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## The stability of pigments in the thalli and extracts of the Barents Sea fucus algae

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### Abstract

Fucus algae of the Barents Sea are a promising source of biologically active substances (BAS), including carotenoid and chlorophyll pigments. The main direction of processing these types of brown algae is aimed at obtaining polysaccharides, and therefore pigments can be lost during the purification stages of the target components. Freezing as a type of preservation of algae is becoming more common, while the biochemical processes during storage of this type of raw material remain poorly understood. The study shows the effect of long-term storage (6 months) in the freezer of frozen thalli of such types of brown algae as *Fucus vesiculosus*, *Fucus distichus* and *Ascophyllum nodosum* on the content of fucoxanthin and chlorophyll a in them. Analysis of the pigment content has been performed by HPLC, spectroscopy – in the visible and UV spectral area. In the course of the work it has been revealed that during the storage of algal thalli pigment degradation do not occur, in addition in some cases their content in the extract has increased 1.5–2 times. On the example of ethanol extract from frozen *Ascophyllum* thalli the stability of pigments during storage in the refrigerating chamber during the 2nd, 3rd, 4th, 10th and 30th days of storage has been studied. It has been established that the content of fucoxanthin and chlorophyll a practically do not change during the studied period. It has been also shown that the concentration of the *Ascophyllum* extract under vacuum at a temperature of 50 °C does not affect the quantitative content of fucoxanthin, however, the amount of chlorophyll a decreases 40 times. The results of the work can contribute to the development of new ways of processing promising types of algae in the Barents Sea with obtaining therapeutic and preventive food products.

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## Изучение стабильности пигментов в талломах и экстрактах фукусовых водорослей Баренцева моря

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Фукусовые водоросли Баренцева моря являются перспективным источником биологически активных веществ (БАВ), включая пигменты каротиноиды и хлорофиллы. Основная цель переработки этих видов бурых водорослей – получение полисахаридов, в связи с чем пигменты могут теряться на этапах очистки целевых компонентов. Замораживание как вид консервации водорослей становится все более распространенным, при этом биохимические процессы при хранении данного вида сырья остаются малоизученными. Проведенное исследование впервые показывает воздействие длительного хранения (6 мес) в морозильной камере мороженных талломов таких видов бурых водорослей, как *Fucus vesiculosus*, *Fucus distichus* и *Ascophyllum nodosum*, на содержание в них фукоксантина и хлорофилла а. Анализ содержания пигментов выполняли методом ВЭЖХ, спектроскопию проводили в видимой и УФ области спектра. В ходе работы выявлено, что в течение срока хранения талломов водорослей деградации пигментов не произошло, кроме того в некоторых случаях их экстрактивность увеличилась в 1,5–2 раза. На примере этанольного экстракта из мороженных талломов аскофиллума изучена стабильность пигментов при хранении в холодильной камере в течение 2-х, 3-х, 4-х, 10-х и 30-х суток хранения. Установлено, что содержание фукоксантина и хлорофилла а практически не изменилось на протяжении исследуемого периода. Также показано, что концентрирование экстракта аскофиллума под вакуумом при температуре 50 °C не сказывается на количественном содержании фукоксантина, однако при этом количество хлорофилла а уменьшилось в 40 раз. Результаты работы могут способствовать появлению новых способов переработки перспективных видов водорослей Баренцева моря с получением лечебно-профилактической пищевой продукции.

### Для цитирования

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## Introduction

At present, the issues of storing plant materials are becoming important, especially for food products and medicines. Various raw materials need a certain storage mode depending on the composition of the raw materials, the properties and intensity of the processes occurring in it. Freezing is a conservation method in which the preservation of quality is achieved due to low temperatures that lead to the blocking of redox processes, the reduction of microbiological activity, as well as the activity of free water found in vegetable raw materials by transferring it to crystalline ice. This method of storage allows preserving up to 75–80 % of biologically active substances (BAS) for a long time (*Stryukova et al., 2013*). The shelf life of frozen foods is measured in months and even years. To date, the processes occurring in brown algae during storage in the freezer and influencing on the content of biologically active substances (BAS) remain poorly understood.

