



# Functional instant beverages

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## Abstract:

Brown algae are a source of hydrothermal extracts that can serve as an effective raw material for instant beverages. This article offers new formulations of functional instant beverages made of concentrated fruit juices and algal extracts of *Saccharina japonica* and *Sargassum miyabei* Yendo. The research objective was to define their bioactive and antioxidant profiles. The research featured *S. miyabei* Yendo and *S. japonica* brown algae from the Far East of Russia, their dry hydrothermal extracts, and instant drinks based on these extracts combined with concentrated juices of cranberry, sea buckthorn, and chokeberry. The list of methods included spectrophotometry, high-performance liquid chromatography, and gas chromatography. The hydrothermal algal extracts of *S. miyabei* and *S. japonica* were rich in fucoidan, phenolic compounds, and iodine. The new instant beverages underwent a sensory evaluation. They contained iodine, phenolic compounds, vitamins (ascorbic acid), fucoidan, pectin, flavonoids, anthocyanins, catechins, carotenoids, and tocopherols. All the samples could be classified as functional, but the best antiradical properties belonged to the sample with black chokeberry juice and *S. miyabei*. The new functional instant beverages had a high radical-binding activity, which reached 96.3%. One portion (200 mL) covered 27–30% of the recommended daily intake of iodine and 22–50% of vitamin C. The obtained results prove that instant beverages made of *S. japonica* and *S. miyabei* Yendo can be used as functional products.

**Keywords:** Brown algae, *Sargassum miyabei*, *Saccharina japonica*, instant beverages, iodine, fucoidan, cranberry, sea buckthorn, black chokeberry

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## INTRODUCTION

As an integral part of human diet, beverages are responsible for water and electrolyte balance. They are consumed by all population strata. Beyond the scope of standard nutrition, they are part of therapeutic and preventive diets [1]. Non-alcoholic beverages include fruit, vegetable, and berry juices, mineral water, tonic extracts, decoctions, etc. Drinks can be easily modified to expand the product line with bioactive and antioxidant functional products.

Instant beverages possess a number of serious advantages, e.g., a convenient commodity form, a long shelf life, a multicomponent composition, etc. They can be used in almost any conditions, which makes them an important part of meals ready to eat for people who work in extreme conditions, e.g., the military, emergency responders, oilfield workers, etc. Instant beverages both replenish the water balance and provide

valuable biologically active substances. Functional beverages can improve certain deficiencies, e.g., those of iodine, selenium, etc.

As a rule, instant beverages are based on land plants, e.g., fruits, vegetables, and berries that possess good sensory properties and are rich in vitamins, minerals, and antioxidants [2–4]. Seaweeds, or kelps, are known to contain a lot of biologically active substances with various antioxidant, antibacterial, anti-inflammatory, and anticarcinogenic properties [5–7]. However, kelps have a very limited application in beverage production. This research offers new formulations of functional beverages that combine biologically active substances of both marine and land origin.

Unfortunately, algal extracts have a very specific smell and taste. These sensory indicators have to be optimized for this raw material to find a wider application in beverage industry. Therefore, a functional

beverage that would combine algal biologically active substances with an attractive sensory profile is an urgent task of the contemporary food science.

*Saccharina japonica* of the *Laminariales* family is brown algae that are widespread in the Sea of Japan. They develop in thickets at 0.5–12 m below the sea surface. *S. japonica* has a thallus, holdfast, stipe, and blade. This kelp contains numerous micro- and macro-elements, e.g., iodine, calcium, zinc, magnesium, iron, and selenium, as well as vitamins and such polysaccharides as fucoidan, laminarins, alginic acids, etc. [8].

*Sargassum miyabei* of the *Sargassaceae* (*Decne*) *Kütz* family is another common kelp that grows in the Seas of Japan and Okhotsk in the Russian Far East. Its thallus is as long as 2.0–2.5 m and can weigh as much as 7 kg. *S. miyabei* is used in the food industry and pharmacy as a source of food and feed additives. This kelp is rich in vitamins A and B ( $B_1$ ,  $B_6$ , and  $B_{12}$ ), as well as in immunostimulatory and antitumor minerals and polysaccharides [9].

Hydrothermal *S. miyabei* extracts obtained by boiling and autoclaving demonstrated a significant content of phenolic substances and excellent antiradical properties [10].

Berry and fruit juices can be converted into functional beverages that possess an attractive sensory profile and contain biologically active substances. Sea buckthorn, cranberry, and chokeberry can serve as prospective raw material for such drinks. Concentrated juices are especially effective. They are obtained by vacuum evaporation or freezing to 44–62% solids and require no extra sugar. The resulting substance is thick and rich in color, which is usually similar to the color of the original juice. In addition, concentrated juices retain the original biologically active substances and vitamins.

