The fundamental cycle "structure - functional nature properties" of phlorotannins from Arctic brown algae

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Abstract. The paper presents the results of a study devoted to the isolation, fractionation and characterization of Arctic brown algae polyphenols. New analytical data on the structure, functional nature, and polymolecular properties of brown algal phlorotannins were obtained using modern analytical methods (size exclusion chromatography, tandem mass spectrometry, MALDI mass spectrometry), a preliminary assessment of the tendency of the change of antioxidant activity in dependence of polymolecular properties was performed.

1. Introduction

Brown algae accumulate a large number of polyphenolic compounds, mainly florotannins. One of the richest sources of algal polyphenol complex in the Arctic region is the algae of the species Fucus vesiculosus (the content of polyphenols reaches 19%). The pharmacological significance of polyphenols is related to their structure and, in particular, to the degree of polymerization. However, it should be noted that the relationship between the molecular weight and antioxidant activity (AOA) of brown algal florotannins is still the subject of study [1]. Thus, Ferreres [2] and co-authors noted a higher antioxidant activity of high molecular weight florotannins compared with a low molecular weight polyphenol fraction, while studies [3 - 6] have shown that an increase in the molecular weight of isolated florotannins leads to a decrease in antioxidant capacity. In carrying out similar studies, Wang et al. [1] did not find a clear relationship between the antioxidant capacity and the molecular weight of florotannins.

The aim of the research is to determine the dependence of antioxidant activity on the molecular weight of florotannins from Arctic brown algae.

2. Experimental

Algae of the species Fucus vesiculosus were sampled in July 2017 in the water area of the Big Solovetsky Island of the White Sea. The algal polyphenol complex was isolated according to the scheme developed by the authors (Figure 1).

The total content of the polyphenolic fraction was determined by the colorimetric method using a Folin-Ciocalteu reagent. As a standard, phloroglucin was used. The freeze-dried polyphenol fraction was treated by gel filtration on a Sephadex LH-20 sorbent. Substance was dissolved in 50% ethanol and immersed in a column with Sephadex LH-20 (column parameters: 15 g sorbent, V = 57 cm3, l = 32 cm) and stepwise elution with mixtures of ethyl alcohol (E): water (W) and ethyl alcohol: acetone (A) (the eluents are shown in Table 1, the volume of each mixture of solvent is 120 ml). At the output, 12 Sephadex sub-fractions were obtained.
Determination of antioxidant activity of extracts was carried out by spectrophotometric method, estimating the degree of decolorization of DPPH solution. The samples were analyzed by exclusive high-performance liquid chromatography using LC-20 Prominence HPLC (Shimadzu, Japan).

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MALDI mass spectrometric analysis was performed using an AximaResonanse time-of-flight mass spectrometer (Shimadzu Biotech, UK).

MS analysis of the polyphenol compounds was carried out using a LCMS-8030 triple quadrupole mass spectrometer (Shimadzu, Japan) with direct sample entry.

FIGURE 1. Scheme of isolation of polyphenol compounds from brown algae

3. Results And Discussion

Experimental results have shown that the most optimal extractant for extracting polyphenols from aqueous solution III is a 4:1 mixture of ethyl acetate and butanol, which results in 68% of the phlorotannins passing from the aqueous phase to the organic phase.

The composition of the polyphenol fraction was studied by preparative chromatography on a column packed with Sephadex LH-20 sorbent. The relative yields and content of polyphenols of the Sephadex subfractions of the polyphenol fraction are shown in Table 1.
TABLE 1. Characteristics of the initial polyphenol fraction and Sephadex subfractions.

| Fraction                  | Eluent     | Polyphenol yield, % | Polyphenol content, g per 100g dry extract | Mw, Da  | Antioxidant activity, Mg ascorbic acid per 100g dry extract |
|---------------------------|------------|---------------------|---------------------------------------------|---------|----------------------------------------------------------|
| Initial polyphenol fraction | -          | -                   | 76,2±3,2                                    | 4620    | 553±24                                                   |
| LH-1                      | E/W (2:3)  | 32,2±2,3            | 52,0±2,0                                    | 7370    | 317±19                                                   |
| LH-2                      | E/W (1:1)  | 5,7±0,4             | 85,2±1,5                                    | 2050    | 523±33                                                   |
| LH-3                      | E/W (2:1)  | 4,0±0,1             | 97,0±3,0                                    | 2650    | 733±30                                                   |
| LH-4                      | E/W (3:1)  | 3,1±0,3             | 97,6±1,9                                    | 2870    | 698±16                                                   |
| LH-5                      | E/W (4:1)  | 1,1±0,2             | 96,0±2,1                                    | 2820    | 894±40                                                   |
| LH-6                      | E/W (5:1)  | 0,9±0,1             | 94,5±1,9                                    | 2760    | 917±29                                                   |
| LH-7                      | E          | 0,4±0,1             | 91,9±2,7                                    | 2610    | 891±28                                                   |
| LH-8                      | E/A (5:1)  | 2,3±0,2             | 94,4±1,8                                    | 3030    | 744±38                                                   |
| LH-9                      | E/A (4:1)  | 2,8±0,2             | 97,9±2,6                                    | 3660    | 798±16                                                   |
| LH-10                     | E/A (3:1)  | 5,7±0,5             | 95,8±3,0                                    | 4470    | 659±35                                                   |
| LH-11                     | E/A (2:1)  | 9,2±0,8             | 92,6±2,5                                    | 5670    | 686±21                                                   |
| LH-12                     | E/A (1:1)  | 19,3±1,1            | 97,5±1,4                                    | 6770    | 584±22                                                   |

The yield is calculated as a percentage of the content of polyphenols in the fraction taken for separation on the sorbent; E – ethanol; W – water; A – acetone.

The molecular weight of polyphenols in subfractions № 3-7, eluted by the ethanol / water solvent system, varies slightly (from 2610 to 2870 Da), while a significant increase in molecular weight from 3030 to 6770 Da is observed with the ethanol / acetone system.

The presence of fractions of phlorotannins in the mass range from 373 Da (trimers) to 994 Da (octamers), differing by 124 was detected. Components with a higher molecular weight elute with an increase of the organic solvent. In the last two fractions obtained using an eluent with a high proportion of acetone, no oligomers were detected.

The change in the component composition of polyphenols in subfractions leads to difference in AOA. So high AOA is observed with the content of pentamers and hexamers in the subfraction. The AOA decrease when molecular weight of polyphenols was greater than pentamers has.

The subfractions № 3-7 have an average molecular weight of 2740 ± 140 Da and AOA of 827 ± 29 mg of ascorbic acid / g extract, so for the clarity we combine it to the one point on the schedule.

The dependence of AOA on the molecular mass is shown in Fig. 2. The triangle indicates the mean value for subfractions No. 3-7. The obtained dependence is observed at a range of masses from 3000 Da.
4. Conclusions

The dependence of the antioxidant activity of the polyphenol fraction on the average molecular weight was revealed. The most active polyphenol molecules have an average mass ranging from 2000 to 4000 Da. With further increase in the molecular weight, the antioxidant activity of the fractions decreases.

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