DEVELOPMENT OF A FUNCTIONAL BASIS OF PHYTO-BEVERAGES WITH AN INCREASED ANTIOXIDANT ACTIVITY FOR THE CORRECTION OF NUTRITION OF PATIENTS WITH DIABETES MELLITUS

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Received March 30, 2017; Accepted in revised form July 10, 2017; Published December 26, 2017

Abstract: Diabetes mellitus is the most threatening form of human metabolic disorder and of malnutrution as one of the main causes of its development. The purpose of the studies is the development of a functional basis of phyto-beverages based on herbal medicinal raw materials with a high antioxidant activity for the correction of nutrition for diabetic patients. The object of the studies was plant medicinal raw materials that grow mainly in the territory of the Kemerovo region, various vegetative parts of plants were used: leaves (of cranberry, knotgrass, common St. John's wort, blindweed, common plantain and common horsetail); roots (of elecampane, common burdock, common dandelion); valves (common beans); shoots (of blueberry). The antioxidant activity of water extracts from plant raw materials mixtures was determined using a spectrophotometric method based on the determination of concentration of malonic dialdehyde in biological material. The following compositions of mixtures have been experimentally determined: 1) common St. John's wort, great nettle, common dandelion, blueberry, common horsetail; 2) knotgrass, great nettle, blueberry, common horsetail; as well as the following extraction parameters: the degree of grinding of raw material is 5–8 mm, the ratio of solid (medicinal plant raw material) and liquid (water) phases is 1 : 7, the temperature is 50 ± 1°C, the extraction time is 6 hours. A possibility of preserving finished extracts with alcohol up to 8% of the volume fraction has been determined, which allows to keep them for 30 days providing the alcohol content in the beverage on the basis of the extract of not more than 0.5%, according to the applicable requirements. It has been shown that the created extracts have an antioxidant activity.

Keywords: Antioxidant activity, phyto-beverages, diabetes mellitus, nutrition correction

DOI 10.21603/2308-4057-2017-2-178-188 *Foods and Raw Materials*, 2017, vol. 5, no. 2, pp. 178–188.

INTRODUCTION

The modern way of life of the majority of population of the developed countries often leads to disturbances in the metabolic processes of the human body. Carbohydrate metabolism disorders, in particular diabetes mellitus (DM), have become one of the most serious forms of metabolic disorders in recent decades, both in terms of the scale and the level of disability. Since 1980, the global incidence of diabetes has nearly doubled, having risen from 4.7% to 8.5% among the adult population. According to the predictions of the World Health Organization (WHO), diabetes will have ranked seventh among the causes of death by 2030 [1]. Over the past 10 years, the number of people with diabetes mellitus (DM) in the world has grown more than twice, having reached 415 million by the end of 2015. According to the predictions of the International Diabetes Federation, 642 million people will have suffered from diabetes by 2040 [2]. Figure 1 presents a comparative rating of the countries that are leaders in terms of the number of people aged 20–79 with diagnosed diabetes mellitus, compiled from the data for 2011 and 2015. [12]. At the same time, in most cases there is a significant increase in the number of the diseased for the period from 2011 to 2015: in particular, China is in the lead not only in terms of the number of people suffering from DM, which is explained by the largest population, but also in terms of the maximum rate of an increase in incidence - + 21% in 2015 compared to 2011.

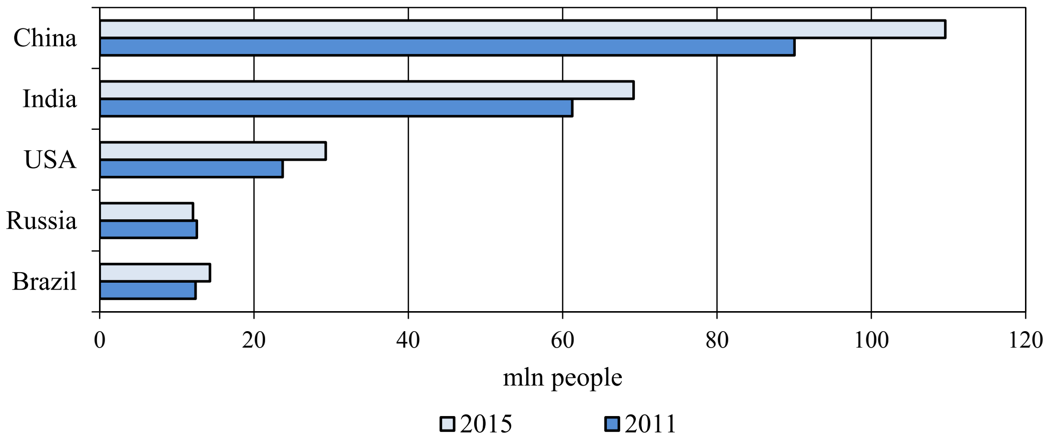

Fig. 1. Number of the people aged 20–79 with diagnosed diabetes mellitus [1, 2].

Despite the fact that the situation with the incidence of diabetes in Russia looks more stable against the background of other countries, the data of the studies show that among the adult population of Russia aged 20–79 years, 19.3% had prediabetes and 5.4% of the population had type 2 diabetes (which is the most common) [3]. At the same time, there is an opinion that the true number of patients with diabetes is approximately 3–4 times higher than the officially registered number, and reaches about 7% of the population.

The main cause of the development of type 2 diabetes is often nutrition disorders, i.e. this disease is alimentary-dependent. One of the most effective measures to prevent alimentary-dependent diseases is the use of the functional foods that can correct the diet. As a rule, the diseases that have a high level of spread are prevented by the production and sales of functional everyday food products consumed by all the population groups. Traditionally, in Russia, such products include grain products, bread and bakery products. There is an experience of enriching them with the micronutrients deficient in the diet of most Russians [4, 5]. In diabetes, the consumption of products of this group is recommended to be limited, therefore it is necessary to seek other ways of solving the problem.

The results of domestic and foreign studies indicate the advisability of supplementing the diet of patients with products with a high content of biologically active substances (BAS), including antioxidants, to increase the efficiency of therapy in patients with diabetes. The additional intake of antioxidants contributes to the prevention of complications, normalizes oxidation-reduction processes and increases the adaptive capacity of the body [6, 7, 8]. An effective source of natural antioxidants is plant raw materials, including medicinal plant raw materials. Some significant positive experience in its use in the complex therapy of diabetes mellitus has been accumulated [9, 10, 11, 12, 13, 14, 15]. Currently, there are more than 150 species of antidiabetic plants belonging to 50 families.