In this research, the choice of fruit and berry juices depended on the content of biologically active substances. Sea buckthorn berries contain 3.5% sugars represented by glucose and fructose; organic acids represented by ascorbic, malic, and citric acids; vitamins  $B_1$ ,  $B_2$ , E, and P; such trace elements as iron, boron, and manganese, as well as pectins, oils, and tannins [11]. Sea buckthorn (*Hippophae* L.) is a rare multivitamin plant in terms of water-soluble and fat-soluble substances, e.g., ascorbic acid, phenolic compounds, carotenoids (vitamin A), and tocopherols (vitamin E) [12]. Sea buckthorn berries are known for their anticarcinogenic and immunomodulatory properties [13, 14].

Cranberries (*Viburnum opulus* L.) are a source of vitamins C, K, and P, as well as various microelements [15]. They contain such biologically active substances as carotenoids and flavonoids that are well-known capillary strengtheners and cholagogues. They also contain antitoxic pectins. The content of tannins in *V. opulus* is 2.01–4.15% [16]. Tannins possess P-vitamin properties, which makes them powerful antioxidants. The content of pectins in cranberries can reach 2.75% [17]. Their ability to bind with harmful substances make them excellent intestinal sorbents.

Black chokeberries (*Aronia melanocarpa* L.) are also rich in biologically active substances, which makes them a popular raw material for pharmaceuticals and food products. They contain carotenoids, bioflavonoids, pectins, organic acids, ascorbic acid, minerals, etc. Black chokeberries also contain such trace elements as boron, fluorine, iron, copper, manganese, and molybdenum. In addition, 100 g of fresh chokeberries contains 6–10  $\mu\text{g}$  of iodine compounds. Also, chokeberries are a source of flavonoids that are known for their antioxidant properties and a beneficial effect on the cardiovascular system [18]. In fact, *Aronia* bioflavonoids proved to be an outstanding natural cardioprotector. A clinical study of 44 patients demonstrated that a chokeberry extract could reduce blood pressure and the level of low-density lipoproteins in the blood. The extract also appeared to be an effective antiaggregant and homocysteine inhibitor. According to some rat studies, chokeberry reduced the concentration of triglycerides and total cholesterol in the blood [19].

The present research objective was to develop granular instant beverages based on various combinations of algal extracts of *S. japonica* and *S. miyabei* and concentrated fruit juices. The resulting mixes were tested for biologically active substances and antioxidant properties.

## STUDY OBJECTS AND METHODS

The research featured hydrothermal extracts of *Sargassum miyabei* and *Saccharina japonica*, as well as new instant beverages based on these extracts combined with concentrated sea buckthorn, cranberry, and black chokeberry juices.

The samples of *S. miyabei* and *S. japonica* were harvested in May 2020 in Nakhodka Bay of the Sea of Japan. They were placed in plastic bags, cooled with ice, and delivered to the laboratory, where they were washed with running tap water to remove salt, sand, and epiphytes. After that, the samples were soaked twice in distilled water. Each thallus sample was ground using a Waring mill and stored in a sealed container at  $-20^\circ\text{C}$ . The hydrothermal extracts were obtained by boiling in water for 60 min. The ratio of raw material to water was 1:2. The suspension was filtered through three layers of gauze. The resulting filtrate underwent infrared drying until  $\leq 8\%$  residual moisture. The pulsed heating-cooling mode of the infrared drying was as follows: irradiation time – 7–11 s, cooling time – 14–22 s, specific energy flux density – 12–15  $\text{kW/m}^2$ , limit temperature –  $85\text{--}95^\circ\text{C}$ , total processing time – 8 h. After that, the dry extract was ground.

The samples of 1,1-diphenyl-2-picrylhydrazyl (DPPH), BHT-2,6-ditertbutyl-4-methylphenol (ionol), and tannic acid were purchased from Sigma-Aldrich (USA). The Folin-Ciocalteu phenolic reagent was obtained from Fluka (Switzerland). Other reagents were of analytical grade.

To determine the ash content, we removed organic matter from the sample, burned it, and measured the

ash weight. We used the titrimetric method to define the iodine content according to the colored complex compound that iodine produced with sodium nitrite in an acidic medium. The Kjeldahl method helped determine the protein content. The content of alginic acid was determined by the titrimetric method. The excess sodium hydroxide after the alginic acid reaction in the test sample was titrated with sulfuric acid. The content of mannitol was determined using a UV-1800 scanning spectrophotometer (Shimadzu, Japan).

