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RESEARCH OF THE SAFETY INDICATORS OF BERRY SAUCES WITH SEAWEED'S RAW MATERIALS

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Abstract. The current thing now is the matter regarding enrichment of food products with organic iodine, which is caused by iodine deficiency in the food ration of most of the country's population and by the country-wide radionuclide pollution, which leads to a hundredfold increase of risks of the thyroid body radiation. In this regard, there were developed the technologies of the berry sauces with the addition of the seaweed's raw materials, as the iodine-concentrating component. The technologies of blueberry-cranberry sauces with guelder-rose juice, cornel-bilberry sauce with guelder-rose juice and blueberry-buckthorn sauce with guelder-rose juice were developed. This work is dedicated to a research of safety indicators of the developed sauces. There were used the hydrated seaweeds of Laminaria, of Fucus, of Undaria pinnatifida as iodinated additives. The following microbiological indicators were determined: the quality of mesophilic aerobic and optionally anaerobic microorganisms (MAOAnM), mold, yeast and lactic acid bacteria, and the presence of the coliform bacteria (CB) bacteria. The study results showed, that there are no microorganisms in the finished products, which may result the damage of the product after pasteurization. It was identified, that the own organic acids of the prepared sauces will be sufficient to ensure the microbiological clearness and the use of the additional preserving agents does not make sense. Toxicological indicators, namely the mass fractions of lead, cadmium, arsenic and mercury, have been studied. It has been established that the content of toxic elements in test samples is hundreds of times less than permissible. The toxicological indicators were studied, namely, the mass fractions of lead, cadmium, arsenic and mercury. It was identified, that the content of toxic elements in test samples is hundreds of times less than permissible. Thus, it is proved that the sauces made according to the developed technology, comply with the requirements of the regulatory documents according to the safety indicators. Moreover, it is proved, that that the addition of the seaweed's raw materials does not worsen these indicators. To confirm the obtained data, the sample with the highest seaweed content was examined by an independent laboratory accredited according to DSTU ISO/IEC 17025 with the issuance of the appropriate test report.

Key words: berry raw materials, sauce, iodine-concentrating additives, seaweed's raw materials, microbiological quality indicators, toxicological indicators.

ДОСЛІДЖЕННЯ ПОКАЗНИКІВ БЕЗПЕЧНОСТІ ЯГІДНИХ СОУСІВ ІЗ ВОДОРОСТЕВОЮ СИРОВИНОЮ

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Анотація. Актуальним у теперішній час є питання збагачення харчових продуктів органічним йодом, що обумовлено йододефіцитом у харчових раціонах більшої частини населення країни та повсюдним радіонуклідним засміченням, що призводить до стократного збільшення ризиків опромінення щитоподібної залози. У зв'язку з цим розроблено технології ягідних соусів із додаванням водоростевої сировини в якості йодозбагачувального компонента. Розроблено технології соусів чорнично-журавлиного з соком калини, кизилово-чорничного з соком калини та чорнично-обліпихового з соком калини. Дану роботу присвячено дослідженню показників безпечності розроблених соусів. В якості йодвміщуючих добавок використовувались гідратовані водорості ламінарії, фукусу та ундарії перистої. Визначено наступні мікробіологічні показники: кількість мезофільних аеробних та факультативно анаеробних мікроорганізмів (МАФАнМ), плісняви, дріжджів та молочнокислих бактерій і наявність бактерій групи кишкової палички (БГКП). Результати дослідження показали, що в готових продуктах відсутні мікроорганізми, що можуть спричини псування продукту після пастеризації. Встановлено, що власних органічних кислот готових розроблених соусів буде достатньо для забезпечення мікробіологічної чистоти та використання додаткових консервантів не має сенсу. Досліджено токсикологічні показники, а саме масові частки свинцю, кадмію, миш'яку та ртуті. Встановлено, що вміст токсичних елементів у дослідних зразках у сотні разів менший за допустимий. Таким чином, доведено, що соуси, виготовлені за розробленою технологією, відповідають вимогам нормативних документів за показниками безпеки. Крім того, доведено, що додавання водоростевої сировини не погіршує ці показники. Для підтвердження отриманих даних, зразок з найбільшим вмістом водоростей досліджено незалежною акредитованою за ДСТУ ISO/IEC 17025 лабораторією з видачею відповідного протоколу випробування.