There are several opinions on the mode of hypoglycemic action of medicinal plants in modern preventive medicine:

(1) Plant raw materials supply alkaline radicals to the body. In a weakly alkaline solution in the presence of calcium hydroxide ($Ca(OH)_2$), glucose is spontaneously converted to fructose and mannose, resulting in an increase in the body's alkaline reserve, which can improve the uptake of glucose by tissue [7].

(2) Flavonoids contained in a lot of plants have a hypoglycemic effect, the mechanism of which is to increase the concentration of calcium that stimulates the secretion of insulin by pancreatic cells using flavonoids. Favonoids also act as vitamin P - they strengthen the walls of capillaries and contribute to an increase in the body resistance [8].

(3) Some plants (mainly that of aster family) contain inulin which is called "plant insulin." Inulin affects normal glycemia, reduces a high blood sugar level; regulates carbohydrate and lipid metabolism; improves the availability of minerals (zinc and copper), which have a hypoglycemic effect; in general, it positively affects the human immune system [7].

The use of medicinal plant raw materials as a source of antioxidants in the production of foodstuffs places a number of limitations in the choice of a product which is a functional carrier. This is due to both the flavor profile of source raw material and the need for preliminary extraction of the functional basis. This fact determines the priority of such a homogeneous group of products as non-alcoholic beverages in terms of the formation of organoleptic indicators and technological characteristics.

Non-alcoholic beverages are a popular and mass consumer product, however, the traditional formulation implies in most cases the use of sugar in quite large quantities. A relation between the use of a number of non-alcoholic beverages and different raw materials and the increased risk of development of type 2 diabetes mellitus has been revealed [16, 17]. In this regard, the studies aimed at producing functional beverages for people with carbohydrate metabolism disorders are relevant. The studies in this area are being performed [18, 19], but the assortment of these beverages in the market is insufficient.

The paper aims at developing the functional basis of phyto-beverages based on plant medicinal raw materials with a high antioxidant activity for the correction of diabetic patients.

OBJECTS AND METHODS OF STUDY

The plant medicinal raw materials that grow on the territory of the Kemerovo region (except for cranberry and blueberry) have been used as the study objects, gathering and harvesting were performed when the corresponding vegetative part of the plant, which is a functional carrier, grew ripe:

– cranberry (leaf), from Latin *Vaccínium vítis–idaéa*;
– knotgrass (leaf), from Latin *Polýgonum aviculáre*;
– elecampane (root), from Latin *Ínula helénium*;
– common St. John's wort (leaf), from Latin *Hypericum*;
– great nettle (leaf), from Latin *Urtíca dióica*;
– common burdock (root), from Latin *Arctium láppa*;
– common dandelion (root), from Latin *Taráxacum officinále*;
– blindweed (leaf), from Latin *Capsélla búrsa–pastóris*;
– common plantain (leaf), from Latin *Plantágo májor*;
– kidney bean (valves), from Latin *Phaséolus*;
– blueberry (shoots), from Latin *Vaccínium myrtíllus*;
– common horsetail (leaf), from Latin *Equisetum arvense*.

All the raw materials were used in the dry state. In addition, phytomixtures and their aqueous extracts were the study objects at different stages of the study (see Table 1 for the composition).

Research methods. The actual nutrition of people suffering from carbohydrate metabolism disorders (type 2 diabetes mellitus) was studied by means of a survey using specially developed questionnaires in accordance with the methodological guidelines "Rationalization of nutrition of the population in the sanitary and epidemiological service" on the basis of Kemerovo Municipal Clinical Hospital No. 3. The questionnaires were processed using a computer program developed by Kemerovo Institute of Food Science and Technology (University), developed using a database of the chemical composition of foods and prepared meals and taking into account nutrient losses during cooking.

The organoleptic evaluation of plant extracts was carried out in accordance with GOST 6687.5 "Non-alcoholic industry products. Methods for determination of organoleptic indices and products volume". In the finished extracts, an appearance, transparency, color, flavor and taste were determined in terms of the possibility of developing beverages with high consumer properties on their basis (an attractive appearance, a harmonious taste and flavor).

The content of solids in plant extracts was determined using a standard refractometric method.

The content of ascorbic acid in plant extracts and non-alcoholic beverages was determined by means of the titration of ascorbic acid with Tillmans paint (2,6-dichlorophenolindophenol), the principle of which is based on the ability of ascorbic acid to quantitatively reduce 2,6-dichlorphenolindophenol.

The content of polyphenolic compounds (in terms of catechine) in dry plant raw materials and plant extracts was determined according to the pharmacopoeial Leventhal method based on the light oxidability of polyphenolic compounds with a solution of 0.1 mole/dm^3 of potassium permanganate in an acid medium in the presence of an indicator and an indigosulfonic acid catalyst.

Currently, due to the relevance of the studies aimed at determining the antioxidant activity in food systems, various electrochemical [17, 18, 19], fluorescent methods of analysis are used [20]. In this paper, the antioxidant activity of water extracts from the mixtures of plant raw materials was determined using a spectrophotometric method based on the determination of the concentration of malonic dialdehyde using thiobarbituric acid in the biological material, which is more often used in medical practice. The method is based on the reaction between malonic dialdehyde and thiobarbituric acid, which proceeds, at a high temperature and a low value of pH, with a change in the colored trimethine complex that contains one molecule of malonic dialdehyde and two molecules of thiobarbituric acid. The maximum absorption of the complex accrued to 532 nm. Therefor, a test standard serum of a healthy person (taken from one healthy person as a standard test object) was used and, adding the test substances to the selected aliquot from the serum volume, malonic dialdehyde was determined after incubation. The dissolution of thiobarbituric acid and the incubation of the sample was carried out in the presence of Triton X-100, the mixture was stirred at a constant oscillation frequency, the reaction was arrested by the addition of the extract under study. Before the determination of the optical density of the sample, Trilon B and a mixture of ethanol and chloroform were added. The concentration of malonic dialdehyde in serum was calculated using Formula 1.

$$\mathrm{C_{mda}} = \frac{(D1 - D2) \times U2}{E \times L \times U2}, \quad (1)$$

where C_{mda} is the concentration of malonic dialdehyde, mmol/dm^3; D_1 is the optical density of the sample with serum; D_2 is the optical density of the control sample; U_1 is the volume of serum taken for calculation, ml; U_2 is the final volume of the mixture, ml; L is the length of the cuvette, cm; E is the extinction coefficient (156 mM^{-1}cm^{-1}).