The amount of fucose in the kelp was determined spectrophotometrically by the color reaction between fucose, L-cysteine, and sulfuric acid. To determine the amount of fucoidan in the biomass, the fucose content was multiplied by two, based on the average fucoidan content in fucoidan, which was 50% [20].

The spectrophotometry method with the Folin-Ciocalteu reagent made it possible to determine the total content of phenolic compounds. A mix of phosphotungstic and phosphomolybdic acids was restored in an alkaline medium to determine the total content of phenols in medicinal plant raw materials and food products [21]. The experiment involved a UV-1800 scanning spectrophotometer (Shimadzu, Japan).

The quantitative content of carotenoids was determined on a UV-1800 scanning spectrophotometer (Shimadzu, Japan) in an acetone extract at a wavelength of 450 nm [22].

We used the titrimetric method to define the content of vitamin C [22].

The content of anthocyanins was determined spectrophotometrically with buffer solutions with pH of 1.0 and 4.5 [23]. The flavonoid profile was described according to the method introduced by Calado *et al.* [24]. The pectins were studied by the titrimetric method based on alkali titration of isolated pectin substances before and after hydrolysis. The quantitative content of catechins was determined spectrophotometrically at a wavelength of 504 nm [25]. The content of tocopherols was determined by high-efficiency liquid chromatography [26].

The sensory evaluation of the finished products included appearance, color, taste, and smell. The maximal score was eight points for appearance and color and nine points for taste and smell. The obtained score was ranked as follows: “excellent” – 22–25 points, “good” – 18–21 points, “fair” – 14–17 points, “not satisfactory”  $\leq$  14 points.

The nitrosamine test relied on the complex gas chromatography method with a fast chemiluminescent detector as described in [27]. The polychlorinated biphenyl test followed the gas-liquid chromatography procedure designed by Zabelina *et al.* [28].

We also tested the samples for such toxic elements as lead, cadmium, copper, and arsenic using an AA-7000 atomic absorption spectrophotometer (Shimadzu, Japan) according to the standard procedure [29]. The samples were mineralized with potassium permanganate and a mix of nitric and sulfuric acids. The detection

limits for mercury, lead, cadmium, and arsenic were 0.01 mg/kg. We used the method of flameless atomic absorption to define the content of mercury in an Hg-1 mercury analyzer (Hiranuma, Japan). The qualitative and quantitative elemental composition was described by the method of atomic absorption spectrometry in an AA-7000 spectrophotometer (Shimadzu, Japan) with a graphite cuvette and a deuterium lamp background corrector. An average assay included three samples pre-dried at 80°C and mineralized with nitric acid. The elemental solutions were standard, state-certified, and registered by the Association of Official Analytical Chemists [29].

To define the content of Cesium-137 and Strontium-90, we measured their specific activity using a Progress-Gamma scintillator (Russia).

The radical test for 2,2-diphenyl-1-picrylhydrazyl (DPPH) included the following procedures. The antiradical properties of the finished products were evaluated using the stable free DPPH radical *in vitro* [30]. The optical density was measured at  $\lambda = 517$  nm using a UV-1800 scanning spectrophotometer (Shimadzu, Japan) in 1-cm cuvettes at 25°C.

The radical-binding properties were described based on the radical-binding activity and the effective concentration which could scavenge 50% of DPPH free radicals ( $EC_{50}$ ), mg/mL.

The radical-binding activity (RBA, %) was calculated according to the following formula:

$$RBA = [A_0 - A_1] / A_0 \times 100 \quad (1)$$

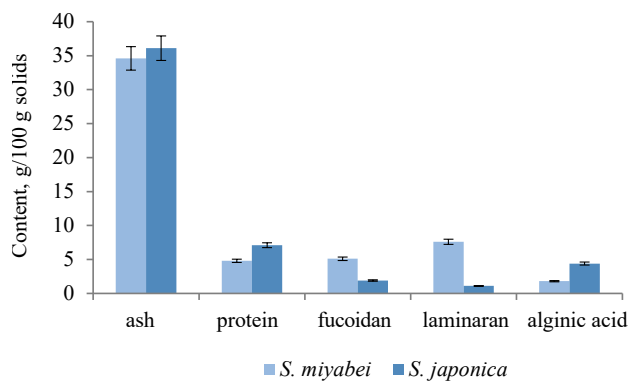
where  $A_0$  was the optical density of the control solution;  $A_1$  was the optical density of the test extract.