Ключові слова: ягідна сировина, соус, йодвміщуючі добавки, водоростева сировина, мікробіологічні показники якості, токсикологічні показники.

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Introduction. Formulation of the problem

The safety and quality of food are the most important factors determining the state of human health. A significant amount of domestic researchers pay attention to iodic insufficiency of food rations of the majority of Ukrainian population, which leads to a hundred-fold risks of radiation exposure of the thyroid body with radionuclides, which fell to the ground as a result of the accident on Chernobyl nuclear-power plant and on the First Fukusima Atomic Power Station [1]. The biological role of iodine is connected with its participation in the structure of hormones of a thyroid body (thyroxine, triiodotyrosine) and it is as their irreplaceable component. The micro-element controls the metabolism, increases the immunity and activity of some sex hormones. Iodine is useful as it burns excess fat, promotes normal growth, improves mental ability, makes our skin, teeth, hair and nails healthy [2]. The insufficiency of iodine, except vulnerability to radiation and induction diseases of a thyroid body, is dangerous, as it leads to development of such diseases as an endemic and nodal goiter, a hypothyroidism, cretinism, recurrent pregnancy loss, intellectual and physical retardation, failure of development at children. The global statistics shows, that the iodine deficiency is the most common reason of a brain damage and mental development disorders and it is the only reason, which can be prevented [3].

Analysis of recent research and publications

The most studied and widely applied way to solve the problems with iodine deficiency in the people's nutrition is the introduction of iodized kitchen salt [4-7].

However, the other variants to increase the iodine consumption will become more and more important within the next several years as a result of a policy accepted by many countries to reduce salt intake to 5 grams per day to prevent hypertension and cardiovascular diseases [8]. It potentially leads to a contradiction between two main objectives: decrease of the average consumption of table salt by the population and fight against iodine deficiency through salt iodination [2].

Now, there are options of iodination also of the other products, such as bread, milk and other milk products, oil, etc. [9-12]. However, they have a number of disadvantages. For example, iodization of bread found its effectiveness only in the regions with moderate and light iodine deficiency [9], the use of iodine-containing dairy products can't be recommended for people with indigestion of milk proteins [13]. In addition, it is necessary to pay attention to the fact that most of the proposed options for products iodizing occur by adding of inorganic iodine, which quickly evaporates when heated [14].

After conduction of the analytical literature review, it was revealed that the greatest amount of organic iodine is contained in hydrobionts, the leaders of which are edible brownish seaweeds [15-20]. It is known that in them up to 95% of iodine is in the form of organic compounds, besides its total content can reach hundreds of milligrams per one gram of alga [16]. Taking into account this and the fact, that the recommended daily rate of iodine consumption is 90 mkg for an age group of 0-59 months, 120 mkg for an age group of 6–12 years, 150 mkg for teenagers and adults and 250 mkg for women during pregnancy and during lactation [2], it can be concluded that the brownish seaweeds can be used as an effective mean in fight against iodine deficiency states. In addition, it should be noted that the results of researches for the last years show, that seaweeds have a lot of: proteins, polysaccharides – alginates and pectins, vitamins – tocopherol, thiamin, ascorbic acid, riboflavin, animal protein factor, nicotinamide etc.; macro- and microelements – calcium, sulfur, phosphorus, iron, selenium, copper, cobalt, etc. [17-19]. The use of algae has a positive effect on metabolism, on reduction of accumulation of radionuclides of strontium and caesium and salts of heavy metals such as lead, mercury, cadmium, on normalization of a condition of the haematogenic, digestive, endocrine and immune systems [17,20].

