Conference Paper

Biologically Active Complex with High Antioxidant Properties Based on Macrophytes of the Azov-Black Sea Basin

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Abstract

The article presents the results of studying the chemical composition of commercial macrophytesfrom Azov-Black Sea basin -- brown algae Cystoseiraspp.andSea grass of the family Zosteraceae. It is shown that the objects selected for the study are sources of alginic acid, fucoidan, pectins, water and fat soluble vitamins, macro-and microelements. A high content of iodine and selenium is also noted. The data are given on physico-chemical characteristics of the selected Zosterin (methoxyl component, acetyl group, complexing and jelly-forming ability), the feasibility of expanding its use in the production of food products of functional purpose is substantiated. The technological applicability of dark-colored grape varieties as a raw source of compounds with antioxidant properties has been experimentally proved. Chemical composition of developed biologically active complexes of ANTIOXI VITA and ZOSTERA VITAis shown. Their suitability for inclusion in the diet of modern human as a means of high antioxidant and detoxifying propertiesis substantiated.

Keywords: macrophytes of the Azov-Black Sea basin, zosterin, anthocyanins, antioxidant properties

1. Introduction

Modern medicine pays great attention to the relationship between human health and diet. In recent years, food has been increasingly seen not only as a means of satiation and a source of energy, but also as a factor determining the normal functioning of all body systems, and as a means of preventing diseases.

In order to fulfill these functions, nutrition has to be balanced, that is, the food has to provide the body the necessary set of vitamins, minerals, enzymes and trace elements for normal functioning

Scientists have established that the so-called "diseases of civilization", such as obesity, hypertension, coronary heart disease, cancer, allergiesand diabetes are largely the result of malnutrition.

In many countries, dietary supplements (taken an addition to food), which can easily and quickly fill the deficit in vital nutrients, are increasingly used a means of prevention and to improve the population's health.

Based on international experience, there is no direct relationship between the population's provision in micronutrients and the availability of food. The necessary reduction in the amount of eatenfood does not allow saturating the body with all the necessary nutrients, especially micronutrients. In addition, the refining, cooking, canning and long-term storage of products lead to a significant reduction in the content of vitamins, macro- and microelements, and other micronutrients. The situation is aggravated by the presence of synthetic additives in food: dyes, flavors, preservatives, etc.As a consequence, even in developed countries, chronic nutritional deficiencies are becoming commonplace. For example, vitamin C and some macro- and microelements such as calcium, potassium, iodine, selenium, and zinc are often in shortage. According to many experts, the fastest, acceptable economical, and scientifically sound way to solve this problem is to create and widely use (preferably natural) dietary supplements in everyday life, including those with high antioxidant properties[1].

Recently, scientists of different specialties have concluded that the same phenomenon---damage to cell to cell walls and other structures within the cell from oxygen free radicals --- is the basis for many pathological processes in the body, leading to various diseases and, ultimately, to aging. Depending on which structures are damaged (the hereditary substance (DNA) or the outer membrane) either cancer develops or other disorders are observed.As the body agesthe activity of free radical increases, as does the risk of various age related diseases.Now, that the cause of these negative changes is known, in order to increase the duration and quality of life, it is urgent to develop biologically active complexes, which by their composition can resist the action of free radicals.

With this view, Macrophytes of the Azov-Black Sea basin and grapes of dark-colored varieties were chosen as objects of research, for an evaluation as sources of food nutrients.

It is known that the hydrological and climatic regimes of the Black sea are determined by the geographical location, the conditions of atmospheric circulation above it, water exchange with the Marmara and Azov seas, and the flow of fresh water from the land. It is located in fairly low latitudes, which causes a large influx of solar energy. According to experts, the flora of the Black Sea includes 80 species of green algae, 76 -- brown and 169 species of red algae[2].

One of the most common commercial species of brown algae in the Black Sea is cistozira (Cystoseiraspp.).It is the largest algae in the Black sea, with an average length of which is 0.6 -- 0.7 m., and its growth continues year round [3].

Among marine commercial herbs in the Black Sea, the most interesting is zoster[4].

Macrophyte flora of the Azov Sea, which is characterized by low salinity, shallow depth and predominance of soft soils, is represented by depleted flora of the Black Sea. It has 45 species of algae [2]. Throughout the coastal Eastern and Northern part the Sea is dominated by Zostera.