All the tests were conducted in triplicates. The experimental data were presented as  $M \pm m$ . The obtained data were processed in Excel and Statistica 7.0. Statistical significance was tested by the Student's t-test at a 95% significance level.

## RESULTS AND DISCUSSION

The method of hydrothermal extraction means that water-soluble substances move from the original raw materials to the extract. Figure 1 shows the chemical composition and content of biologically active substances in the algal extracts of *Sargassum miyabei* and *Saccharina japonica*. The hydrothermal extracts were salty, brown-green, and smelled of kelp. The extracts were highly soluble in water and formed a clear light green liquid with a slightly algal smell and salty taste.

The algal extracts contained a lot of combustible matter, especially the sample with *S. japonica*. The protein content in both extracts was low, especially in the *S. miyabei* sample, because it was low in the initial raw material. As for biologically active substances, both samples contained fucoidan, laminaran, and alginic acid. The content of fucoidan was especially high and exceeded 5.1 g/100 g of solids in the *S. miyabei* sample;



**Figure 1** Chemical composition and bioactive profile of *Sargassum miyabei* and *Saccharina japonica* algal extracts

**Table 1** Macro- and microelement profile of algal extracts of *Sargassum miyabei* and *Saccharina japonica* (solids)

Element	Content, g/100 g	
	Extract <i>Sargassum miyabei</i>	Extract <i>Saccharina japonica</i>
Na	0.049 ± 0.002	0.040 ± 0.002
K	0.172 ± 0.008	0.246 ± 0.0102
Ca	0.013 ± 0.0006	0.025 ± 0.0011
Mg	0.012 ± 0.0006	0.010 ± 0.004
Cr	0.00003 ± 0.000001	0.00004 ± 0.000002
Mn	0.00008 ± 0.000004	0.00005 ± 0.000002
Ni	0.000007 ± 0.0000003	0.000005 ± 0.0000002
Fe	0.005 ± 0.0002	0.004 ± 0.0002
Co	0.000001 ± 0.0000005	0.000002 ± 0.0000001
Zn	0.0000008 ± 0.00000008	0.000009 ± 0.0000003
Mo	0.000001 ± 0.0000005	0.000009 ± 0.0000004
Se	0.000005 ± 0.0000002	0.000003 ± 0.0000001

however, it was 2.7 times lower in the *S. japonica* sample. Fucoidan is a sulfated heteropolysaccharide with an extremely wide range of biological activities, e.g., antitumor, immunomodulatory, antibacterial, anti-inflammatory, etc. [31–33]. Fucoidan is a natural anticoagulant. Its effect is similar to that of heparin, but the mechanism is different [35]. In addition, fucoidan possesses an antiviral effect [36]. It is a powerful natural antioxidant that protects cells from damage by free radicals [37, 38]. Fucoidan has no cytotoxic effect. It affects both the primary tumor focus and its metastases, even distant ones [39].

The content of iodine was  $\geq 0.0019$  g/100 g of solids. The *S. japonica* sample contained 1.8 times more iodine than the *S. miyabei* sample. Iodine is an essential element that affects the metabolism of proteins, fats, and carbohydrates. It controls the balance of syntheses and catabolisms in the body. Iodine is important for the thyroid gland because it is responsible for such hormones as thyroxine and triiodothyronine [40]. In general, iodine facilitates the growth and differentiation of cells of all tissues, as well as transports sodium and hormones. Iodine deficiency triggers such conditions

as endemic goiter, hypothyroidism, and arterial hypotension. It inhibits metabolism and cognitive development in children. Iodine deficiency also affects the reproductive system in women: it may cause miscarriages and stillbirths. Moreover, iodine balance is crucial for pre-natal and post-natal development [41, 42].

Phenols were another class of biologically active substances registered in the algal extracts. Phenolic compounds are often found in plants. If consumed regularly, phenols reduce the risk of cardiovascular diseases. Plant phenolic compounds owe their high biological activity to their antioxidant properties. In addition, phenols can affect the enzymic activity of xenobiotic metabolism [43].

The content of phenolic compounds in the *S. miyabei* and *S. japonica* extracts was 251 and 196 mg/g of solids in terms of tannic acid, respectively. The list of phenolic compounds included chlorogenic, coffee, 2,5-dihydroxybenzoic, coumaric, ferulic, salicylic, and syringic acids, as well as epigallocatechin gallate, epicatechin, and epicatechin gallate.