Considering the fact that the use of several grams of seaweed can satisfy the daily human need for micronutrients such as iodine, they can be used as food additives for food iodization.

In confirmation of this, it can be indicated a number of existing developments. First of all, the works of Korzun V.N., who contributed to the scientific substantiation of the principles of nutrition in the conditions of a large-scale accident and its consequences, can be noted. It belongs to him the development of the use of brownish seaweed of Fucus, Cystoseira, Laminaria in the technology of baked yeast rolls, bread, dumplings, pancakes, fish cutlets, salads from fresh vegetables, etc. [15]. It was Peresichnyi M.I, who made a significant contribution in the direction of the seaweeds use, as food additives. It was conducted, under his watch, a number of different developments, such as vegetable and vegetable and meat minces, other meat products, biscuit semi-finished products, cheesecakes, smoothie, bars with sour-milk filling and other in which the seaweed of a Laminaria and Fucus were used [21]. It should be noted, that now the majority of developments, in which the seaweeds are used as food additives, belong to the production of products from raw materials of animal origin. In such a way, Ahunova L.V. and Vinnikova L.H. were engaged in the development of technology of liver mash for functional purpose with adding of Fucus, Akhmedova T.P. – of meat cutlets with addition of a Laminaria, Ochkolias O.M. and Lebska T.K. – of butter with Laminaria and Fucus [22-24]. Berhilevych O.M., Kaluhina I.M., Korshunova H.F., Kryzhova Yu.P., Cofradesa S., Hollender R., Gallaher J.J., López-Lópeza I., Moroney N.C. worked in this direction [25-29]. Thus, it is obvious that the use of brownish seaweeds, as a means to prevent and eliminate the iodine deficiency conditions is extremely relevant and promising scientific direction.

We have developed technologies of three berry sauces with seaweeds additives, namely cornel-bilberry with guelder rose juice, bilberry-cranberry with guelder rose juice, blueberry-buckthorn with guelder rose juice. From a position of influence on organoleptic indicators of quality, it is a reasonably rational concentration of seaweeds of Laminaria, Undaria pinnatifida and Fucus as the part of recipes of these sauces [30-31]. The previous calculated studies show that consuming up to 100 g of one of the proposed sauces will help to satisfy the daily human need for iodine.

Considering the fact, that safety of food is one of the main requirements, which are provided to them, the **purpose** of this article is the research of influence seaweed's raw materials on microbiological indicators of safety of the developed sauces and establishment of content of toxic elements.

The research of influence of additives seaweed's raw materials on safety indicators was conducted on the example of bilberry and cranberry sauce with guelder-rose juice without addition and with addition of different percentage composition of seaweeds.

To achieve this goal, it was necessary to solve a number of **tasks**, namely:

– To identify by what microbiological and toxicological indicators and in accordance with which methods, it is reasonably to conduct research;
– To determine, which requirements the samples of research have to meet;
– To conduct directly the microbiological studies;
– To identify the necessity to use additional preserving agents;
– To determine directly the mass fraction of toxic elements;
– According to the obtained data, to make a conclusions about influence seaweed's raw materials on microbiological and toxicological indicators of the developed sauces;
– To confirm the received results with independent examination.

Research materials and methods

Preparation of sauces and direct researches were carried out in laboratory conditions on the basis of Oles Honchar Dnipro National University. Samples collection for microbiological analysis was performed according to DSTU 8051:2015, preparation of samples according to DSTU 7963:2015.

Considering the requirements of MBT No. 5061, the tests were carried out in accordance with the state standards according to the following microbiological indicators:

– mesophilic aerobic and optional anaerobic microorganisms (MAOAnM) – DSTU 8446:2015;
– coliform bacteria (CB) – GOST 30518-97;
– mold fungi and yeast – DSTU 8447:2015;
– lactic acid bacteria – DSTU 7999:2015.

The prepared samples of sauces were examined in five stages:

– immediately after preparation;
– 1 day after preparation;
– 7 days after preparation;
– 10 days after preparation;
– 14 days after preparation.