Macrophytesare a potential source of vitamins A, C, D, E, K and most substances from group B.TAlso, these aquatic "plants" also contain many micro- and macroelements, but especially iodine. In addition, these organisms are rich in polyunsaturated fatty acids, chlorophyll, phenolic compounds, phytosterols, plant enzymes, as well as lignins, pectin, and other biologically valuable components.

The Sea grass of the Zosteraceae family is a source of marine pectin (Zosterin), fiber, and polyphenolic compounds.

Pectin substances are high-molecular polysaccharides present in soluble (soluble pectin) or insoluble (protopectin) form in all terrestrial plants and in a number of algae. Sea grasses synthesize and accumulate polysaccharides that are not found in higher plants and have unique properties that are not fully studied. Sea pectin (Zosterin) has the formula $C_6H_80_6$ and by 90-95% is a mixture of polygalacturonase and polyglycerol acids. Having in the molecule free carboxyl groups, it forms salts-- zosterat [5].

We selected dark-colored grape varieties as a source of anthocyanins.

It is known that due to its phenolic structure anthocyanins are directly capable of binding active oxygen radicals: superoxide radical (O_2 -), singlet oxygen (`O_2), peroxide radical (ROO -), hydrogen peroxide (H_2O_2), hydroxyl radical (OH). Antioxidant activity of anthocyanins is mainly due to the presence of hydroxyl groups in their carbon rings.

Along with antioxidant properties, anthocyanins are able to influence tumor growth by affecting some basic mechanisms.

2. Methods

Brown algae Cystoseirabarbata and sea grass Zostera marina collected in the coastal zone of the Black and Azov Seas in June--July 2018; Pinot Gris, Pinot Fran, Ancellotta, Merlot, Cabernet Sauvignon, Syrah were used as objects of research.

Studies of the chemical composition were performed using standard methods adopted in a comprehensive chemical analysis: the content of total nitrogen by the Kjeldahl method using an analyzer from the company FOSS; the content of alginates by the spectrophotometric method; the monosaccharide composition by GLC after complete acid hydrolysis.

The mineral content was determined gravimetrically, after burning at a temperature of 600 -- 700 °C, using a fiber analyzer from the company FOSS. Polysaccharide from Sea grass Zosteramarinawas extracted by the method for determining pectin in terrestrial plants (Donchenko, 2000). It determined the mass fraction of moisture, methoxyl component, acetyl groups and the degree of esterification by conductometric titration.

The jelly-forming ability of the isolated pectin was determined by the estimation of the strength of the prepared gel with a mass fraction of dry sodiumzosterat 1 %, 60 % sugar and 0.3% tartaric acid; complexing capacity was determined by Tetrasodium EDTA titration.

Quantitative determination of polyphenolic compounds and anthocyanins was performed by method of high efficiency liquid chromatography[6], mineral content -- by capillary electrophoresis; vitamin content-by HPLC and fluorometry.

3. Results

The results of the study of the chemical composition of brown algae of the Black Sea are presented in Table 1.

Table 1: Chemical composition ofCystoseirabarbata, % dry substance.

Total quantity	69,90
Alginic acid	21,30
Fucoidan	9,11
Mannitol	6,14
Nitrogen substances	7,2
Mineral substances	22,7
Iodine	0,008

From the presented data it follows that the chemical composition of brown algae of the Black Sea is represented by carbohydrate, protein and mineral complex. At the same time, alginic acid predominates in the composition of carbohydrate components. The content of nitrogenous substances is on average 7.2 %, which is typical for these types of algae [7].

The amount of minerals averages 22.7%. The iodine content is 0.008%.

Fractional composition of monosaccharides in brown algae is presented in Figure 1.

The monosaccharide composition of brown algae is represented by glucose, xylose, glucose, galactose and mannitol. Fucose predominates-4.3 %.

Since traditionally macrophytesare considered as sources of vitamins and minerals, we conducted studies to determine the content of micro-and macronutrients in the studied sample of Cystoseirabarbata.

The data obtained are shown in Table 2.

The results of the study showed that the vitamin composition of the sample is represented mainly by water-soluble vitamins of group B. Calcium, magnesium, sodium, potassium, phosphorus, manganese, copper, zinc, iron, selenium are present from the minerals.

