Isolation of a protein-polysaccharide complex from brown algae biomass

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Abstract. This article presents new comparative data on the chemical composition of Arctic brown algae and algae from the Yellow sea. The effectiveness of the use of the extraction scheme to obtain the protein-polysaccharide complex (which has a double effect: enterosorption and immunomodulating) is estimated.

1. Introduction

Arctic brown algae are a complex mixture of chemical substances and many of these substances exhibit high biological activity [1-3]. Therefore, they are valuable raw materials for the production of various kinds of biologically active additives, components of cosmetics and pharmaceuticals. The industry is most interested in polysaccharides, such as fucoidan, laminarin as well as alginic acids, which are widely used as bio-additives, sorbents, and food additives.

It was previously established that components of brown algae such as polyphenols and cellulose also have a number of important properties that allow them to be used as sorbents, antioxidants, and antibacterial drugs [4].

Among the entire scope of studies on the processing of brown algae, the least attention has been paid to their protein component. The isolation of pure protein from the biomass of brown algae is complicated by a large number of intermolecular bonds with polysaccharides which form the strong matrix of a macrophyte cell wall. In the aggregate, with a low protein content in the biomass of brown algae (3-20%), isolation becomes difficult and inappropriate.

Proteins are not as highly promising pharmacological substances as their degradation products – peptides. They possess a number of important properties such as antioxidant, antiproliferative, antihypertensive, and antimicrobial activity, etc. [5-8]. Therefore, it will not be rational to isolate the protein in its pure form, but it is possible to isolate it in combination with the polysaccharides matrix (cellulose). Thus, the target substance may possess double activity: enterosorbent properties due to the presence of a cellulose matrix [9] and an immunomodulation activity due to the protein component.

In accordance with that mentioned above, the purpose of this study is to compare the total chemical composition of Arctic brown algae with samples from the Yellow Sea (South Korea) in order to identify the most promising species and to obtain a protein-polysaccharide complex (PPC) possessing double-activity.
2. Experimental

Four species of brown algae (Laminaria digitata (L.d.), Laminaria saccharina (L.s.), Fucus vesiculosus (F.v.) и Ascophyllum nodosum (A.n.)) were collected in the summer of 2015 from the water near the Big Solovetsky Island in the White Sea. Ecklonia cava (E.c.) and Undaria pinnatifida (U.p.) were collected in October of 2019 from the Yellow Sea (Jeju isl.).

A protein-polysaccharide complex was obtained according to the scheme developed by the authors (Figure 1).

The chemical composition was determined according to standard protocols [10-14]. Ash was determined gravimetrically after combustion in a muffle furnace at 550 °C. Mannitol was determined spectrophotometrically by measuring the absorbance of mannitol-Cu ions blue complexes. Before measuring EHP (easily hydrolysable polysaccharides) biomass and extracts were hydrolyzed with 6N HCl at 110 °C. Released sugars were determined by iodometric titration. Polyphenols in all extracts were measured spectrophotometrically after reaction with Folin-Ciocalteu reagent in the alkali medium. Alginic acids in biomass were determined by titration of the excess amount of alkali after reaction with alginate acids. They were precipitated from alkali extracts with HCl, washed, dried and weighed. Cellulose was determined gravimetrically according to Kurschner's method. Elemental analysis was carried out on an EuroEA 3000 (EuroVector, Italy).

FTIR spectra were recorded on an IR Prestige 21 IR Fourier spectrophotometer (Shimadzu, Japan) with an attachment for impaired total internal reflection MIRacle with a ZnSe prism (Pike, USA).

Figure 1. Isolation scheme of the protein-polysaccharide complex from brown algae.

3. Results and Discussion

In order to determine the most promising species for the production of the protein-polysaccharide complex, the chemical composition of six brown algae species was evaluated. Protein content was calculated based on the amount of total nitrogen in the samples (Table 1) by multiplying total nitrogen with the specific coefficient for brown algae.

| Algae                        | C     | H     | N      |
|------------------------------|-------|-------|--------|
| Ascophyllum nodosum          | 34.81±1.23 | 5.04±0.14 | 1.84±0.07 |
| Fucus vesiculosus            | 29.04±1.07 | 3.95±0.11 | 1.41±0.06 |
| Laminaria digitata           | 33.98±1.21 | 5.35±0.15 | 1.45±0.05 |
| Laminaria saccharina         | 32.27±1.10 | 5.51±0.17 | 1.65±0.06 |
| Ecklonia cava                | 37.74±1.22 | 5.03±0.15 | 1.85±0.07 |
| Undaria pinnatifida          | 36.62±1.20 | 5.58±0.21 | 3.75±0.15 |