The sauces were kept in a tightly closed, pre-disinfected container at a temperature of 3–6°C and with relative humidity, which is not higher than 75%.

All tests were carried out three times.

To detect the MAOAnM, lactic acid bacteria, mold fungi and yeast, it was used a poured plate.

The number of MAOAnM was determined by transfering of the first and the second cultivation on the medium of meat-peptone agar and further thermostating at 37°C during 72 hours.

The identification of mold fungi and yeast was carried out by transferring of a clean product and the first cultivation on the Saburo environment with laevomycetin.

The temperature control occurred at a temperature of 24°C. Primary the calculations were performed after 3 days, final – after 5 days.

The presence (absence) of lactic bacteria was detected by transferring of a clean product on a cabbage agar and further thermostating at 30°C within 5 days.

To detect *E. coli* (coliform) at the first stage, the clean product was placed into the Kessler's medium of accumulation with glucose, thermostatically controlled at 37°C within 24 hours, and in the absence of gas formation – once more under the same conditions. Further, in order to confirm the received conclusion, they made a surface transfer to Endo's medium. Table 1 shows the microbiological indicators, which are normalized in sauces according to DSTU 6087:2009 and MBT 5061-89, and their means in accordance with the specified regulatory documents.

Table 1 – Requirements to the microbiological indicators of the sauces

Identification of indicators by the regulatory document	Meaning of indicators by the regulatory document
Number of mesophilic aerobic and optionally-anaerobic microorganisms, CFU in 1 g of the product, not more:	50
E. coli (coliform) in 1 g of the product:	Are not permitted
Mold fungi, CFU in 1 g of the product, not more than:	50
Yeast, CFU in 1 g of the product, not more than:	50
The number of lactic acid bacteria, CFU in 1 g of product:	Are not permitted

The preparation of sauces for toxicological tests were carried out in laboratory conditions on the basis of Oles Honchar Dnipro National University, and directly the toxicological tests – in the laboratory of the Testing and research and development centre of Food and Industrial Products of the State Enterprise "Dnipro Regional State Scientific and Technical Center for Standardization, Metrology and Certification". The sample preparation for the measurement was carried out in compliance with the requirements of DSTU 7670:2014. The determination of the content of these toxic elements was carried out according to the methodology of MVV DTsSMS 9/32-00, which is included in the current field of laboratory accreditation. The measurement technique is implemented on an atomic and absorption spectrophotometer with flame and electro-thermal atomization, and also it is used a device for steam generation.

The steam generation is an extremely sensitive procedure, which is used to determine the content in samples of mercury and arsenic. The detection limit of the content of toxic elements is: 0.0040 mg/kg for lead, 0.0010 mg/kg for cadmium, 0.0010 mg/kg for arsenic, 0.0001 mg/kg for mercury.

According to the requirements of DSTU 6087:2009 it is normalized in sauces:

– Weight percentage of the lead, not more than 0.40 mg/kg;
– Weight percentage of the cadmium, not more than 0.03 mg/kg;
– Weight percentage of the arsenic, not more than 0.20 mg/kg;
– Weight percentage of the mercury, not more than 0.02 mg/kg.

Results of the research and their discussion

To determine the influence of the iodine-containing additives on the safety indicators of the sauces with from wild-growing and cultivated berries, we conducted tests on the study samples of the products. Namely, there were made the samples of bilberry-cranberry sauce with guilder-rose juice according to the developed recipes without the content of the seaweed's raw materials and with the content of hydrated seaweeds: Undaria pinnatifida – 3%; Fucus – 3%, 5%; Laminaria – 3%, 5% and 8%.

During conduction of the microbiological researches, we were guided by provisions of MBT No. 5061. The choice of this normative document allows to control the influence of the seaweed's raw materials on the microbiological indicators, which can lead to the damage of a product after further pasteurization.