As they live in sea water, kelps possess a selective ability to accumulate macro- and microelements in much larger concentrations than in their environment. The mineral profile of kelps includes potassium, calcium, magnesium, iron, manganese, selenium, etc. (Table 1).

As for macro-elements, potassium predominated in both extracts, but its content in the *S. japonica* sample exceeded that in the *S. miyabei* sample by 43%. The *S. japonica* sample had a 92% greater calcium content than the *S. miyabei* extract. The content of magnesium and sodium was similar in both extracts. As for micro-elements, the extracts contained eight trace elements where iron and manganese predominated, especially in the *S. miyabei* sample. The content of nickel, cobalt, chromium, zinc, and selenium was similar in both extracts. However, the *S. japonica* extract had nine times more molybdenum.

The research included a traditional technology for non-alcoholic granulated instant drinks, which consisted of three stages: preparation of raw materials, mixing and drying, and dry granulation. The algal extract was mixed with powdered sugar in a mixer for 10–15 min at 100 rpm. After that, it was mixed with a certain amount of concentrated berry juice at 100 rpm for 10–15 min. The resulting mix was loaded into the dryer tank and dried in a fluidized bed at 35–40°C for 30–40 min until the residual moisture fell below 7%. After drying, the mass was granulated through a sieve with a mesh of 2.0 or 3.0 mm. The finished product was packaged in 10-g bags. After that, 10 g of substance was dissolved in 200 mL of drinking water at 25–40°C. The drink was restored by stirring for 1–2 min.

Table 2 illustrates the mix ratios of the instant fortified beverages. The experimental formulations were based on the best sensory evaluation, where the absence of specific algal smell and taste was the main requirement.

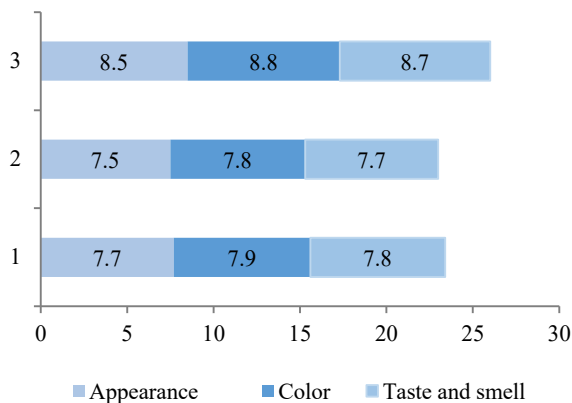


**Table 2** Formulations of instant drinks based on a combination of algal extracts of *Sargassum miyabei* or *Saccharina japonica* and concentrated berry juices

Component	Content, per 100 kg of finished product			
	Powdered sugar, kg	<i>Sargassum miyabei</i> extract, kg	<i>Saccharina japonica</i> extract, kg	Concentrated berry juice, L
Concentrated black chokeberry juice	40	–	24	36
		26	–	34
Concentrated cranberry juice	44	–	23	31
		25	–	33
Concentrated sea buckthorn juice	42	–	21	37
		23	–	35

**Table 3** Sensory profile of dissolved instant beverages based on a combination of algal extracts of *Sargassum miyabei* or *Saccharina japonica* and concentrated berry juices

	Sea buckthorn sample	Cranberry sample	Black chokeberry sample
Appearance	Transparent; no sediment	Transparent; no sediment	Opaque; no sediment
Color and taste	Orange-yellow, typical of sea buckthorn berries; sweet and sour	Burgundy-red, typical of cranberries; sour and tannic	Purple-red, typical of chokeberries; sweetish and tannic
Smell	Typical of sea buckthorn berries	Typical of cranberries	Typical of chokeberries



**Figure 2** Sensory evaluation of dissolved instant beverages based on a combination of algal extracts of *Sargassum miyabei* or *Saccharina japonica* and concentrated berry juices: 1 – cranberry juice, 2 – chokeberry juice, 3 – sea buckthorn juice

The cranberry sample required the highest content of powdered sugar, which was at its lowest in the formulation with chokeberry juice. The content of algal extracts stayed below 24% to suppress the specific algal taste and smell. The optimal content of concentrated fruit juice was between 31 and 37%.

The dissolved concentrates were tested for such traditional sensory properties as appearance, color, taste, and smell (Table 3).

When dissolved in water, the new instant beverages were transparent or opaque; they maintained the taste and smell of the berries that were part of the formulation.

Figure 2 illustrates the sensory evaluation results of the developed functional beverages.