In addition to the target substance, we obtained and analyzed acid, alkaline, and aqueous extracts to describe the effectiveness of the extraction. The chemical composition of the extracts is shown in Table 2. Water-soluble components (mannitol, polysaccharides, polyphenols, and mineral compounds) mainly leave during the acid and alkaline extractions. These extracts contain most of the mannitol (up to 96%) as well as easily hydrolysable polysaccharides (up to 95%). Alginic acids are extracted from Laminaria
algae only with an alkali solution, while for Fucus spp, alginic acids were removed by aqueous extraction.

| Extract | % d.w. | Mannitol | EHP | Polyphenols | Alginic acid | Ash |
|---------|-------|---------|-----|-------------|--------------|-----|
| Ascomycum nodosum |      |         |     |             |              |     |
| Acidic mass | 6.32±0.30 | 9.58±0.45 | 6.40±0.32 | - | 8.92±0.46 |
| rel* | 74.79±3.75 | 65.62±3.22 | 79.01±3.96 | - | 31.53±1.59 |
| Alkali mass | 1.15±0.07 | 1.49±0.09 | 1.30±0.08 | 19.46±0.99 | 9.20±0.47 |
| rel* | 13.61±0.69 | 10.21±0.55 | 16.05±0.88 | 76.31±3.85 | 32.52±1.64 |
| Water mass | 0.77±0.05 | 3.10±0.18 | 0.34±0.06 | 3.10±0.14 | 8.96±0.48 |
| rel* | 9.11±0.48 | 21.23±1.07 | 4.20±0.25 | 12.16±0.58 | 31.67±1.58 |
| Fucus vesiculosus |      |         |     |             |              |     |
| Acidic mass | 7.02±0.33 | 12.58±0.66 | 5.60±0.29 | - | 7.85±0.39 |
| rel* | 75.48±3.75 | 60.48±3.05 | 82.35±4.10 | - | 32.93±1.66 |
| Alkali mass | 1.70±0.11 | 4.36±0.24 | 1.10±0.08 | 16.30±0.85 | 7.69±0.41 |
| rel* | 18.28±0.93 | 20.96±1.08 | 16.18±0.84 | 70.26±3.52 | 32.26±1.63 |
| Water mass | 0.37±0.04 | 3.30±0.17 | 0.10±0.03 | 3.90±0.21 | 7.32±0.35 |
| rel* | 3.98±0.21 | 15.87±0.79 | 1.47±0.08 | 16.81±0.85 | 30.70±1.52 |
| Laminaria digitata |      |         |     |             |              |     |
| Acidic mass | 16.93±0.82 | 16.50±0.84 | 0.20±0.02 | - | 6.10±0.30 |
| rel* | 88.22±4.38 | 78.20±3.92 | 44.54±2.24 | - | 43.32±2.14 |
| Alkali mass | 1.37±0.08 | 3.00±0.13 | 0.20±0.02 | 29.72±1.48 | 3.70±0.17 |
| rel* | 7.14±0.37 | 14.22±0.69 | 44.54±2.25 | 94.65±4.73 | 26.28±1.32 |
| Water mass | 0.48±0.03 | 1.10±0.07 | 0.04±0.01 | - | 3.94±0.20 |
| rel* | 2.50±0.15 | 5.21±0.27 | 8.91±0.46 | - | 27.98±1.41 |
| Laminaria saccharina |      |         |     |             |              |     |
| Acidic mass | 17.85±0.90 | 16.40±0.80 | 0.30±0.03 | - | 5.20±0.24 |
| rel* | 90.15±4.51 | 73.87±3.71 | 55.56±2.79 | - | 43.33±2.15 |
| Alkali mass | 1.22±0.06 | 3.83±0.21 | 0.20±0.01 | 28.78±1.42 | 2.40±0.13 |
| rel* | 6.16±0.32 | 17.25±0.88 | 37.04±1.83 | 93.90±4.67 | 20.00±1.02 |
| Water mass | 0.34±0.03 | 1.48±0.08 | 0.03±0.01 | - | 4.26±0.21 |
| rel* | 1.72±0.10 | 6.67±0.34 | 5.56±0.29 | - | 35.50±1.79 |
| Ecklonia cava |      |         |     |             |              |     |
| Acidic mass | 14.38±0.71 | 7.59±0.39 | 4.22±0.22 | - | 3.37±0.16 |
| rel* | 71.51±3.55 | 57.46±2.88 | 55.45±2.78 | - | 25.80±1.27 |
| Alkali mass | 4.79±0.21 | 5.03±0.24 | 3.06±0.13 | 25.11±1.24 | 3.05±0.15 |
| rel* | 23.82±1.19 | 38.08±1.89 | 40.21±2.01 | 83.67±4.17 | 23.35±1.17 |
| Water mass | 0.46±0.03 | 0.26±0.02 | 0.33±0.02 | - | 2.71±0.16 |
| rel* | 2.29±0.14 | 1.97±0.15 | 4.34±0.22 | - | 20.75±1.07 |
| Undaria pinnatifida |      |         |     |             |              |     |
| Acidic mass | 0.25±0.01 | 6.31±0.33 | 0.10±0.01 | - | 7.22±0.36 |
| rel* | 17.24±0.88 | 63.42±3.16 | 13.70±0.69 | - | 30.66±1.52 |
| Alkali mass | 0.54±0.03 | 3.15±0.15 | 0.28±0.01 | 32.81±1.66 | 4.75±0.25 |
| rel* | 37.24±1.89 | 31.66±1.59 | 38.36±1.95 | 86.66±4.32 | 20.17±1.03 |
| Water mass | 0.47±0.02 | 0.19±0.01 | 0.35±0.02 | - | 7.88±0.40 |
| rel* | 32.41±1.66 | 1.91±0.11 | 47.95±2.41 | - | 33.46±1.68 |