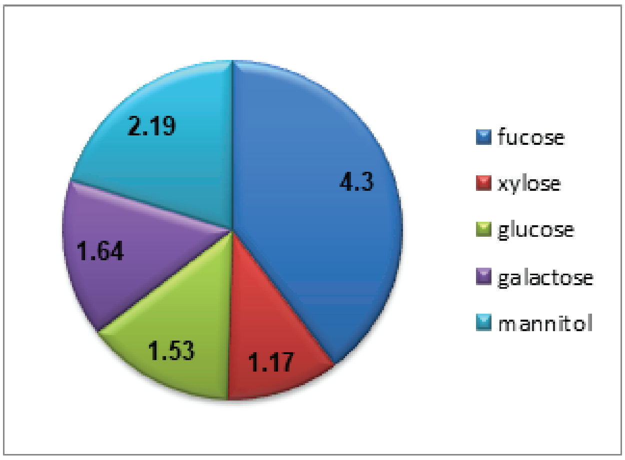

Figure 1: Fractional composition of biomass monosaccharidesCystoseirabarbata, %.

The composition of dry substances of sea grasses is represented by mineral and organic compounds.

Comparative data on the content of the main components of Zostera biomass are given in Table 3.

From the presented data it can be seen that the content of minerals in Sea grass is less than in brown algae [8].

Table 2: Content of vitamins and mineral substances in Cystoseirabarbata.

Vitamin	Content	Mineral substances	Content
Vitamin C, mg	3,00	Sodium, mg	229
Vitamin B_1, mg	0,05	Potassium, mg	90
Vitamin B_2, mg	0,14	Calcium, mg	170
Vitamin B_5, mg	0,65	Magnesium, mg	120
Vitamin B_6, mg	0,002	Phosphorus, mg	45
Vitamin B_9, mcg	170	Copper, mg	130
Beta carotene, mg	0,06	Zinc, mg	1,30
Vitamin A, mcg	6,00	Manganese, mg	0,20
Vitamin E, mg	0,85	Iron, mg	2,90
Vitamin K, mcg	61	Selenium, mics	0,70
Vitamin PP, mg	0,42		
Choline, mg	12,50		

Table 3: Chemical composition of sea grass Zostera marina collected in the coastal zone of the Black and Azov seas.

Name of Indicator	Coastal zone of the Black Sea	Coastal zone of the Azov Sea
Mass % of moisture	10,2	9,8
Mineral substances, % absolute dry substance	16,2	15,9
Organic substances, % absolute dry substance	83,9	82,7
Nitrogenous substances, % absolute dry substance	9,4	8,9
Cellulose, % absolute dry substance	12,8	12,9
Pectin substances, % absolute dry substance	12,2,	11,9

Organic substances of Sea grass are presented by carbohydrates and nitrogenous substances,their content fluctuates within 82,7 -- 83,9 %. The fiber content varies between 12,8-12,9 %.

The content of pectin is 11,9-12,2 %. We have studied the basic physical and chemical characteristics of the isolated zosterol (Table 4), confirming properies consistent with lowesterified pectin, (beet pectin is an example of lowesterified pectin) [5].

Table 4: Physical and chemical parameters of pectin substances isolated from sea grass Zostera marina collected in the coastal zone of the Black and Azov Seas.

NameofIndicator	Coastal zone of the Black Sea	Coastal Zone of the Azov Sea
Mass % of moistire	10,5	10,7
Degree of esterification, %	49,5	48,9
Metaxylene component, %	7,8	8,1
Acetylene Groups, %	0,1	0,09
Complexing ability, mg Pb^{2+}/g	270	280
Jelly-forming ability, g	12,2,	11,9

The substances of phenolic nature are quite diverse and occur in plants in the form of Monomeric, oligomeric and polymeric compounds. The results of targeted studies of phenolic compounds of grapes can significantly expand the understanding of the composition and properties of this extremely important group of substances, characterized previously as a total of tannins (tannids or tannins) and dyes (anthocyanins)[9].

Anthocyanins are a class of polyphenolic compounds synthesized only in plants. They are predominant among the flavonoids of fruits and vegetables, and are glycosides containing glucose, galactose, rhamnose, xylose, or arabinose bound to aglicone via the C3 hydroxyl group in the carbon ring. Anthocyanins are water-soluble, and, depending on the acidity and the presence of chelated metal ions, are intensely colored blue, crimson or red. Therefore, the total content of phenolic compounds and anthocyanins in the juice of the studied grape varieties was determined. (Table 5).

From the table data it is clear that grapes of the Syrah variety stand out in terms of the content of both phenolic compounds and anthocyanins. In other varieties, the total content of is in the range of 458 -- 640 mg/dm^3 for phenolic compounds and from 2,4 to 12,3 mg/dm^3 for anthocyanins.

The mass concentration of polyphenolic compounds is shown in Figure 2.