Brown algae (*Phaeophyceae*) are the largest of the known algae, characterized by a huge variety of shapes and sizes. They are a valuable source of various BAS for industry. Brown algae contain a significant amount of pigments, which are usually removed in the production of polysaccharides. However, recent studies have shown the biofunctionality of pigments. For example, fucoxanthin can be used as an antioxidant, chemoprophylactic and chemotherapeutic, reducing fat mass, and also as an anti-inflammatory agent (*Indrawati et al., 2015*). Chlorophyll a stimulates tissue growth, prevents the spread of bacteria and accelerates the healing process of wounds (*Hosikian et al., 2010*).

In the production of pigment extracts, it is important to take into account that chlorophylls and carotenoids easily decompose when exposed to heat and light, as well as during chemical treatment (acid-base, redox) (*Indrawati et al., 2015*). Due to their instability, special extraction approaches are required to maximize the release of these pigments from algae. It is necessary to develop a new approach that can be used to create pigment-containing products from commercial and promising species of brown algae, which are still not used in industry.

For the production of pigment extracts, it is necessary to investigate the stability of pigments in raw materials during storage as well as in extracts.

The purpose of this research is to study the quantitative content of fucoxanthin and chlorophyll a in frozen thalli of *Fucus* algae of the Barents Sea and extracts from them during storage and heat treatment.

## Materials and methods

*Fucus* algae at the age of 4+ ... 7+ years of the following species were used in the work: *Fucus vesiculosus* L., *Fucus distichus* L., *Ascophyllum nodosum* L. Algae samples from the southern part of the Teriberskaya Bay in the Zavalishin Bay of the Barents Sea were collected in August 2018 at low tide.

Fresh algae were delivered to the laboratory 3 hours after collection, thoroughly cleaned from epiphytes and sand, frozen in a freezer at  $-25 \pm 2$  °C.

Extraction of pigments was carried out according to the modified method described in our previous works (*Gerasimenko et al., 2010; Daurtseva, 2018*). Briefly, 3 consecutive extractions with 96 % ethanol with a duration of 5, 10, 10 minutes and then an exhaustive extraction by percolation was made. All extractions were performed at room temperature in a darkened room.

To obtain an extract enriched with fucoxanthin, frozen *A. nodosum* stored for 6 months were used as raw materials. Before extraction, the algae were thawed and crushed on a Redmond grinder (China). Extraction was carried out for 5 minutes, then the extract was filtered and placed in a refrigerator at +4 °C. The total storage time of the extract was 35 days. Then the extract was evaporated to dryness on rotary evaporator IR-1 (Russia) under vacuum at a temperature of 50 °C. To determine the pigment content, the enriched dry extract was dissolved in 96 % ethanol until the original volume was restored.

Fucoxanthin content in the extracts was determined on Shimadzu LC-20AD Prominence liquid chromatograph (Japan) with a Shimadzu SPD-M20A Prominence photodiode array detector and Supelco (250 × 4.6, C18, 5 μm) chromatographic column (USA). Methanol : acetonitrile was used as the mobile phase. Selection of chromatographic parameters was carried out experimentally. To assess the purity of the pigment extracts, spectra were obtained in the visible and UV region by spectrophotometry. The spectra were obtained on Nicolett Evolution 500 spectrophotometer by Spectronic Unicam (Great Britain).

Fucoxanthin (Sigma-Aldrich) and chlorophyll a (Sigma-Aldrich) were used as standard samples for spectrophotometry and HPLC.

Absolutely dry mass of algae samples was determined according to the generally accepted method<sup>1</sup>.

All data were obtained in 3 replicates and processed in the program Statistica.

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<sup>1</sup> GOST 26185-84. Seaweeds, sea-grasses and its processed products. Methods of physical and chemical analysis. M., 2004.

## Results and discussion

In accordance with the current regulatory documentation<sup>2</sup>, the recommended shelf life of frozen seaweed from the date of production is 12 months at a storage temperature not higher than  $-18^{\circ}\text{C}$ . At the same time, only organoleptic properties are indicators of the quality of this type of food products, and only the amount of mineral impurities normalizes the physicochemical properties. Pharmacopoeias of various countries, including Russia, standardize the content of polysaccharides and iodine in the dried thalli of brown algae, setting a shelf life of 3 years<sup>3</sup>. However, there is no document where the content of pigments normalized for both dry and frozen algal thalli. Considering the prospect of freezing as the best way to preserve native algal BAS, as well as the timing of possible collecting of brown algae in the conditions of the Murmansk coast of the Barents Sea, a study was conducted on the stability of pigments (fucoxanthin and chlorophyll a), which have the greatest practical value, during 6 months of storage in the freezer chamber at a temperature of  $-25^{\circ}\text{C}$  (Fig. 1).

