Red alga *Polysiphonia fucoides* growing in community with *Ahnfeltia plicata*, its influence on the yield and quality of agar

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**Abstract.** This article presents the results of studies on the chemical composition of *Ahnfeltia plicata* and *Polysiphonia fucoides*. The influence of the quantitative ratio of these algae in the mixture on the yield and quality indicators of agar is shown. It was found that *A. plicata* contains 13% more sulfated polysaccharides than *P. fucoides*. It was shown that an increase in the mass fraction of *P. fucoides* in the mixture does not affect the total and fractional yield of agar, but leads to a deterioration in its quality characteristics. Therefore, it is recommended to remove *P. fucoides* from the red algae mixture when collecting *A. plicata* red algae.

1. **Introduction**

Agar is a commercially important gelling polysaccharide used in the food, pharmaceutical, microbiological and cosmetic industries [1]. In the Russian Federation, agar is obtained from *A. plicata* (Hudson) growing in the White Sea and *A. tobuchiensis* (Kanno & Matsubara) - in the Sea of Japan [2, 3]. The quality of the produced agar depends not only on the technology of its production, but also on the raw materials used [3]. It is known that certain algae often grow in community with other species [2, 4, 5]. The use of a mixture of algae in the production of agar can adversely affect the quality of the resulting polysaccharide and its gelling properties. In the coastal zone of the Republic of Karelia (White Sea), the red algae *A. plicata* grows, which is a raw material for the production of agar. Studies have established that in the coastal zone of the White Sea near the village of Rabocheostrovsk, Kemsky District, *A. plicata* grows in a community with *P. fucoides* (Hudson), while the mass fraction of *P. fucoides* in this community reaches 30%. In this regard, it is advisable to study the influence of the composition of the *A. plicata* community with *P. fucoides* on the yield and quality indicators of agar.

2. **Materials and methods**

As objects of research, we used red algae *A. plicata* and *P. fucoides* harvested in the coastal zone of the village of Rabocheostrovsk, Kemsky District of the Republic of Karelia (White Sea) from 13 to 22 July 2020, and their model mixtures.

2.1. Getting agar

Weighed portions of algae (*A. plicata*, *P. fucoides* or their mixtures, where the mass fraction of *P. fucoides* was 10, 20, 30%) was filled with water in a ratio of algae to water 1:30 with the addition of calcium oxide in an amount of 5% to the weight of raw material and kept within 1 hour at a temperature of 20±2 °C. Then the algae were washed with water to remove mechanical impurities.
(shells, sand, silt, etc.). The prepared algae were placed in a container and filled with water at a temperature of 20±2 °C at a ratio of algae: water of 1:40. Agar was extracted at a temperature of 97±2 °C for 5 hours. Then the extract was poured off, and the algal residue was again poured with water, and a second of agar extraction was carried out at similar process parameters. After each extraction, the extract was filtered through a nylon sieve, centrifuged and combined, and then cooled to 20±2 °C and gelled for 4 hours. The resulting gel was cut into pieces, frozen at minus 20±2 °C, and kept for 24 hours. Then the gel was thawed, the melt water was separated, the coagel was dissolved in water at a temperature of 85-90 °C. A 1% polysaccharide solution was obtained, which was cooled to 20±2 °C and gelled for 4 hours. The gel of a 1% agar solution was used to study its physicochemical properties.

2.2. Determination of water content
The water content in the samples was determined by the change in their mass to constant after drying at a temperature of 103±2 °C.

2.3. Determination of ash content
The ash content was determined by burning a sample at a temperature of 450-500 °C and weighing it.

2.4. Determination of total nitrogen content
The total nitrogen content in the samples was determined by the Kjeldahl method on a Kjeltec Foss-2300 autoanalyzer.

2.5. Determination of fiber content
The fiber content in algae was determined by the method of Kushner and Ganak.

2.6. Melting point determination
The melting point of the gel was determined by visual fixation of the moment of its melting after heating at a rate of 0.5 °C/min.

2.7. Determination of gelation temperature
The gelation temperature of the gel was determined by visual fixation of the moment of its gelation as a result of cooling at a rate of 0.5 °C/min.

2.8. Determination of gel transparency
The transparency of the gel was determined by spectrophotometry. The measurements were carried out at zero light filter against distilled water.