Table 5: Content of phenolic compounds in extract of dark grape varieties, mg/ dm^3.

Name of variety	Total content of phenolic compounds, mg/dm^3	Content anthocyanins, mg/ dm^3
Pinot gris	548	3,6
Pinot franc	542	9,6
Anchellotta	640	2,4
Merlo	520	12,3
Cabernet Sovignon	458	9,2
Syrah	929	25,6

In Figure 2, it can be seen that the total content of polymerics forms of phenolic compounds observed in the studied Syrah grapes is 1852 mg/ dm^3.Taking into account the obtained data we studied biologically active complex ANTIOXI VITA, containing in its composition of grape juice, for example of Syrah variety (50%), extract of Cystoseira-Cystoseirabarbata (30%), rosehip syrup or other syrup (10 %), saturated sugar syrup (10%), and citric acid (0,20 per cent).

A Jelly composition ZOSTERA VITA based on grape juice was prepared, consisting of 2% Zosterin, 0,2 % citric acid and stevia sugar substitute.

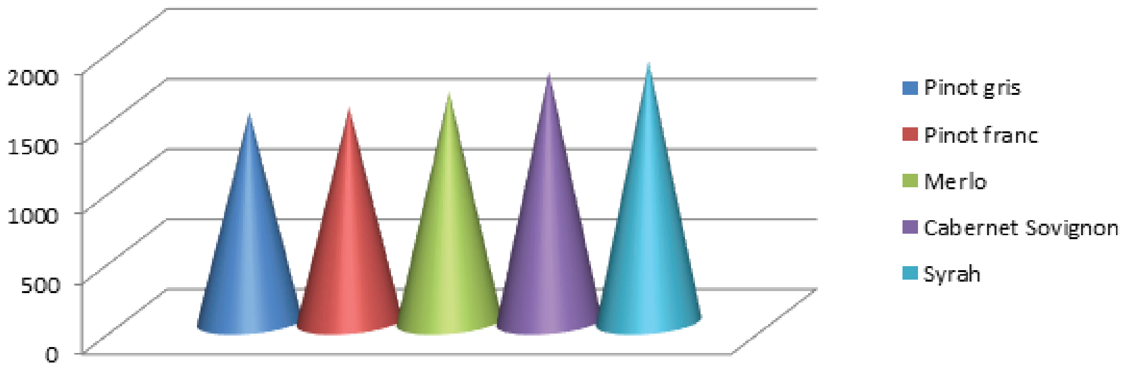

Figure 2: Mass concentration of polyphenolic compounds in grape juice, mg/dm^3.

The characteristic of organoleptic indicators of compositions by the profile method is presented in Figure 3.

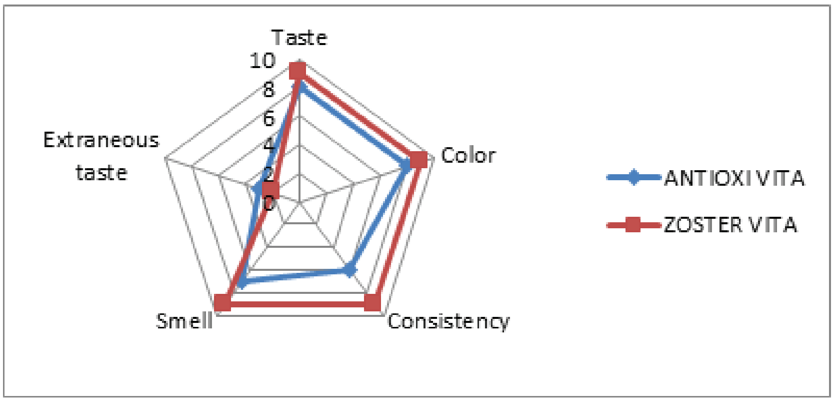

Figure 3: Profilogram of the developed biologically active complexes.

As itcan be seen from the figure, the best score on a ten-point scale has the biologically active complex ZOSTER VITA. It has a pleasant burgundy color, with a pleasant taste and smell of grapes. The liquid composition of ANTIOCH VITA has a homogeneous consistency with smallsediment; the color is light burgundy with a slight greenish tinge. It has a pleasant taste and smell. However, there is a slight taste and smell of algae.

Chemical content of the developed compositions is presented in Table 6.

Table 6: Chemical composition of the developed biologically active complexes.