Consequently, the higher the concentration of malonic dialdehyde in blood serum, the lower the antioxidant activity of the extracts studied.

RESULTS AND DISCUSSION

Any preventive measures to reduce alimentary-dependent diseases are associated with two main activities: the development / introduction of educational programs for groups of people with certain diseases and the development / saturation of the market with functional food products. As a rule, functional food products are designed to optimize the diets of all groups of the population and, in particular, for people who are predisposed to and / or have an alimentary-dependent disease. The first step in the optimization of diets is the study of actual nutrition, the results of which allow us to identify deviations from the norm and to propose the target directions of the studies within the general prevention task.

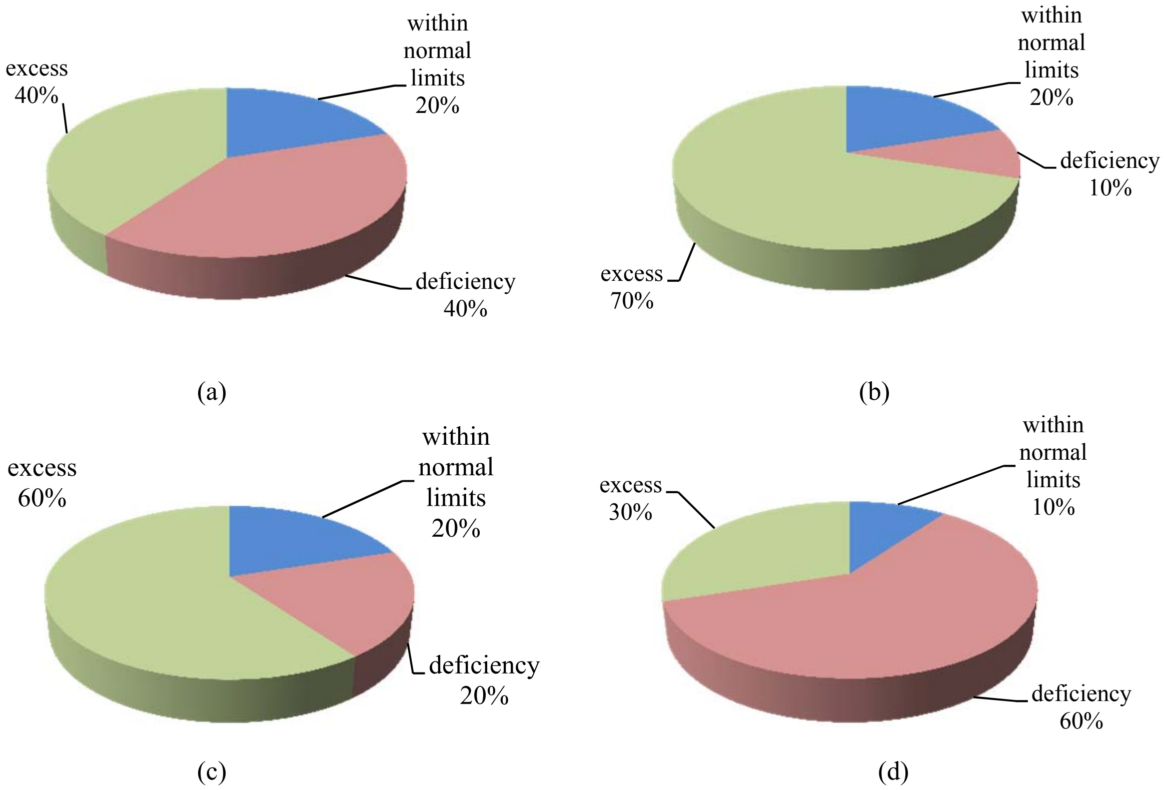

Fig. 2. Compliance of the caloric value and the content of macronutrients to the norms in the diets of the studied group of people with type 2 diabetes mellitus: (a) the compliance of the caloric value of diets, (b) the compliance of protein content in the diet, (c) the compliance of fats content in the diet, (d) the compliance of carbohydrates content in the diet

In the group of respondents in the number of 130 people (52 men and 78 women) with diagnosed diabetes mellitus (DM), diets were analyzed to determine the degree of their compliance with the principles of balanced nutrition and the recommendations of nutritionists.

Figure 2 provides the results of compliance with the norms of the caloric value of diets, as well as the content of the main nutrients.

The results showed that the caloric value of the diets in general for the whole group of the observed people was on average 1905 calories per day (at the rate of 1800–1975). The caloric value was within normal limits in 20% of patients; the caloric value of the diets was reduced on average by 10–17% in 40% of the surveyed people, and the caloric value of the diets of 40% of the men and women surveyed had a caloric value of 2250–2300 kcal.

The consumption of nutrients by respondents is significantly differentiated, which is caused by a number of things: a social status, the economic accessibility of foods, the specific knowledge about nutrition in DM and food culture in general. Thus, the average daily protein intake averaged 75.4 g in the group. The protein intake within normal limits was in 20% of men and women; protein deficiency was in 10% of the observed people, and excess protein - in 70% (up to 50%) of them. Animal protein the content of which should be at least 55% of the total, is important, in fact it was 45.1 g (59.8%) per day. The diets of 55% of respondents showed a significant content of animal protein, mainly caused by a high level of consumption of meat and poultry, sausages, canned meat and eggs. There are almost no meat products in the diets of people with low incomes, the protein intake is mainly due to the consumption of dairy products, herewith, there is lack of protein in the diet.

The average daily fats intake in the observed patients averaged 74.2 g in the group, which, at first glance, corresponds to the physiological needs. However, the use of fats within the norm is only observed in 20%, for the majority of respondents (60%) there is an excess of fat in the diet reaching 30%, and there is lack of lipids in the diet in 20% of respondents. According to medical records, 22% of respondents suffer from atherosclerotic vascular disease, which may be a result of a high content of saturated animal fats in the diet.

Despite the fact that polyunsaturated fatty acids contained in vegetable oils help to lower the cholesterol level in blood, have an anti-sclerotic effect, the percentage of vegetable fats consumption in 70% of the surveyed is reduced and is 5.2 g per day. Only 10% of respondents consume vegetable fats within normal limits, 30% of them had a slight excess of the norm of vegetable fats content in their diets (up to 12.3 g).

There was a decrease on average by 15–30% in the consumption of total of carbohydrates in 60% of respondents. Probably, this fact is due to the willful restriction of carbohydrate-containing foods within the prescribed diet. However, the nutrition of 30% of people is characterized by an excessive consumption of carbohydrates due to an excessive use of bread. For the whole of the group, the consumption of carbohydrates was 61.8% of the total caloric intake (with the recommended 50–56%), there is an exclusion of refined carbohydrates from the diet (sugar, confectionery). The respondents use sugar substitutes to give a sweet taste to products.