*rel – based on the content of the component in the original biomass

The efficiency of water extraction is quite low, especially for Laminaria species, because only minor components are removed from the biomass at this stage. Thus, up to 40% of alginic acids remain in the biomass as well as residues of bound polysaccharides such as fucoidan, and laminarin. Concerning
southern species, the removal of large quantities of alginic acids occurs with less efficiency (the final product contains up to 15 %rel. alginic acids).

It's worth noting that the protein content in Laminaria species is slightly higher than for Fucus spp. The obtained values correlate with the data available in the literature on the protein content in brown macrophytes [15]. Despite the high initial protein content in U. pinnatifida only about 40% rel. remains bound with the cell-matrix after extractions.

Table 3 presents general data on the chemical composition of solid samples from the initial macrophytes and protein-polysaccharide complexes.

In order to confirm the effectiveness of the extraction process we recorded the FTIR spectra of the biomass before extraction and the PPC's (Figure 2).

There are changes in the structure (therefore in the composition) of the Arctic brown algae PPC after all extractions.

A wide peak in the region of 3330–3364 cm\(^{-1}\) is present in all of the spectra, and it is characteristic of O–H and C–H group stretching vibrations. Polysaccharides, polyphenols, and mannitol mainly contribute to its intensity. Nearby is a less intense peak at 2920–2934 cm\(^{-1}\). This corresponds to stretching vibrations of N–H.

**Table 3.** Total chemical composition of protein-polysaccharide complexes % dry weight (% d.w.)

| Raw algae       | % d.w.          | Mannitol     | EHP  | Protein | Polyphenols | Alginic acid | Cellulose | Ash  |
|-----------------|-----------------|--------------|------|---------|-------------|--------------|-----------|------|
| A. n.           | 8.45±0.42       | 14.60±0.74   | 6.60±0.33 | 8.10±0.40 | 25.50±1.29 | 5.15±0.27 | 28.29±0.67 |      |
| F. v.           | 9.30±0.48       | 20.80±1.05   | 6.60±0.34 | 6.80±0.34 | 23.20±1.16 | 5.81±0.29 | 23.84±0.59 |      |
| L. d. mass      | 19.19±0.97      | 21.10±1.06   | 7.60±0.38 | 0.45±0.02 | 31.40±1.58 | 6.10±0.31 | 14.08±0.71 |      |
| L. s.           | 19.80±0.99      | 22.20±1.12   | 8.80±0.44 | 0.54±0.03 | 30.65±1.54 | 6.00±0.30 | 12.00±0.59 |      |
| E. c.           | 20.11±1.04      | 13.21±0.69   | 9.60±0.54 | 7.61±0.44 | 30.01±1.59 | 5.15±0.24 | 13.06±0.65 |      |
| U. p.           | 1.45±0.15       | 9.95±0.58    | 19.50±1.08 | 0.73±0.07 | 37.86±1.96 | 4.75±0.26 | 23.55±0.95 |      |