It has been revealed that after 6 months of storage the total content of investigated pigments in thalli from *F. vesiculosus* and *F. distichus* has increased. The total content of fucoxanthin in *A. nodosum* remains unchanged – 0.30 mg/g DW, and the content of chlorophyll a decreases from 0.57 to 0.40 mg/g DW. The received data show that the extraction efficiency of fucoxanthin and chlorophyll a from the thalli of *F. vesiculosus* and *F. distichus* after long-term storage in the freezer is 1.5–2 times higher than from fresh ones (Fig. 1). It can be assumed that during the storage of algae in a frozen state, various physicochemical processes take place, as a result of which the pigments pass into a more accessible form for extraction. It is likely that cellular structures can be destroyed, which leads to a change in the release of BAS (Roshanak et al., 2016; Chang et al., 2006; Arslan et al., 2008). The structure of the thallus of *A. nodosum* and its biochemical composition are significantly different from studied algae of the genus *Fucus*, which could affect the results of the experiment on long-term storage (Obluchinskaya, 2014; Stengel et al., 1998).

It is known that pigments easily decompose under the influence of environmental factors such as temperature, light radiation and oxidizing agents. Fucoxanthin and chlorophyll a are very sensitive to decomposition under the influence of heat, low pH, long shelf life and exposure to light (Mercadante, 2008; Acedo, 2010; Spinardi et al., 2012). When stored in a freezer with unchanged conditions (lack of light and constant temperature), fucoxanthin and chlorophyll a remain better than under conditions with increasing temperature, for example, when stored after drying. According to the literature, the content of carotenoids and chlorophyll a in some species of medicinal plants immediately after freezing remains almost unchanged (Prokof'ev et al., 2014).

The air-dry method of algae dehydration changes the content of BAS, including pigments. In the work of Tkhan Tajk and co-authors (Tajk et al., 2016) it was shown that the content of fucoxanthin in dried *Cylinrotheca* microalgae decreased by almost 2 times during storage for 1.5 months at room temperature. During storing dried algae *Laminaria japonica* under these conditions the content of chlorophylls decreased by 20 %.

The issues of maintaining high values of the quantitative characteristics of BAS of algae are little studied. According to studies (Lin et al., 2005; Hii et al., 2010), the addition of an antioxidant compound, such as ascorbic acid, showed the greatest preservation of fucoxanthin content both in dark and light conditions. The algae of the Barents Sea studied by us contains 100–400 mg/g of vitamin C. Therefore, it can be assumed that this compound could contribute to the preservation of pigments. According to the Kawee-ai (2013) study, fucoxanthin should be stored at a temperature below  $4^{\circ}\text{C}$  in an alkaline environment.

<sup>2</sup> GOST 31583-2012. Sea frozen kale. Specifications. M., 2013.

<sup>3</sup> State Pharmacopoeia. XI ed., Vol. 2, Art. 83. M., Medicine, 1987 ; European Pharmacopoeia. 9th Ed. Suppl. 9.2. Rockville: United States Pharmacopoeial Convention. Inc., 2016. P. 1405–1406.