The buckthorn juice sample achieved the best score for appearance, taste, and smell, while the cranberry sample had the best color. The total sensory scores were

as follows: cranberry sample – 23.4 points, chokeberry sample – 23 points, sea buckthorn sample – 26 points. All the samples were rated as excellent, but the sea buckthorn sample received the highest total score.

Sensory profile is important for prospective consumers. However, for functional foods the biological activity of the food system is more important, i.e., the content of biologically active substances, e.g., vitamins, carotenoids, flavonoids, mineral elements, etc., that produce a proven positive effect on human health. Table 4 demonstrates the content of biologically active substances in the new functional beverages.

The samples revealed a wide range of biologically active substances: iodine, phenolic compounds, ascorbic acid, fucoidan, pectin, flavonoids, anthocyanins, catechins, carotenoids, and tocopherols. The content of iodine was 42–89 µg per 200 mL and depended on the type of the algal extract in the formulation. *S. japonica* contributed more iodine than *S. miyabei*. The content of vitamin C was 20–45 mg per 200 mL and depended on the type of juice. It was maximal in the sea buckthorn sample and minimal in the chokeberry sample. The content of fucoidan was 40–80 mg per 200 mL and depended on the algal extract: *S. miyabei* provided two times more fucoidan than *S. japonica*. The content of pectin ranged from 25 to 63 mg per 200 mL. The maximal content belonged to the cranberry sample. The cranberry sample also demonstrated the biggest amount of flavonoids: up to 20 µg% per 200 mL. The chokeberry sample contained the greatest content of anthocyanins, which reached 2.4 mg% per 200 mL. The sea buckthorn and chokeberry samples proved to be rich in catechins: up to 21 mg% per 200 mL. Carotenoids were especially abundant in the sea buckthorn sample: 10–14 mg per 200 mL. The sea buckthorn sample also had the greatest content of tocopherols.

**Table 4** Biologically active substances in dissolved instant beverages based on a combination of algal extracts of *Sargassum miyabei* or *Saccharina japonica* and concentrated berry juices

Indicator	Content per 200 mL					
	Sea buckthorn sample		Cranberry sample		Black chokeberry sample	
	<i>Sargassum miyabei</i>	<i>Saccharina japonica</i>	<i>Sargassum miyabei</i>	<i>Saccharina japonica</i>	<i>Sargassum miyabei</i>	<i>Saccharina japonica</i>
Iodine, µg	45.1 ± 2.2	89.1 ± 4.4	42.5 ± 2.1	83.3 ± 4.0	43.6 ± 2.1	85.6 ± 4.2
Vitamin C, mg	45.8 ± 1.5	35.0 ± 1.3	37.0 ± 1.6	39.0 ± 1.7	22.0 ± 1.0	20.7 ± 0.9
Fucoidan, mg	75.6 ± 3.2	47.3 ± 2.0	72.1 ± 3.0	46.5 ± 2.2	80.5 ± 3.8	40.3 ± 1.8
Pectin, mg	37.4 ± 1.6	45.7 ± 2.1	63.8 ± 2.9	59.4 ± 2.7	28.3 ± 1.3	25.0 ± 1.2
Flavonoids, mg%	29.3 ± 1.3	19.2 ± 0.9	20.2 ± 1.0	19.2 ± 0.8	20.1 ± 1.0	18.4 ± 0.9
Anthocyanins, mg%	1.6 ± 0.1	1.9 ± 0.1	1.3 ± 0	1.2 ± 0	2.4 ± 0.1	2.2 ± 0.1
Catechins, mg%	7.1 ± 0.3	18.5 ± 0.7	8.3 ± 0.4	8.0 ± 0.3	12.7 ± 0.6	11.7 ± 0.5
Carotenoids, mg	14.8 ± 0.5	10.6 ± 0.4	6.8 ± 0.3	6.0 ± 0.2	7.2 ± 0.3	6.5 ± 0.3
Tocopherols, mg	2.64 ± 0.11	2.31 ± 0.10	1.34 ± 0.06	1.61 ± 0.07	1.50 ± 0.07	1.93 ± 0.08

Mean values ± SD, n = 3

**Table 5** Safety profile of dissolved instant beverages based on a combination of algal extracts of *Sargassum miyabei* or *Saccharina japonica* and concentrated berry juices