Talbe 2 presents the results of studies regarding the number of mesophilic aerobic and optionally anaerobic microorganisms in the test samples of sauces.

The results of studies regarding the content of CB (coliform), mold fungi, yeast and lactic acid bacteria in blueberry-cranberry sauce with guilder rose juice without addition of seaweeds and with addition of the seaweeds of Laminaria – 3%, 5%, 8%, Fucus – 3%, 5%, and Undaria pinnatifida – 3% presented the same results, which are shown in table 3.

According to the data of table 4, firstly, the seaweed's raw materials don't influence on the growth of the indicated microorganisms; secondly, the technological parameters of the developed sauce exclude a possibility of growth of harmful microorganisms.

To confirm the obtained data, the sample with the highest content of the seaweed's raw materials, namely from 8% of the hydrated laminaria, was sent for an independent examination in the Testing and Research and Development Centre of Food and Industrial Products (TRDC FIP) of the State Enterprise "Dniprovskyi regional state Scientific and Technical center of standardization, metrology and certification". The laboratories of TRDC FIP are accredited according to the international standard DSTU/IEC 17025 (accreditation certificate NAAU No. 2H047 dated January 06, 2017, which is valid until June 16, 2019).

The sample with a daily period of storage was subjected to test. Upon termination of independent estimation it was received the Test report of sample No.1483 dated May 03, 2018, which results are specified in table 4.

Data of independent examination certifies about reliability of the received by us results of a research.

It should be noted, that the growth of microorganisms influences first of all by the acidity of the product. Generally, the higher the acidity, the less is probability that it will be damaged by microorganisms. Therefore it is reasonable to indicate the study results of this indicator. The determination of acidity was carried out according to the requirements of DSTU 4957:2008 by titration of experimental samples of the product with the solution of sodium hydroxide (c = 0.1 mol/dm^3) with the presence of phenolphthalein. The calculations were made in terms of apple acid. The obtained data demonstrate, that the acidity indicators of sauces without composition of the seaweed's raw materials and with composition have almost identical results and a mass fraction of titrable acids do not exceed the value of 1.9%, the limits of a possible relative error of measurements are $\Delta \pm 3.0$, $R = 0.95$.

Table 2 – The dynamics of MAOAnM composition in blueberry-cranberry sauce with guilder rose juice depending on the type of used food additive

Name of the studied sample	Term of saving				
	After preparation	1 day	7 days	10 days	14 days
Control (without content of the seaweed additive)	<1.0x10	≤1.0x10	≤1.0x10	1.0x10	>1.0x10^{2*}
Sample with the content: of Undaria pinnatifida 3%	<1.0x10	<1.0x10	≤1.0x10	1.0x10	≥1.0x10^{2*}
of Fucus 3%	<1.0x10	≤1.0x10	1.0x10	≥1.0x10^{2*}	>1.0x10^{2*}
of Fucus 5%	<1.0x10	<1.0x10	≤1.0x10	1.0x10	≥1.0x10^{2*}
of Laminaria 3%	<1.0x10	<1.0x10	≤1.0x10	1.0x10	≥1.0x10^{2*}
of Laminaria 5%	<1.0x10	<1.0x10	≤1.0x10	1.0x10	1.0x10^{2}
of Laminaria 8%	<1.0x10	≤1.0x10	≤1.0x10	1.0x10	≥1.0x10^{2*}

*– results, which are beyond the reference values

Table 3 – Dynamics of microbiological indicators

Identification of indicators by the regulatory documents	Term of saving				
	After preparation	1 day	7 days	10 days	14 days
E. coli (coliform) in 1 g of the product	Wasn't detected	Wasn't detected	Wasn't detected	Wasn't detected	Wasn't detected
Mold fungi, CFU in 1 g of the product, not more than	<1.0x10	<1.0x10	<1.0x10	<1.0x10	<1.0x10
Yeast, CFU in 1 g of the product, not more than	<1.0x10	<1.0x10	<1.0x10	<1.0x10	≤1.0x10
The number of lactic acid bacteria, CFU in 1 g of product	Wasn't detected	Wasn't detected	Wasn't detected	Wasn't detected	Wasn't detected

