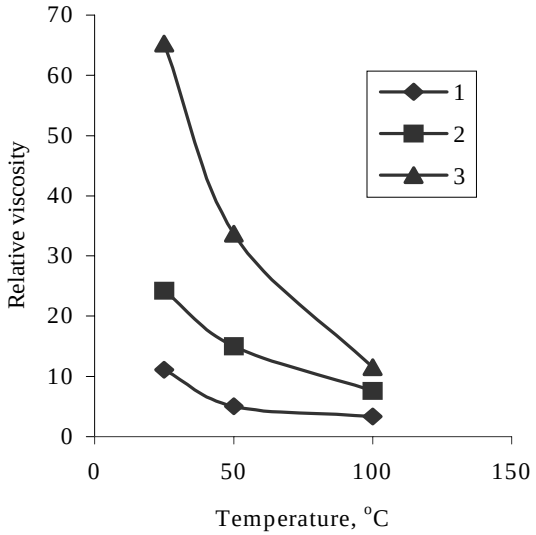

Fig. 2. Dependence of relative viscosity of alginates on processing temperature of *Fucus vesiculosus*. Concentrations of alginate solutions: 1 – 0.5%, 2 – 1%, 3 – 1.5%.

Table 2. Variability of relative viscosity of alginate solutions extracted from *Fucus vesiculosus*

Concentration of Na-alginate solutions, %	Experiment No.					
	1	2	3	4	5	6
0.5	11.0	9.5	10.4	8.4	8.1	11.1
0.75	19.5	15.8	15.7	14.0	14.8	15.8
1.0	28.3	23.5	21.5	19.9	17.9	24.2
1.5	79.7	59.6	59.9	46.2	43.8	65.3

Although the molecular particle size distribution of such biopolymers is quite a complicated problem, the effective molecular mass of the alginates, calculated from the MHS equation on the basis of viscosity data, decreases approximately two orders – from 2×10^6 at room temperature (method a) to 10^4–10^5 (method c).

Apparently, the alginates from *F. vesiculosus* are more sensitive to high isolation temperatures than alginates from other species.

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PRUUNVETIKATE *Fucus vesiculosus* (PÕISADRU) BIOMASSI MINERAALSE JA ALGINAATKOMPONENDI UURIMINE

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On uuritud Läänemere põisadru *Fucus vesiculosus*'e mineraalkoostist (38 elementi) ning alginaatkomponendi struktuuri ja viskoossussõltuvust.

Vetikate biomass on rikas kergete elementide (eriti Mg, K, Ca, Na) ja halogeenide (Br, I) poolest. Suhteliselt suur (kuid tüüpiline Läänemere makrovetikatele) on arseenisisaldus (üle 300 g t^{-1}), teiste toksiliste elementide (Hg, Sb,

Se jt.) sisaldus on üliväike. Biomassi mineralisatsioon ei sõltu oluliselt vetikate kogumise sesoonist.

Põisadrust eraldatud alginaadid on suhteliselt madala viskoossusega, see-juures sõltuvad viskoossusomadused suuresti vetikate säilitamise ja töötlemise tingimustest, eeskätt biomassi ekstraheerimistemperatuurist. *Fucus vesiculosus*'e alginaadid on väga termolabiilsed, mistõttu neid tuleb biomassist isoleerida toatemperatuuril.