| Raw algae       | % d.w.          | Mannitol     | EHP  | Protein | Polyphenols | Alginic acid | Cellulose | Ash  |
|-----------------|-----------------|--------------|------|---------|-------------|--------------|-----------|------|
| A. n. rel*      | 0.20±0.02       | 0.20±0.02    | 1.70±0.09 |           | 2.90±0.16  | 4.91±0.26  | 0.70±0.05 |      |
| F. v. rel*      | 2.37±0.13       | 1.37±0.08    | 25.76±1.29 |           | 11.37±0.57 | 95.34±4.77 | 2.47±0.27 |      |
| L. d. rel*      | 0.20±0.02       | 0.20±0.02    | 2.80±0.15 |           | 2.70±0.14  | 5.60±0.29  | 0.70±0.04 |      |
| L. s. rel*      | 2.15±0.12       | 0.96±0.05    | 42.42±2.14 |           | 11.64±0.59 | 96.39±4.82 | 2.94±0.31 |      |
| E. c. rel*      | 0.20±0.02       | 0.10±0.02    | 2.80±0.16 |           | 1.50±0.08  | 6.04±0.31  | 0.10±0.02 |      |
| U. p. rel*      | 1.04±0.05       | 0.47±0.03    | 36.64±1.85 |           | 4.78±0.25  | 99.02±4.95 | 0.71±0.05 |      |
| Protein-polysaccharide complex | % d.w.          | Mannitol     | EHP  | Protein | Polyphenols | Alginic acid | Cellulose | Ash  |
| A. n.           | 12.81±0.85      | 4.98±0.13    | 38.84±1.53 |           | 13.77±0.49 | 95.34±4.77 | 2.47±0.27 |      |
| F. v.           | 3.80±0.09       | 3.70±0.04    | 12.81±0.85 |           | 12.76±0.49 | 95.34±4.77 | 2.47±0.27 |      |
| L. d.           | 2.17±0.09       | 2.80±0.16    | 36.64±1.85 |           | 4.78±0.25  | 99.02±4.95 | 0.71±0.05 |      |
| L. s.           | 0.80±0.05       | 1.97±0.15    | 82.29±4.16 |           | 15.33±0.79 | 99.42±4.97 | 29.56±1.48 |      |
| U. p.           | 1.17±0.02       | 0.26±0.04    | 7.70±0.45 |           | 4.85±0.23  | 4.69±0.26  | 3.55±0.21 |      |

*rel – based on the content of the component in the original biomass

In the spectra of protein-polysaccharide complexes, marked changes were observed (in comparison with the initial biomass) in the region of 1500-1630 cm\(^{-1}\). After all procedures, a very distinct peak ~1519 cm\(^{-1}\) appeared in the spectra. The occurrence of this peak is probably due to the relative increase in the protein concentration in the sample after the removal of the accompanying components.

The region of 700–1400 cm\(^{-1}\) is characteristic of the molecular vibrations of polysaccharides, including alginates, fucoidans, laminarins, and cellulose. A peak at 1100 cm\(^{-1}\) is characteristic, including for mannuronic acid [16], which indicates the presence of alginic acid in the final product. We can also note the disappearance of peaks in the region of 800–900 cm\(^{-1}\) (especially for L. digitata and L. saccharina), which are characteristic of sulfated polysaccharides. This confirms the removal of these components from the biomass.

In general, the spectra of protein-polysaccharide complexes look similar, which indicates the achievement of uniformity in the composition of all studied brown algae complexes.

Comparing the composition, extraction efficiency, and FTIR spectra of the Arctic and southern brown algae, we can conclude that from the point of view of obtaining PPC, it is impossible to unequivocally give preference to certain types of brown algae. On one hand, we have high protein content in the initial biomass of southern algae. However, this decreases to values comparable with the
data of Arctic species during processing. On the other hand, in algae samples of *E. cava* and *U. pinnatifida*, alginic acid remains bound to the final product in significant quantities, which negatively affects the purity of the substance.

Figure 2. FTIR spectra of initial biomass and PPC of brown algae.

4. Conclusions
The chemical composition of brown algae of the southern seas in comparison with the Arctic species has been studied. Protein-polysaccharide complexes were obtained from six types of brown algae. Probably, due to structural and morphological features, the proposed scheme cannot be effectively applied to extract PPC from samples of southern algae. Thus, the most promising species for obtaining double-acting preparations are the brown algae of the Arctic seas.

Acknowledgements
The study was funded by state assignment, project No. 0793-2020-0005. This work was carried out using scientific equipment of the FCIARctic RAS and the Korean Polar Institute. The authors would like to express special gratitude to the employees of the Korean Polar Research Institute for the brown algae samples and their help during the experiment in the framework of the Arctic Science Fellowship Program.
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