The total content of cellulose and pectin in the diets corresponds to the physiological norms: the average dietary fiber intake was 13.7 g, including fiber - 10.3 g and pectin - 3.4 g. However, these amounts are not enough to prevent complications and correct the behavior of DM.

On average, the level of intake of ascorbic acid with food corresponds to the recommendations. At the same time, 20% of respondents get vitamin C within normal limits, 40% feel lack of it, and 40% have a slight excess of it. The content of vitamin A in the diets has on average been reduced by almost 50% compared with the norm, and the content of vitamin PP - by 15–25%. There is lack of vitamin A in 90% of the respondents, and lack of vitamin PP - in 70%. The content of vitamin B_1 in the diet exceeds the norm by 0.5–12% in 60% of people with carbohydrate metabolism disorders, 20% feel lack of this vitamin. The average daily intake of vitamin B_2 corresponds to nutritional standards and averages 1.44 mg only in 40% of respondents. It should be noted that the imbalance in the intake of vitamins can reduce the efficiency of prevention and treatment of DM.

The study of the mineral composition of diets showed that the average daily calcium and magnesium content in diets of men and women is in most cases below the recommended level, there is only a small excess of calcium in 20% of respondents and a small excess of magnesium - in 10%. There is a high iron intake - to 27.1 mg per day - in 40% of the observed people, a low iron intake - to 12.4 mg - in 30%, and iron intake was only normal in 30%. However, it should be noted that the main part is iron from plant raw materials with a low digestibility. The level of phosphorus intake corresponds to the recommendations on average in 30%, 40% of people feel lack of phosphorus, and 44% (up to 1510 mg) - an excess of it. The ratio of Ca and P is upset in favor of phosphorus in the studied diets as well, and the ratio of Ca and Mg is upset in favor of Mg.

Thus, the analysis of diets of people with diagnosed type 2 diabetes mellitus indicates an imbalance in the consumption of nutrients and energy. In general, there is an increased energy contribution of the fat component (mainly due to animal fats), a low consumption of vegetable fats and a number of vitamins and minerals. These factors reduce the efficiency of prevention and treatment of DM and contribute to various aggravating violations.

As noted above, non-alcoholic beverages are one of the most advanced groups of food products for manufacturing functional products, which is explained by its popularity with the population, all-season consumption, quite a simple production process and a high digestibility of biologically active substances (BAS) of raw materials. In this regard, the products of this group should be used in the diet of people with diabetes mellitus as well - with the aim of optimizing it. The substantiated choice of the functional basis will allow:

– firstly, to exclude food additives from the formulation (dyes and flavors),

– secondly, to make the product therapeutic and preventive, in the context of this paper - with a high antioxidant activity.

Thus, the joint use of traditional and proven methods used in the treatment of diabetes mellitus - phytotherapy and diet therapy - was used during the study, which is justified from both the medical and preventive and the socio-economic points of view. An important aspect was a steady tendency observed in Russia to increase consumer interest in a healthy lifestyle, including interest in food products that contain natural biologically active substances.

The expediency of using medicinal plants in the production of beverages for DM is due to the fact that the considerable list of them has a hypoglycemic effect. In addition, these plants have a positive effect on the body in general, since there are a large number of complications of different organs of the body in DM.

At the first stage of the study, an analysis of scientific and technical literature containing recommendations of practical medicine for collecting medicinal plants that have a hypoglycemic effect was made. When choosing mixtures for the studies, we followed the data on the chemical composition (the absence of potent substances, the high content of vitamins, antioxidants), the proven pharmacological properties, the abundance and availability of raw materials for procurement on an industrial scale, and therefore we followed the raw material base of the Kemerovo region. It is necessary to note the experience of a number of small enterprises for processing plant raw materials and producing functional beverages with a scientific support of scientists from KemIFST and KSMU.

An important factor in the choice of medicinal raw materials was also the presence and diversity of taste and flavor characteristics, as well as coloring agents, since the use of plants that contain bitters and a lot of tannins to create beverages is undesirable.

Taking into account the above, of all the variety of the mixtures used in medical practice 10 collections that have a hypoglycemic effect in diabetes mellitus and have the organoleptic characteristics adequate for the preparation of the beverage were preferred (Table 1).

As can be seen from Table 1, various vegetative parts of plants (leaves, flowers, fruits, etc.) were used:

– leaves (of cranberry, knotgrass, common St. John's wort, blindweed, common plantain and common horsetail);

– roots (elecampane, common burdock, common dandelion);

– valves (common beans);

– shoots (of blueberry).

Table 1. Composition of the antidiabetic mixtures selected for the studies

No. of mixture	Medicinal plant raw materials											
	Cranberry (leaves) from Latin *Vaccínium vitis-idaéa*	Knotgrass (leaves) from Latin *Polýgonum aviculáre*	Elecampane (root) from Latin *Ínula helénium*	St. John's wort (leaves) from Latin *Hypericum*	Great nettle (leaves) from Latin *Urtíca dióica*	Common burdock (root) from Latin *Arctium láppa*	Common dandelion (roots) from Latin *Taráxacum officinále*	Blindweed (leaves), *from Latin Capsélla búrsa-pastóris*	Common plantain (leaves) from Latin *Plantágo májor*	Kidney beans (valves) from Latin *Phaséolus*	Blueberry (shoots), *from* Latin *Vaccínium myrtíllus*	Common horsetail (leaves) from Latin *Equisetum arvense*
1					+		+				+	
2				+	+						+	
3				+	+		+				+	+
4					+		+		+		+	
5	+				+						+	
6		+	+		+		+				+	
7						+				+	+	
8		+			+		+				+	
9		+			+						+	+
10		+			+			+				+

All the selected plants contain a large number of flavonoids, tannins, organic acids, vitamins and microelements. Elecampane, common burdock and dandelion contain inulin, have a choleretic, diuretic, antiseptic, general tonic and antioxidant effect, strengthen the walls of capillaries and regulate metabolism, in particular, have a positive effect on carbohydrate and lipid metabolism. Knotgrass, common St. John's wort, common dandelion, blueberry and common burdock are also recommended by Methodical recommendations MR 2.3.1.1915–04 "Recommended levels of consumption of food and biologically active substances" as alternative sources of flavonoids intaken by the body. Most of these plants are also widely used in other branches of the food industry.