2.9. Determination of gel color
The color of the gel was determined spectrophotometrically at a wavelength of 670 nm.

2.10. Determination of water-binding capacity
A weighed portion of dried algae was soaked in water at a temperature of 22±3 °C for 1 h. Then, the water content in the swollen algae was determined. The water-binding capacity was calculated from the ratio of the water content to the dry matter content of the swollen sample.

2.11. Determination of swelling
A weighed portion of dried crushed algae was placed in a cylinder and poured with water at a temperature of 22±3 °C and infused for 1 h. The swelling capacity was calculated as a percentage from the ratio of the volume of the weighed portion after swelling to its initial volume [6].

Some algae are known to frequently grow in community with other species

In order to comprehensively assess the quality of agar, each indicator of its characteristics was transformed into values of a dimensionless desirability scale using the Harrington logistic function [7].
The choice of numerical marks when constructing the desirability scale was carried out according to the values of the indicators obtained during the research.

3. Results and discussion
Analysis of the chemical composition of algae showed that the differences in the content of protein and fiber in *A. plicata* and *P. fucoides* averaged 1.0-1.5%. The ash content is twice as high in *P. fucoides* than in *A. plicata*. The difference in the content of the main carbohydrate component of algae (agar and agar-like substances) is about 13%. When soaked, dried *P. fucoides* algae absorb 3 times more water than *A. plicata*, which in general leads to a more significant increase in the volume of Polysiphonia during swelling (table 1).

Table 1. Chemical composition and technological properties of *A. plicata* and *P. fucoides*.

| Component name                        | Units       | Species of algae |   |   |
|---------------------------------------|-------------|------------------|---|---|
| Water                                 | %           | *A. plicata*     | 9.50 | 6.80 |
| Protein (N_{total}*6.25)              | % dry substances | *A. plicata* | 19.09 | 18.25 |
| Ash                                   |             | *P. fucoides*    | 12.20 | 28.59 |
| Polysaccharides                       |             | *A. plicata*     | 68.71 | 53.16 |
| including: fiber                      |             | *P. fucoides*    | 14.30 | 11.80 |
| agar and agar-like substances         |             | *A. plicata*     | 54.41 | 41.36 |
| Volume swelling                       | %           | *P. fucoides*    | 150 | 271 |
| Water binding capacity                | g of water per 1 g of dry matter of algae | *A. plicata* | 2.6 | 6.3 |

For the experiment, model mixtures were compiled, consisting of *A. plicata* and *P. fucoides*, where the mass fraction of the latter was 10, 20, 30%. Comparison of the properties of the studied polysaccharides was carried out with respect to agar isolated from *A. plicata*, which did not contain impurities of other algae. Comparison of the values of the yield of agar from *A. plicata* and its mixture with *P. fucoides* showed that the total yield of the polysaccharide does not depend on the composition of the mixture. About 60-65% of the total yield of agar is extracted during the first extraction and does not change depending on the composition of the mixture (table 2).

Table 2. The yield and physicochemical properties of agar isolated from *A. plicata*, *P. fucoides* and their mixture

| Sample № | The amount of algae in the mixture, % | *A. plicata* | *P. fucoides* | Agar yield after 1 extraction, % | Agar yield after 2 extractions, % | Total agar yield, % | Gel strength of 1% agar solution, g/cm² | Transparency, % light transmission | Color, units optical density | Temperature, °C | Content |
|-----------|-------------------------------------|-------------|---------------|-------------------------------|---------------------------------|-------------------|---------------------------------|---------------------------------|-----------------------------|----------------|---------|
| 1         | 100                                 | 0           | 9.3           | 5.4                           | 14.6                           | 245                 | 90                              | 0.046                           | 80-81                       | 33-34             | 5.18 0.46 |
| 2         | 90                                  | 10          | 8.6           | 5.8                           | 14.4                           | 155                 | 89                              | 0.054                           | 76-77                       | 32-33             | 8.78 0.46 |
| 3         | 80                                  | 20          | 8.9           | 5.3                           | 14.2                           | 135                 | 87                              | 0.060                           | 74-75                       | 31-32             | 10.28 0.54 |
| 4         | 70                                  | 30          | 8.7           | 4.8                           | 13.5                           | 85                  | 86                              | 0.063                           | 66-67                       | 29-30             | 12.05 0.56 |
| 5         | 0                                   | 100         | 9.1           | 4.9                           | 14.0                           | 45                  | 85                              | 0.069                           | 53-54                       | 27-28             | 22.73 0.40 |
An increase in the mass fraction of *P. fucoides* in the mixture leads to a 5-fold decrease in the strength of the agar gel, from 245 to 45 g/cm² and the values of melting and gelation points to 53-54 °C and 27-28 °C, respectively, while the color of the gel varies from light beige to dark brown. An increase in the amount of *P. fucoides* in the mixture also leads to a 4-fold increase in the ash content in the agar, while the total nitrogen content remains practically unchanged.