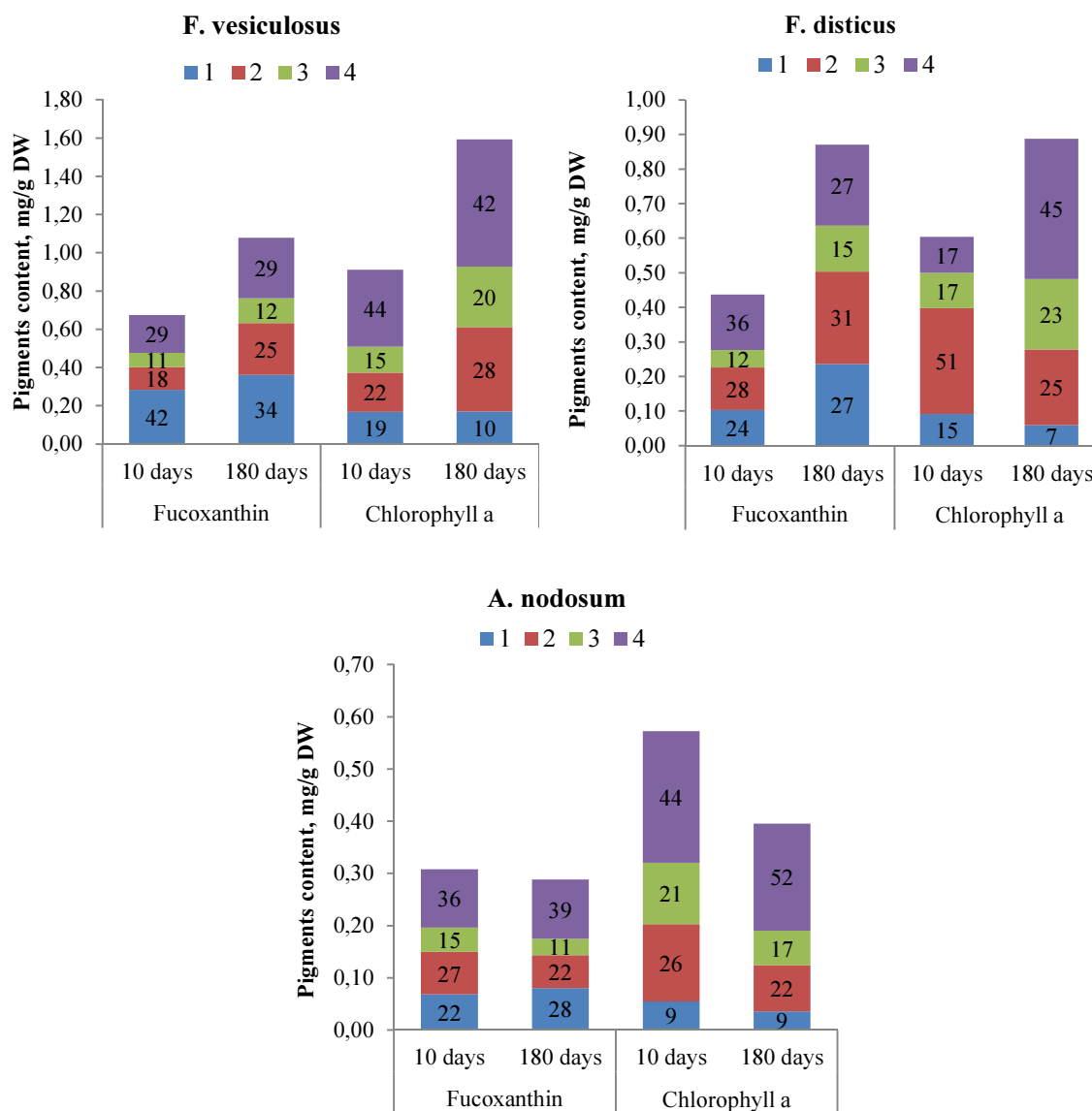


Fig. 1. The content of fucoxanthin and chlorophyll a after 10 and 180 days of storage in extracts from *F. vesiculosus*, *F. distichus*, *A. nodosum*, mg/g DW, with indicating the percentage of pigment output for each extraction stage (1–4)

Рис. 1. Содержание фукоксантина и хлорофилла а через 10 и 180 дней в экстрактах из *F. vesiculosus*, *F. distichus*, *A. nodosum*, мг/г абсолютно сухой массы сырья, с указанием процентной доли выхода пигментов для каждого этапа экстрагирования

When determining the content of pigments in 4 obtained extracts from fresh and frozen algae, it was possible to establish interesting patterns. The first extraction with a duration of 5 minutes made it possible to extract 30–40 % of fucoxanthin (in terms of its total content in algae) from the thalli of *F. vesiculosus*. For *F. distichus* and *A. nodosum*, under these conditions, 20–28 % of fucoxanthin was transferred to the extract. Chlorophyll a in these conditions extracted in smaller quantities, namely 10–20 % for all studied in this work members of the *Fucaceae*. At the subsequent stages of short-term extraction it was possible to isolate 60–70 % of the total fucoxanthin content from both fresh and long-stored algae. The extractability of chlorophyll a depended on the type of raw material and differed for each stage in fresh and frozen samples. Thus, the content of this pigment in *F. distichus* samples reached 51 % in the second stage of extraction of fresh frozen raw materials, and for long-stored samples a similar value (45 %) was extracted only by exhaustive percolation. Chlorophyll a content in *F. vesiculosus* and *A. nodosum* is the lowest for the first short-term extraction (10–20 %) and the highest for exhaustive percolation (42–52 %) regardless of the storage duration of the raw material. Thus, by varying the processing time of each type of raw material it is possible to obtain extracts