Table 4 – Microbiological indicators of the sample of the blueberry-cranberry sauce with guilder-rose juice with 8% of Laminaria content according to the independent examination results

Indication of the regulatory documents	Indication of indicators according to the normative document	Actual value of indicators	Number of the products samples	
			Checked, kg	What doesn't correspond, kg
DSTU 8446:2015	Number of mesophilic aerobic and optionally-anaerobic microorganisms, CFU in 1 g of the product, not more	1.0x10	1.0	–
GOST 30518-97	*E. coli* (coliform) in 1 g of the product	Wasn't detected	1.0	–
DSTU 8447:2015	Mold fungi, CFU in 1 g of the product, not more than	less than 1.0x10	1.0	–
	Yeast, CFU in 1 g of the product, not more than	less than 1.0x10	1.0	–
DSTU 7999:2015	The number of lactic acid bacteria, CFU in 1 g of product	Wasn't detected	1.0	–

According to the received results of above-mentioned studies, it can be assumed, that the addition of the preserving agents to the recipe of prepared sauces does not make sense, as it is enough the own food acids of the source raw to preserve the microbiological purity of the product.

Considering that, in Ukraine as a whole, from 1.5 to 10% of food samples contain toxic elements, including lead, cadmium, arsenic, mercury, of which from 2.5 to 5% in the concentrations, which exceed the maximum permissible levels [32], the next stage of the study was the determination of the content of these indicators in the test samples of the products according to the requirements of DSTU 6087: 2009.

Taking into account the limit of sensitivity of the specified technique, which allows determining of the content of elements, which is one hundred times less the admissible contents according to the standard, the results of a research will allow to make not only compliance with the requirements of regulatory documentation, but also to determine quantitatively how safe is the content of the toxic elements. The results of the study are shown in table 5.

Table 5 – The content of toxic elements in the study sample of the products

Name of the studied sample	Mass fraction of the toxic element, mg/kg			
	lead	cadmium	arcenic	mercury
Control (without content of the seaweed additive)	<0.0040	<0.0010	<0.0010	<0.0001
Sample with the content:				
of Undaria pinnatifida 3%	<0.0040	<0.0010	<0.0010	<0.0001
of Fucus 3%	<0.0040	<0.0010	<0.0010	<0.0001
of Fucus 5%	<0.0040	<0.0010	<0.0010	<0.0001
of Laminaria 3%	<0.0040	<0.0010	<0.0010	<0.0001
of Laminaria 5%	<0.0040	<0.0010	<0.0010	<0.0001
of Laminaria 8%	<0.0040	<0.0010	<0.0010	<0.0001

The study of product samples without addition of the seaweed's raw materials and with its addition, showed the results, which are less than the possible indicators' detection limits. From the data of table 5, you can see, that the content of lead is at least in 100 times, of arsenic and mercury in 200 times, of cadmium in 300 times less than the permissible rate. The received results certify that the composition of the toxic elements in the studied samples of the products corresponds to the standard requirements.

It should be also noted, that by developing of the technology and by preparation of sauces, it was used the raw materials, which in accordance with the declarations of conformity and conclusions of the state sanitary and epidemiological expertise meets the requirements of the regulatory documentation.

Conclusion

The berry sauces with seaweed's raw materials, prepared by the developed technology, meet the requirements of the regulatory documents according to the most influential safety indicators. The addition of the seaweed's raw materials to the recipe does not affect the microbiological indicators of the sauce and the content of toxic elements. Considering the results of microbiological studies and the content of organic acids, the addition of extraneous preservative agents to the recipe does not make sense. It is provided, that the next stage of the study will be the determination of the other indicators of safety and quality of the developed berry sauces, namely, foreign substances, vegetable-based substances, the mass fraction of mineral substances, the mass fraction of radioactive elements.

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