It should be noted that the total content of plants in mixtures does not exceed five, which is optimal from the point of view of the possibility of predicting the projected functional properties of beverages. In addition, it is this number of plants that is recommended for the production of phyto-beverages. The purpose of experimental studies was to confirm the hypothesis of a hypoglycemic effect in mixtures and to rank mixtures by the quantitative content of BAS-antioxidants.

Determination of regimes of extraction of biologically active substances. The biologically active substances (BAS), which cause the antioxidant and hypoglycemic effect, transit to a solution while being extracted from plants. In this regard, the next step was to select the parameters of extraction of biologically active substances from the antidiabetic mixtures. The following criteria were set to select an extraction method:

– the harmonious organoleptic properties of the extract,
– the output of biologically active substances that determines the phyto-effect;
– the high antioxidant activity of the extract (AOA);
– the availability of the technology of the selected method of extraction of biologically active substances.

The most common is the method of BAS extraction in both the food industry and in medicine. The efficiency of the extraction process is effected by a number of factors: the nature of the extractant, the temperature and duration of extraction, the concentration of substances in the system and hydrodynamic conditions, the anatomical structure and the degree of grinding of plant material, the water absorption coefficient and the raw material-extractant ratio.

The following requirements were imposed on the extractant: the used extractant must be safe and accessible, and also provide the maximum yield of the necessary compounds. Based on the analysis of the existing experience and recommendations, preference is given to the aqueous extraction of plant raw materials, which fully meets the above requirements: it is available and completely safe, and makes possible the transition to a solution not only for antioxidants, but also for the compounds that provide a taste and flavor composition of the beverage (polysaccharides, tannins, organic acids, etc.). Purified water is pharmacologically indifferent, has a large diffuse capacity and high desorption properties.

The optimal raw materials- extractant ratio was selected taking into account the water absorption (Wa) coefficient. Providing that the common coefficient is 1.5 for roots; 2.0 for flowers and herbs, and there are both of them in mixtures, the calculated coefficient was 1.75.

The ratios of the liquid and solid phases were experimentally determined. To determine the ratio of the liquid and solid phases, the weighed quantity of the studied raw materials was filled with water, which is 1 cm higher than the upper layer of the raw materials. When using excessively large amounts of water, extracts have a low content of solids; when the volume of the liquid phase is insufficient, the process of diffusion of substances into an extractant deteriorates and the extraction of extractive substances will be incomplete. To optimize the extraction process in production, it is recommended to carry out the process of extracting BAS from the gathered raw materials, not from the individual components.

It has been experimentally determined that the optimum ratio of the solid (medicinal plant raw materials) and liquid (water) phases is 1 : 7.

An extraction temperature regime has been determined, which is (50 ± 1) °C - this temperature provides a high yield of taste and flavor substances of raw materials and the maintenance of activity of the most valuable biologically active compounds. This temperature provides an increase in the solubility of tannins, starch, pectin and other substances, and an increase in diffusion.

It is known that the phase contact area, the efficiency of release of substances from the plant cell due to tissue rupture, the rate of penetration of the extractant and of dissolution of substances depend on the degree of grinding of plant raw materials. When determining the necessary degree of grinding of the raw materials used, we followed the fact that coarse grinding (particles of more than 10 mm in size) leads to an insufficient extraction of biologically active substances - antioxidants. Grinding raw material to particles of less than 1 mm contributes to the appearance of a large number of deteriorated cells, from which nonnutritive fibers, insoluble particles and colloids transit into the extract, which impairs the organoleptic characteristics of the extract, and the resulting liquid is subsequently difficult to purify. Thus, the degree of grinding of raw materials was 5–8 mm in the studied mixtures.

To determine the duration of extraction with a certain periodicity, a yield of solids was measured. The measurements were carried out every hour using the refractometric method. The optimum extraction time was determined based on the maximum content of substances in the extract, which was 5 hours for mixtures No. 1, 4, 7 and 10 (the maximum solids content was within 2.7–3.2%), and 6 hours for mixtures from No. 2, 3, 5, 6, 8 and 9 (3.0–3.2%) (Fig. 3 and 4). The obtained values of the content of solids can be considered rational in accordance with the technology of extraction of raw materials and the recommendations of All-Union Research Institute of Brewing, Non-Alcoholic and Wine Industry (Moscow). Extracts of plant raw materials with the content of solids of 1.5% and lower are considered ineffective.

The recommended time of extraction of the plant raw materials under study for production was determined using the maximum yield of solids and the organoleptic characteristics of the extracts. It was 6 hours for all the types of the studied plant raw materials. The process cycle of extraction with duration of 6 hours is caused not only by the output of the maximum amount of extract, but also by the "ripening" of the extract, which is expressed in providing fullness and harmony for taste.

After extraction, the liquid phase was separated and the yield of the extract was determined. The yield of extracts was from 65.0 to 78.6% for various mixtures, the minimum yield of extracts being 8, 9, 2 and 10, the maximum - 3, 4, 5, 6 and 1. Valid correlation dependences of the effect of individual plants on the yield of the extract were not revealed. Different yields of extracts are explained by a different qualitative and quantitative composition of mixtures, but in general it is effective for industrial production.

In the non-alcoholic industry, to produce beverages from plant raw materials in order to increase the shelf life, alcoholic extracts preserved to a volume fraction of alcohol of 16–20 are widely used. When adding the extracts preserved in alcohol to a volume fraction of 16% or more to the beverage, in the amounts necessary to have a preventive effect, the alcohol content in the finished beverage will exceed the norm, which should not be more than 0.5% (according to GOST 28188–2014 "Non-alcoholic drinks. General specifications"), by several times. Based on the literature data and the results of our own studies on the detection of bacteriostatic properties of certain types of plant raw materials obtained earlier, a hypothesis has been put forward on the possibility of reducing the alcohol content in an alcohol extract.