enriched with the target pigment. The result obtained is well illustrated by the absorption spectra of the extracts from frozen *F. vesiculosus* thalli that have been stored for 6 months (Fig. 2).

The study also examined the effect of storage and heat treatment on the ethanol extract of *A. nodosum*. During algae processing in order to obtain BAS, it may be necessary to store intermediate products, such as ethanol extracts. It is important to determine the possible shelf life of such a product in terms of typical cold rooms used in manufacturing. The maximum shelf life of pigment extracts was limited to 30 days, as most often required. To study the effect of thermal processing of the extract vacuum evaporation was used – the most common method of concentrating ethanol extracts in the galenic industry.

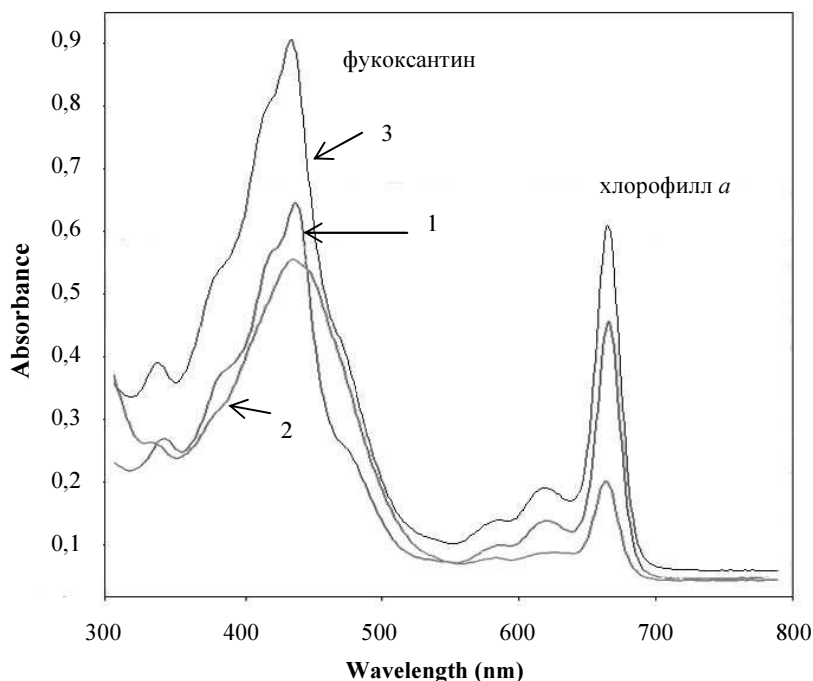


Fig. 2. Absorption spectrum of *F. vesiculosus* extracts: 1 – first extraction (5 min), 2 – second extraction (10 min), 3 – exhaustive extraction

Рис. 2. Спектр поглощения экстрактов *F. vesiculosus*: 1 – первая экстракция 5 мин, 2 – вторая экстракция – 10 мин, 3 – исчерпывающая экстракция

The study was performed with the extract obtained in the first stage of extraction at 5 minute exposure. The pigment content was determined by HPLC immediately after receiving the extracts, and then on the 2nd, 3rd, 4th, 10th, and 30th day of storage. It was established that in the studied extract stored in the refrigerator for 30 days the amount of fucoxanthin and chlorophyll a did not change. In addition, after evaporation of the extract under vacuum on a rotary evaporator at 50 °C, the amount of fucoxanthin also practically did not change, and the amount of chlorophyll a significantly decreased (Fig. 3). This is also shown on pigment chromatograms (Fig. 4). This method can be used to obtain a purer fucoxanthin fraction without using additional purification steps and other organic solvents.