Indicator	Regulatory value (TR CU 021/2011)	Actual value					
		Sea buckthorn sample		Cranberry sample		Black chokeberry sample	
		<i>Sargassum miyabei</i>	<i>Saccharina japonica</i>	<i>Sargassum miyabei</i>	<i>Saccharina japonica</i>	<i>Sargassum miyabei</i>	<i>Saccharina japonica</i>
Nitrosamines, mg/kg	≤ 0.003	< 0.0002*	< 0.0001*	< 0.0002*	< 0.0001*	< 0.0001*	< 0.0002*
Polychlorinated biphenyls, mg/kg	≤ 2.0	< 0.001*	< 0.0001*	< 0.0002*	< 0.0001*	< 0.0002*	< 0.0001*
Toxic elements, mg/kg							
Lead	≤ 0.1	0.039	0.025	0.034	0.030	0.048	0.040
Arsenic	≤ 0.1	< 0.04*	< 0.04*	< 0.04*	< 0.04*	< 0.04*	< 0.04*
Cadmium	≤ 0.05	< 0.003*	< 0.003*	< 0.003*	< 0.003*	< 0.003*	< 0.003*
Mercury	≤ 0.03	< 0.004*	< 0.004*	< 0.004*	< 0.004*	< 0.004*	< 0.004*
Iron	≤ 5.0	0.7	1.0	0.9	0.85	1.18	0.59
Copper	≤ 0.4	< 0.03*	< 0.03*	< 0.03*	< 0.03*	< 0.03*	< 0.03*
Radionuclides: permissible levels, Bq/L							
Cesium-137: specific activity	≤ 40	5.01	8.05	6.47	4.82	7.54	8.45
Strontium-90: specific activity	≤ 80	3.26	7.14	5.96	3.93	8.08	10.1

\* < detection limit

Since the new formulations were based on algal extracts, we tested the samples for food safety (Table 5).

The new instant beverages proved safe for food use as the content of nitrosamines, polychlorinated biphenyls, toxic elements, and radionuclides did not exceed the regulatory values.

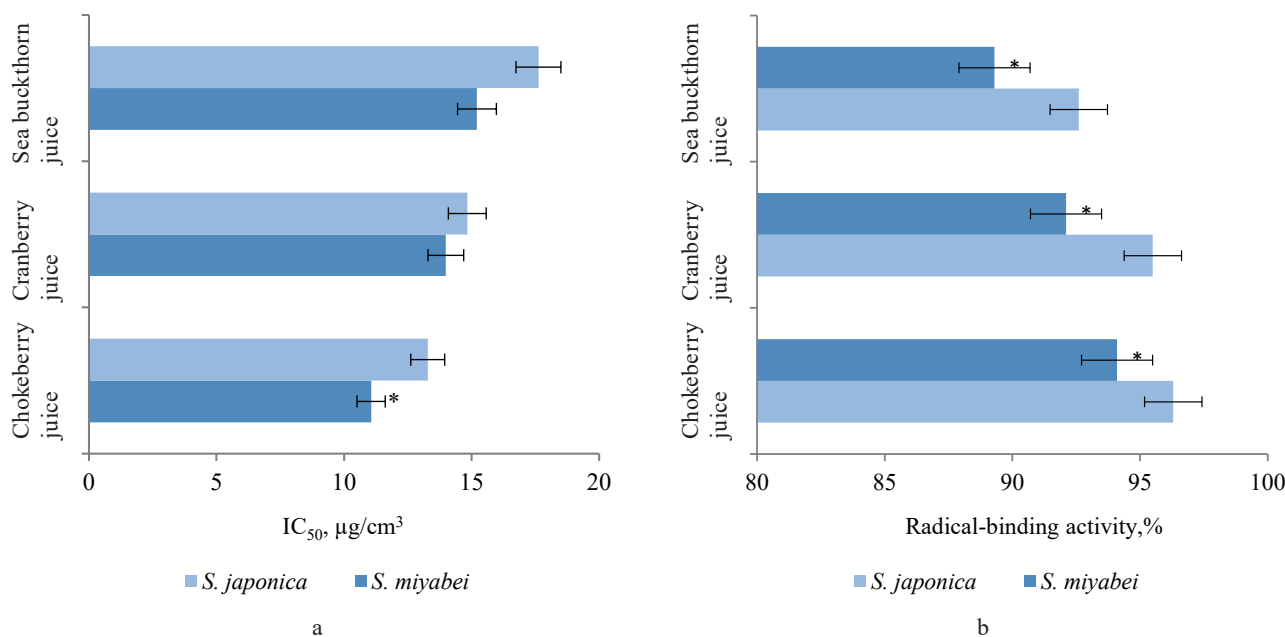
To prove that the developed beverages can be classified as functional, we calculated the percentage of the recommended daily intake for iodine and vitamin C since their biological activity is a scientifically ascertained fact (Table 6). The data on the recommended daily intake came from the standards published by the Russian Federal Agency for Oversight of Natural Resource Usage (MP 2.3.1.0253-21).