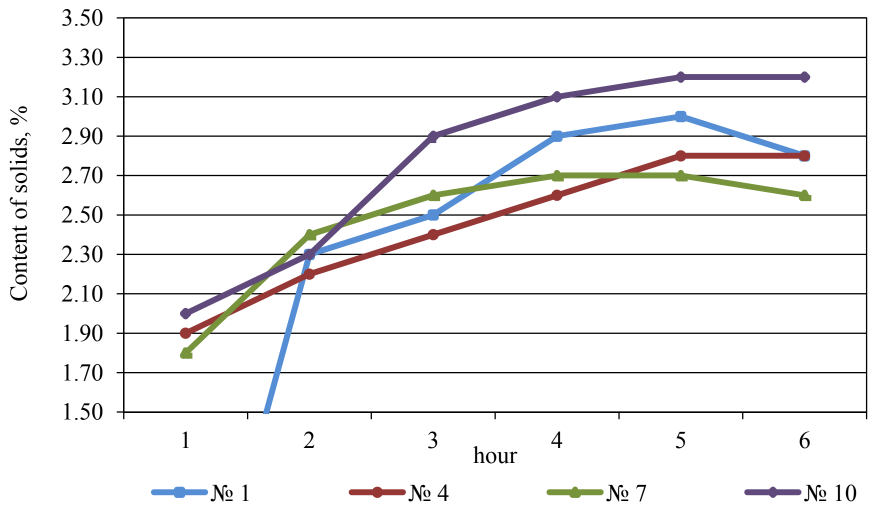

Fig. 3. Dynamics of accumulation of solids in the extracts from mixtures No. 1, 4, 7 and 10.

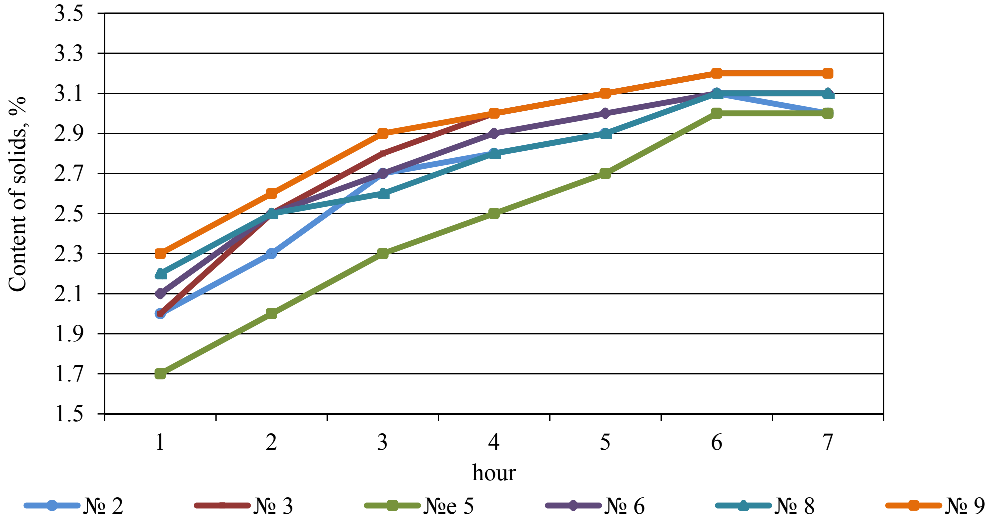

Fig. 4. Dynamics of accumulation of solids during the extraction of mixtures No. 2, 3, 5, 6, 8 and 9.

The theoretical calculation showed that when an alcoholic extract is added in the amount of 8 cm^3 to 100 cm^3 of the finished beverage, with the alcohol content of not more than 0.5% in it, the extract can only be fortified to a volume fraction of alcohol of 8%. Samples of extract from Mixture No. 3 containing 8% of volume fraction of alcohol were prepared and subjected to microbiological testing (Table 2).

The results obtained show that coliforms, pathogenic microorganisms, yeast and molds are not detected in the extract. However, the content of mesophilic aerobic and facultative anaerobic microorganisms increases as the extract is stored and it exceeds the norm on the 39th day of storage - 5.6×10^4 CFU / g at a rate of 5.0×10^4 CFU/g, which, taking into account the safety coefficient, suggests a possible shelf life of 30 days for the extract. According to GOST 28188-2014 "Non-alcoholic drinks. General specifications", the shelf life of non-alcoholic beverages of specific names, the storage and transportation conditions are established by the manufacturer in technological instructions or formulations based on the results of the studies of a pilot batch of beverages, allowing to correct the quantitative indicators of products obtained in laboratory conditions. In this regard, the results of laboratory studies of obtaining extracts and the recommendations are basic for the development of a project of technological documentation.

Study of antioxidant activity of the extracts from plant raw materials. The value of antioxidant activity (AOA) of the studied mixtures is a complex indicator that characterizes the total content of a number of biologically active substances. The analysis of reference data showed that the studied samples are rich in flavonoids, among which quercetin, avicularin, luteolin, kaempferol, hyperoside, rutin and other substances are the most common, contain ascorbic acid, catechins, tannin, sterols, free organic acids, etc.

The bioflavonoids, classified as vitamin P, form a single oxidation-reduction system together with vitamin C. Within the framework of this paper, it is proposed to use the aqueous extraction of plant raw materials and it can be stated that of the whole range of the bioflavonoids present in the analyzed raw materials water-soluble polyphenols (catechin, epicatechingallate, epicatechin, etc.) will mainly transit to an extract, and, in this regard, the antioxidant activity of the extracts will mainly be determined by the content of phenolic compounds and ascorbic acid.

According to the above, a content of phenolic compounds (in terms of catechine) was determined in the extracts. The studied mixtures can be ranked by the content of phenolic substances in the following order (Fig. 5): No. 3, 9, 2, 8, 10, 5, 6, 7, 4, 1. By the content of ascorbic acid, respectively: No. 9, 8, 3, 5, 6, 10, 4, 2, 7, 1.

Based on the results of the organoleptic evaluation of the extracts obtained, we predicted their effect on the taste and flavor of the phytonoproteins based on them. Table 3 presents total of the obtained results, five most promising extracts for the production of treatment and prophylactic beverages have been selected based on the analysis of the data presented - No. 2, 3, 8, 9 and 10.