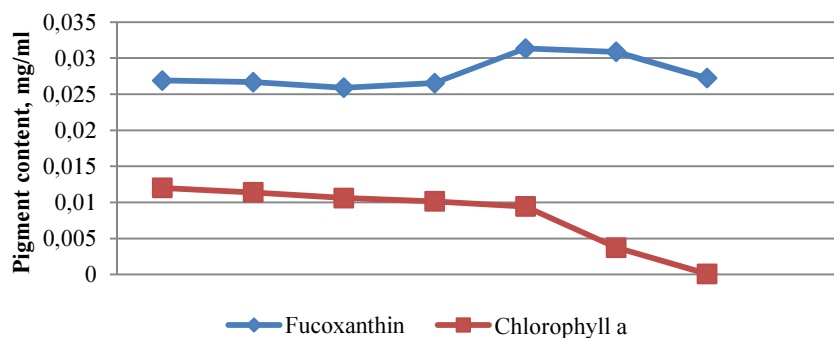


Fig. 3. The pigments content in the extract of *A. nodosum* during storage at 4 °C and after vacuum evaporation at 50 °C, mg/ml

Рис. 3. Содержание пигментов в экстракте из водорослей *A. nodosum* в процессе хранения при 4 °C и вакуум-выпаривании при 50 °C, мг/мл

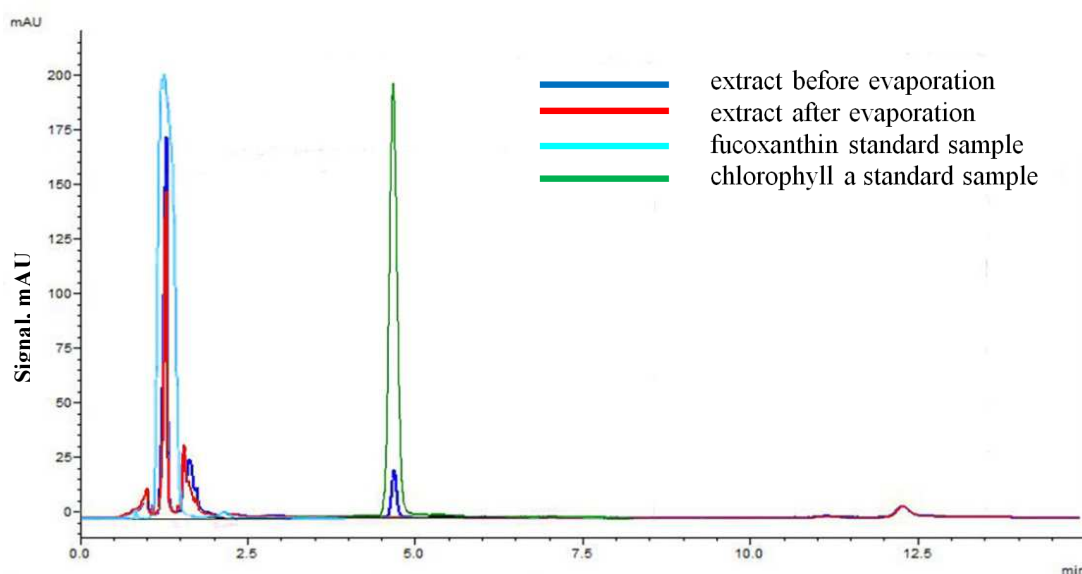


Fig. 4. Chromatogram of *A. nodosum* extracts before and after vacuum evaporation at 50 °C, mg/ml  
Рис. 4. Хроматограммы экстрактов из водорослей *A. nodosum* до и после вакуум-выпаривания при 50 °C, мг/мл

## Conclusions

In the course of the study, it has been revealed that the storage of the thalli of algae *F. vesiculosus* L., *F. distichus* L., *A. nodosum* L. of the Barents Sea in the freezer at –25 °C for 6 months does not only affect the stability and quantitative content of pigments, such as fucoxanthin and chlorophyll a, but also allows to increase their extractability from raw materials, which can be used to optimize the extraction process. It has been also shown that when *A. nodosum* ethanol extract is stored in a dark place at 4 °C for a month, the amount of fucoxanthin and chlorophyll a remains unchanged, and evaporation in vacuum at 50 °C leads to the destruction of chlorophyll a and does not affect the stability of fucoxanthin.

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