When conducting research for agar, seven main indicators of its quality were determined. However, for a comprehensive assessment of the quality of agar, it is advisable to transform data on each of the seven characteristics into a single integral indicator. Table 3 shows the developed desirability scale.

**Table 3.** Basic values of desirability functions (scale) and corresponding values of indicators.

| Indicator name                  | Indicator value | Desirability scale score | Polysaccharide quality |
|---------------------------------|-----------------|--------------------------|------------------------|
| Gel strength, g/cm²             | 250             | 0.8                      | Good                   |
|                                  | 40              | 0.2                      | Bad                    |
| Gel transparency, % light transmission | 90               | 0.8                      | Good                   |
|                                  | 85              | 0.2                      | Bad                    |
| Gel color, units optical density | 0.046           | 0.8                      | Good                   |
|                                  | 0.075           | 0.2                      | Bad                    |
| Melting point of gel, °C        | 80              | 0.8                      | Good                   |
|                                  | 50              | 0.2                      | Bad                    |
| Gelation temperature, gel, °C    | 34              | 0.8                      | Good                   |
|                                  | 25              | 0.2                      | Bad                    |
| Ash content, % dry substances    | 4.5             | 0.8                      | Good                   |
|                                  | 25.0            | 0.2                      | Bad                    |
| Total nitrogen content, % dry substances | 0.2             | 0.8                      | Good                   |
|                                  | 0.6             | 0.2                      | Bad                    |

Using the developed desirability scale and the values of each indicator, the data of particular desirability functions were obtained (table 4).

**Table 4.** Values of partial desirability functions for agar isolated from red algae and their mixtures.

| Indicator name                  | Experiment number⁴ |
|---------------------------------|---------------------|
|                                | 1  | 2   | 3   | 4   | 5   |
| Gel strength                   | 0.816 | 0.606 | 0.542 | 0.364 | 0.222 |
| Gel transparency                | 0.802 | 0.721 | 0.487 | 0.344 | 0.205 |
| Gel color                      | 0.802 | 0.683 | 0.564 | 0.496 | 0.349 |
| Melting point of gel           | 0.835 | 0.787 | 0.759 | 0.618 | 0.302 |
| Gelation temperature, gel       | 0.805 | 0.764 | 0.714 | 0.593 | 0.445 |
| Ash content                    | 0.804 | 0.731 | 0.695 | 0.648 | 0.283 |
| Total nitrogen content         | 0.452 | 0.452 | 0.308 | 0.272 | 0.553 |

⁴The experiment number corresponds to the experiment number in table 2.

Based on the values of the partial desirability functions, a single integral indicator (generalized desirability function) of agar quality was calculated (figure 1).
Figure 1. Change in the generalized desirability function depending on the amount of P. fucoides in the model algae mixture.

From the data of the graphical dependence of the generalized desirability function, it can be seen that with an increase in the proportion of P. fucoides in the mixture, it leads to a decrease in the quality indicators of agar. Thus, in order to produce high quality agar, it is necessary to remove P. fucoides from the mixture of these algae when harvesting A. plicata.

4. Conclusion

Analysis of the chemical composition of red algae samples showed that A. plicata contains 13% more sulfated polysaccharides than P. fucoides.

It was found that the total yield of agar and the yield of agar by fractions do not depend on the composition of the mixture of algae.

Using a comprehensive assessment of the quality of agar obtained from a mixture of P. fucoides and A. plicata algae and agar isolated from A. plicata, it was proved that an increase in the mass fraction of P. fucoides in the mixture leads to a noticeable deterioration in the quality characteristics of the gel-forming polysaccharide and, in connection with this, the necessity of removing this algae from raw materials during its preparation for the production of agar has been determined.

References
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