One portion (200 mL) of the new functional beverages satisfied 19–49% of iodine recommended daily

intake. It was maximal in the samples with *S. japonica*. One portion (200 mL) satisfied 22–38% of vitamin C recommended daily intake. The maximal result belonged to the cranberry sample. The sea buckthorn samples (200 mL) provided 15–18% of the physiological daily need in tocopherols. The chokeberry and sea buckthorn samples demonstrated good results in catechins. The results for anthocyanins appeared to be quite low: 12–15% of the recommended daily intake. The obvious functionality of the new beverages means that they can be used as correcting food systems for iodine and vitamin C deficiency. In addition, brown algae contain iodine in its organic form, which has a greater absorption capacity. The new instant beverages could serve as sources of tocopherols, catechins, and anthocyanins.

**Table 6** Percentage of recommended daily intake: iodine and vitamin C in the functional instant beverages based on a combination of algal extracts of *Sargassum miyabei* or *Saccharina japonica* and concentrated berry juices

Biologically active substances	Physiological need for adults	Percentage of recommended daily intake per 200 mL, %					
		Sea buckthorn sample		Cranberry sample		Black chokeberry sample	
		<i>Sargassum miyabei</i>	<i>Saccharina japonica</i>	<i>Sargassum miyabei</i>	<i>Saccharina japonica</i>	<i>Sargassum miyabei</i>	<i>Saccharina japonica</i>
Iodine	150 µg/24 h	19	48	28	47	27	49
Vitamin C	100 mg/24 h	29	28	38	34	22	25
Tocopherols	15 mg toc. equivalent/24 h	18	15	9	11	10	13
Flavan-3-ols (catechins)	200 mg/24 h	14	19	14	14	16	16
Anthocyanins	50 mg/24 h	13	14	13	12	15	14



**Figure 3** Antiradical properties of the functional instant beverages based on a combination of algal extracts of *Sargassum miyabei* or *Saccharina japonica* and concentrated berry juices: a – IC<sub>50</sub> effective concentration, b – radical-binding activity. Mean values ± SD, n = 3

The antioxidant and anti-radical properties of food systems are an important manifestation of their biological activity. Such foods can neutralize free radicals in body cells, which helps prevent certain diseases. The antiradical activity of the new functional beverages comes from two sources: the biologically active substances in the algal extracts, which received scientific confirmation, and the biologically active substances in the concentrated berry juices, e.g., catechins, anthocyanins, and ascorbic acid [10]. The composition and bioactive profile in the new functional beverages demonstrated a potentially high antiradical activity. Figure 3 shows the antiradical properties of the new instant drinks.

The chokeberry sample had a higher radical-binding activity, which was at its lowest in the sea buckthorn sample. However, all the samples demonstrated a very high radical-binding activity, which amounted to 92.6–96.3%. IC<sub>50</sub> is the concentration of an antioxidant-containing substance required to scavenge 50% of the

initial DPPH radicals. The chokeberry sample also showed the maximal radical-binding properties, which were the lowest in the sea buckthorn sample. The cranberry sample demonstrated intermediate radical-binding activity and EC<sub>50</sub>. The chokeberry sample had the highest content of anthocyanins, flavonoids, and catechins. The results suggested a high correlation between the content of biologically active substances and the antiradical properties. The *Sargassum miyabei* samples possessed higher antiradical properties than the samples based on *Saccharina japonica*, possibly due to more fucoidan.

### CONCLUSION

The new functional instant beverages were based on a combination of algal extracts of *Sargassum miyabei* or *Saccharina japonica* and concentrated berry juices. All the samples had an excellent sensory profile, but the sea buckthorn sample obtained the highest sensory score.

The beverages contained a wide range of biologically active substances, e.g., iodine, phenolic compounds, vitamins (ascorbic acid), fucoidan, pectin, flavonoids, anthocyanins, catechins, carotenoids, tocopherols, etc. As a result, the new instant beverages can be classified as functional products. One portion of the novel beverage (200 mL) satisfied 27–30% of recommended daily intake of iodine and 22–50% of vitamin C. All the samples revealed a high radical-binding activity, which reached 96.3%. The sample based on chokeberry juice and *S. miyabei* extract showed the highest antiradical

properties. Therefore, the new functional products have a positive impact on human health.

### CONTRIBUTION

The authors were equally involved in the research and are equally responsible for any potential plagiarism.

### CONFLICT OF INTEREST

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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