Name of indicator	Biologically active complexes	
	ANTIOXI VITA	**ZOSTERA VITA**
Alginic acid, % absolute dry substance	5,96	1,35
Fucoidan, % absolute dry substance	1,72	
Mannitol, % absolute dry substance	1,71	
Iodine, mg %	15,0	6,0
Vitamin C, mg %	0,84	1,8
Vitamin B_1, mg%	0,01	0,03
Vitamin B_2, mg%	0,04	0,07
VitaminB_5, mg%	0,18	0,4
Vitamin B_6, mg%	0,0006	0,001
Vitamin B_9, mcg%	47,6	95,0
Beta carotene, mg %	0,02	
Vitamin A, mcg%	1,7	
Vitamin E, mg%	0,24	
Vitamin K, mcg %	17,0	0,4
VitaminPP, mg	1,2	
Choline, mg%	3,5	
Sodiummg%	63,3	5,0
Potassium, mg%	54,3	104,0
Calcium, mg%	47,9	11,0
Magnesium, mg%	34,2	10,0
Phosphorus, mg%	12,9	14,0
Copper, mg%	36,4	
Zinc, mg%	0,36	
Manganese, mg%	0,05	0,09
Iron, mg%	0,8	0,3
Selenium, mcg %	0,19	
Phenolic compounds, mg/ dm^3	370	850
Polyphenolic compounds, mg/ dm^3	680	1200
Anthocyans, mg/ dm^3	10,2	23,7
Pectin substances, g	0,2	2,0

4. Discussion

The studies described in this paper have shown brown algae of the Black Sea is chemically composed of carbohydrate, protein and a mineral complex Alginic acid predominates in the composition of carbohydrate components. The predominant monosaccharide is fukoza at 4,3%. Both of these compounds have a high physiological value, showing immunological and antioxidant properties.

Fucoidan, contained in the obtained biologically active complexes, is also a powerful natural antioxidant and protects cells from free radical damage.

The samples of the selected dark-colored grape varietieswere found to contain a notably high content of phenolic acid, including anthocyanins.

The WHO expert Committee on dietary supplements (JECFA) has calculated an acceptable daily dose of anthocyanins (ADI) of 2,5 mg/kg body weight for humans.

According to the recommendations of Russian scientists, the required level of consumption of anthocyanins should be 50-150 mg per day. According to studies conducted in the United States, the average consumption of anthocyanins, is estimated at 12,5 mg per person per day [9].

The results of the study of the chemical composition of the obtained biologically active complexes has shown that the content of phenolic compounds is 370 -- 850, mg/ dm^3, polyphenols -- 680 -- 1200 mg/ dm^3, and anthocyanins -- 10,2 -- 23,7 mg/ dm^3.

In their composition, they also contain fat-and water-soluble vitamins, macro and microelements.

It should be noted that among the water-soluble vitamins, the highest content was noted in vitamins of group B.

It is known that almost all residents of megacities suffer from a deficiency of these vitamins due to the consumption of refined cereals and flour products. In addition, as for all water-soluble vitamins, there is a need for its systematic replenishment in the human body. The compositions of the developed complexes are high in vitamin B_9, which is a strong antioxidant.

The content of macro- and microelements is the highest in the complexwhich is based on the extract from Cystoseirabarbata, which is consistent with theoretical data. This complex has the highest content of sodium (63,3 mg), potassium (54,3 mg), copper (36,4 mg) and calcium (47,9 mg).

Also significant is the content of micronutrients such as iodine, iron and selenium is physiologically significant. Both complexes contain micronutrients, including iodine, iron, and selenium. They have have a relatively high content of iodine (6 -- 15 mg %), which is of interest because it has pronounced antioxidant properties.

A significant role in the formation of the nutritional value of the developed biologically active complexes is played by pectin substances having immunomodulatory and detoxifying properties[11]. WHO recommendspectin as a prophylactic to reduce cholesterol and blood glucose.

5. Conclusion

The results of the research showed that macrophytes of the Black Sea brown algae Cystoseira and Sea grass family Zosteraceae are a source of vitamins, pectins and minerals and other biologically active compounds.

Dark-colored grape varieties Pinot Gris, Pinot Fran, Ancellotta, Merlot, Cabernet Sauvignon and Syrah contain a high content of mono-and polyphenolic compounds, anthocyanins with high antioxidant properties.

The use of macrophytes and grapes as a raw material source for the development of biologically active complexes determined their functional orientation due to their chemical composition.

The results of these studies provide a basis for the conclusion on the feasibility of setting up the production of new biologically active complexes and their inclusion in the diet of modern human.

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Conflict of Interest

The authors have no conflict of interest to declare.

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