Table 2. Microbiological indicators of the herbal extract preserved to the volume fraction of alcohol of 8%

Microbiological indicators	Admissible levels	Content of microorganisms						
		Day 7	Day 14	Day 21	Day 28	Day 30	Day 35	Day 39
Coliforms per 1 g	not permitted	not detected	not detected	not detected	not detected	not detected	not detected	not detected
Pathogenic, including salmonella, per 25 g	not permitted	not detected	not detected	not detected	not detected	not detected	not detected	not detected
QMAFAnM, CFU/g,	not more than 5.0×10^4	1.8×10^3	2.8×10^3	3.0×10^4	1.8×10^4	3.8×10^4	4.6×10^4	5.6×10^4
Yeasts and molds, CFU/cm^3, not more than	10	not detected	not detected	not detected	not detected	not detected	not detected	not detected

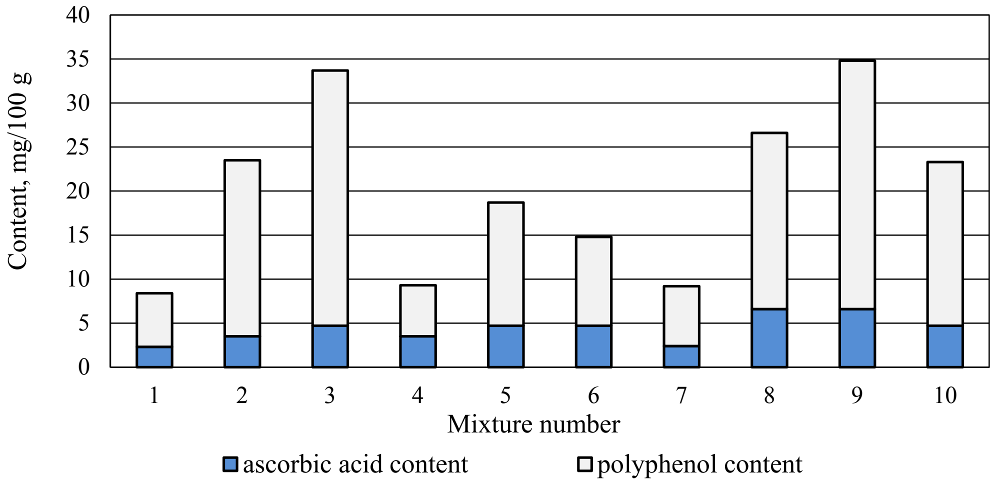

Fig. 5. Content of antioxidants in the extracts from plant raw materials.

Table 3. Organoleptic and physicochemical indicators of the water extracts prepared from phytomixtures

No. of mixture	Content of solids,%	Content of ascorbic acid, mg/100g	Transparency, color and appearance	Flavor and taste
1	3.0 ± 0.2	2.4 ± 0.3	Opaque; dark brown	Slight taste, characteristic of the grass
2	3.1 ± 0.3	3.5 ± 0.4	Opaque; greenish brown	The taste is harmonious, characteristic of the grass
3	3.2 ± 0.2	4.7 ± 0.2	Opaque; brown	The taste is full, characteristic of the grass, with a pleasant note
4	2.8 ± 0.2	3.5 ± 0.4	Opaque; light brown	The taste is blank, the flavor is weak, characteristic of the grass
5	3.0 ± 0.3	4.7 ± 0.4	Opaque; dark brown	The taste is pleasant and poor
6	3.1 ± 0.3	4.7 ± 0.4	Opaque; greenish brown	Needlessly bitter, characteristic of the grass
7	2.7 ± 0.3	2.4 ± 0.3	Opaque; light brown	The taste is imperfect and inharmonious
8	3.1 ± 0.3	6.6 ± 0.3	Opaque; greenish brown	Light, sour-bitter, pleasant
9	3.2 ± 0.2	6.6 ± 0.3	Opaque; dark brown	Slightly astringent, full, with a bit of sourness
10	3.2 ± 0.3	4.7 ± 0.2	Opaque; brownish light green	A light flavor, a pleasant taste

Due to the fact that the used method for determining polyphenol compounds allows us to determine the amount of phenolic substances, not all of which belong to bioflavonoids, the final stage of the studies was the determination of the total antioxidant activity of the extracts of the selected mixtures.

The level of antioxidant activity of extracts was judged by the content of malonic dialdehyde in blood serum, as there is an inverse relation between these indicators (the higher AOA, the lower the content of malonic dialdehyde). Table 4 presents the study results.

As shown by the data presented in Table 4, the administration of the obtained extracts into blood serum makes the lipid peroxidation processes slower, and, consequently, leads to a decrease in the concentration of malonic dialdehyde. According to antioxidant activity, the studied extracts can be ranked as follows: No. 3 > No. 9 > No. 2 > No. 8 > No. 10. The extracts prepared from mixtures of plant raw materials No. 3 and No. 9 have the highest value of antioxidant activity, they are the most promising for use as a functional basis of beverages for the correction of diabetic patients. It should be noted that these extracts previously showed the highest content of ascorbic acid and polyphenols (see Fig. 5), which indicates a direct dependence of total of AOA on them.

Based on the results obtained, formulations of the functional basis (of extracts) of phyto-beverages with a high antioxidant activity have been developed, Table 5 presents the consumption of components per 100 dal.

Table 4. Content of malonic dialdehyde in blood serum

No. of mixture	List of inward raw materials	Concentration of malonic dialdehyde, C_{mda}, mmol/l
No. 2	Common St. John's wort, great nettle, blueberry	0.0188
No. 3	Common St. John's wort, great nettle, common dandelion, blueberry, common horsetail	0.0135
No. 8	Knotgrass, great nettle, common dandelion, blueberry	0.0283
No. 9	Knotgrass, great nettle, blueberry, common horsetail	0.0169
No. 10	Knotgrass, great nettle, blindweed, common horsetail	0.0291

Table 5. Consumption of components per 100 dal (without regard to losses)

Name of food raw material	Units of measurement	Amount of raw material
Extract from Mixture No. 3		
Common St. John's wort (leaves)	kg	33.4
Great nettle (leaves)	kg	33.4
Common dandelion (roots)	kg	33.4
Blueberry (shoots)	kg	33.4
Common horsetail (leaves)	kg	33.4
Drinking water (extractant)	dm^3	1670
Ethyl alcohol, 96% vol (preservation agent)	dm^3	80
Yield	dm^3	1000.0
Extract from Mixture No. 9		
Knotgrass (leaves)	kg	35.8
Great nettle (leaves)	kg	35.8
Blueberry (shoots)	kg	35.8
Common horsetail (leaves)	kg	35.8
Drinking water (extractant)	dm^3	1430
Ethyl alcohol, 96% vol (preservation agent)	dm^3	80
Yield	dm^3	1000.0

Thus, the extracts developed on the basis of medicinal plant raw materials (mainly local raw materials) contain biologically active substances and have an antioxidant activity to varying degrees. The analysis of the functional orientation of the recommended mixtures of medicinal herbs for patients with diabetes mellitus on the basis of the results of the studies has shown the expediency of using mixtures No. 3 and No. 9 for the industrial production of phyto-beverages. A possibility of fortification of the finished extracts to 8% of the volume fraction of alcohol has been determined, due, on the one hand, to their 30-day storage capacity, which is important for industrial production, on the other hand, to the reduction of the quantitative content of the extract in the beverage to the preventive one at a given level of quality, namely, with the alcohol content of not more than 0.5% according to the requirements of regulatory documentation.

REFERENCES

1. Whiting D.R., Guariguata L., Weil C., and Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. *Diabetes Research and Clinical Practice*, 2011, vol. 94, no. 3, pp. 311–321. DOI: 10.1016/j.diabres.2011.10.029.
2. *IDF atlas (7th edition update). Brussels, Belgium. International Diabetes Federation*, 2015. Available at: http://www.diabetesatlas.org/ (accessed 28 April 2017).
3. Dedov I.I., Shestakova M.V., and Galstyan G.R. The prevalence of type 2 diabetes mellitus in the adult population of Russia (NATION study). *Diabetes Mellitus*, 2016, vol. 19, no. 2, pp. 104–112. DOI: 10.14341/DM2004116-17.
4. Davydenko N.I. and Mayurnikova L.A. On the possibility to grow high-selenium wheat in the Kuznetsk Basin. *Food and Raw Materials*, 2014, vol. 2, no. 1, pp. 67–81. DOI: 10.12737/4089.
5. Davydenko N.I. The development of a complex additive for bread enrichment with selenium and iodine. *Food Processing: Techniques and Technology*, 2013, no. 1, pp. 127–132. (In Russian).
6. Kaneto H., Kajimoto Y., Miyagawa J., et al. Beneficial effects of antioxidants in diabetes. *Diabetes*, 1999, vol. 48, no. 12, pp. 2398–2406.
7. Gromnatsky N.I. *Diabetologiya* [Diabetology]. Moscow: All-Russian educational, scientific and methodological center for continuing medical and pharmaceutical education of the Ministry of Health of the Russian Federation, 2002. 256 p.
8. Babenkova I.V. and Teselkin Yu.O. Effect of an antioxidant drug based on bioflavonoids and vitamin C on the antioxidant activity of blood plasma. *Problems of Nutrition*, 1999, no. 3, pp. 9–11. (In Russian).
9. Dadali V.A. and Makarov V.G. Biologicheski aktivnye veshchestva lekarstvennykh rasteniy kak faktor detoksikatsii organizma [Biologically active substances of medicinal plants as a factor of detoxification of organism]. *Problems of Nutrition*, 2003, no. 5, pp. 49–55. (In Russian).
10. Chanda S. and Dave R. In vitro models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: An overview. *African Journal of Microbiology Research*, 2009, 3(13), pp. 981–96.
11. Sulaiman Mohammed, Tijani Hamzat Ibiyeye, Abubakar Bashir Mohammed, et al. An overview of natural plant antioxidants: analysis and evaluation. *African Journal of Microbiology Research*, 2013, 1(4), pp. 64–72. DOI: 10.11648/j.ab.20130104.12.

12. Zhu H., Wang Y., Wang J., et al. Antidiabetic and antioxidant effects of catalpol extracted from rehmannia glutinosa (di huang) on rat diabetes induced by streptozotocin and high-fat, high-sugar feed. *Chinese medicine*, 2016, vol. 11, no. 1, Article number 25. DOI: 10.1186/s13020-016-0096-7.

13. Dzhafarova R.E. Study of action of phytocomplex «Antidiabet» and «Mirfazin» to sugar in blood plasma and glycogen in liver and muscle tissue. *Vestnik of Russian military medical Academy*, 2013, no. 4(44), pp. 172–174. (In Russian).

14. Chang C.L. and Lin C.S. Phytochemical composition, antioxidant activity, and neuroprotective effect of Terminalia chebula Retzius extracts. *Evidence-Based Complementary and Alternative Medicine*, vol. 2012. Available at: https://www.hindawi.com/journals/ecam/2012/125247. (accessed 28 December 2016).

15. Abdille Md.H., Singh R.P., Jayaprakasha G.K., and Jena B.S. Antioxidant activity of the extracts from Dillenia indica fruits. *Food Chemistry*, 2005, vol. 90, no. 4, pp. 891–896. DOI: 10.1016/j.foodchem.2004.09.002.

16. Ma Y., He F.J., Hashem K.M., Macgregor G.A., and Yin Y. Gradual reduction of sugar in soft drinks without substitution as a strategy to reduce overweight, obesity, and type 2 diabetes: a modelling study. *The lancet diabetes and endocrinology*, 2016, vol. 4, no. 2, pp.105–114. DOI: 10.1016/S2213-8587(15)00477-5.

17. Yracheta J.M., Lanaspa M.A., Le M.T., et al. Diabetes and kidney disease in american indians: potential role of sugar-sweetened beverages. *Mayo clinic proceedings*, 2015, vol. 90, no. 6, pp. 813–823. DOI: 10.1016/j.mayocp.2015.03.018.

18. Cherevach E.I., Tenkovskaya L.A., and Pankova M.E. The plant antioxidants for simulating non-alcoholic beverages of bio correctional action. *Beer and beverages*, 2013, no. 4, pp. 70–72. (In Russian).

19. Kiseleva T.F. Optimization of ingredient structure of functional soft drinks. *Beer and beverages*, 2006, no. 4, pp. 62–63. (In Russian).

20. Peyrat-Maillard M.N., Bonnely S., and Berset C. Determination of the antioxidant activity of phenolic compounds by coulometric detection. *Talanta*, 2000, vol. 51, pp. 709–716.

21. Roginsky V. and Lissi E.A. Review of methods to determine Chain-breaking antioxidant activity in food. *Food Chemistry*, 2005, vol. 92, no. 2, pp. 235–254. DOI: 10.1016/j.foodchem.2004.08.004.

22. Yashin A.Ya., Yashin Ya.I., Chernousova N.I., and Pakhomov V.P. Express electro-mechanical method of anti-oxidant activity of food products. *Beer and beverages*, 2004, no. 6, pp. 32–36. (In Russian).

23. Vladimirov G.K., Sergunova E.V., Izmailov D.Yu., and Vladimirov Yu.A. Chemiluminescent determination of total antioxidant capacity in medicinal plant material. *Bulletin of Russian State Medical University*, 2016, no. 2, pp. 65–72. (In Russian).

Please cite this article in press as: Mayurnikova L.A., Zinchuk S.F., Davydenko N.I., and Gilmulina S.A. Development of a Functional Basis of Phyto-beverages with an Increased Antioxidant Activity for the Correction of Nutrition of Patients with Diabetes Mellitus. *Foods and Raw Materials*, 2017, vol. 5, no. 2, pp. 178–188. DOI: 10.21603/2308-4057-2017-2-178-188.