Plant-based functional food ingredients: zinc, chromium and bilberry polyphenols complexes

S N Zorin, V K Mazo, N A Petrov, I S Vorobiova and Yu S Sidorova

Federal research center of nutrition, biotechnology and food safety, 2/14, Ustinsky proezd, Moscow, 109240, Russia

E-mail: sidorovaulia28@mail.ru

Abstract. This work aimed to develop the technology for the production of functional food ingredients (FFI) with hypoglycemic and hypocholesterolemic action. These are organic forms of zinc and chromium and complex of bilberry polyphenols sorbed on brown buckwheat flour. Soy protein isolate hydrolysate was obtained using pancreatin as an enzymatic agent. The hydrolysis was conducted at 50-52°C for 3 hours. The product was then pasteurized at 75°C for 20 min. Organic forms of zinc and chromium were obtained by mixing the hydrolysate with zinc chloride and chromium chloride water solutions followed by micro- and nanofiltration. The bilberry fruits polyphenols were sorbed on the buckwheat flour by mixing them. The mixture was then centrifuged, the precipitate was lyophilized. Organic sources of zinc and chromium were included in the composition of specialized food product (SFP) for patients with metabolic syndrome (MS) as FFI. The introduction of the product into the hypocaloric diet of MS patients led to improvements in lipid metabolism. In essence, lowered total cholesterol decreased low-density lipoproteins and triglycerides levels, lowered atherogenic coefficient. During in vivo study hypoglycemic properties of FFI (bilberry polyphenols complex with buckwheat flour) were established. Experimental mice had significantly lower glucose, glycated haemoglobin and relative liver weight on the background of FFI consumption. Firstly, the obtained data proves the possibility to extract, concentrate and save beneficial properties of polyphenols through their sorption on the biopolymer plant matrices. Secondly, the obtained data proves the prospects of the use of essential microelements organic forms for preventive diet therapy.

1. Introduction

The excess weight and obesity are the leading risk factors for the development and progression of alimentary dependent diseases, like diabetes mellitus type 2 (type 2 diabetes) and metabolic syndrome (MS). Specialized food products (SFP) with modified chemical composition should be used in the nutrition of the population for prevention and diet therapy of obesity. It is important to note that these products contain functional food ingredients (FFI) exerting hypolipidemic and hypoglycaemic effects and being relevant to modern safety and efficacy requirements [1, 2, 3].

The study of food proteins impact on the lipid metabolism disorders (showing as central type obesity, hypertension, dyslipidemia, hyperglycemia and other manifestations) is an important point of clinical and experimental researches for a long time. Soy protein isolates (SPI) and their hydrolysates, obtained from non-modified soy, are traditionally used for prevention and diet therapy of lipid and/or carbohydrate metabolism disorders and relevant diseases [4].
Chromium (III) is necessary for the proper functioning of the carbohydrate metabolism of humans and animals [5, 6]. Chromium (III) stimulates the glucose income into the cells inducing intracellular signalling genes. Chromium (III) can directly interact with insulin, its dimers in particular, thus stabilizing the hormone structure or strengthening its bonds with the receptor. Biochemical and physiological actions of zinc include, among others, the regulation of chronic inflammation status via decreasing inflammation cytokines levels, decrease in oxidative stress impact, participation in lipid and carbohydrate metabolism. Zinc enhances glucose metabolism, insulin sensitivity of type 2 diabetes patients [7] and plays a crucial role in the synthesis, deposition and secretion of insulin in pancreas β-cells. The decrease in zinc level negatively impacts insulin sensitivity and glucose tolerance. Zinc also stimulates glycolysis, inhibits gluconeogenesis and participates in glucose transport in adipocytes. Regarding obesity, the metabolic effects of zinc are bounded to its impact on adipokines are the fat tissue hormones (interleukin 6, tissue necrosis factor TNFα, leptin, adiponectin and others) [8, 9].

To obtain new food sources of chromium and zinc in organically bind highly digestible form, the reactions of complexing of these microelements with enzymatic hydrolysates of food proteins can be used very effectively.

Bilberry fruits and leaves are traditionally used for decreasing the symptoms of carbohydrate metabolism disorders. That is substantiated by the wide range of polyphenolic compounds containing in fruits and leaves, foremost anthocyanins, the hypoglycemic and hypolipidemic properties of which are widely studied in vivo and in clinical researches [10]. The use of bilberry juice for preventive or therapeutic needs is heavily limited by low bioavailability of polyphenolic compounds. Thus, it is necessary to find technological approaches, aimed at FFI production with high polyphenol content for inclusion into the SFP composition. One of such approaches is concentrating polyphenols by their sorption on the protein matrix, which also makes them more resistant to degradation at high temperatures and low pH [11].

The aim of this study was the technology development of FFI based on plant materials: organic forms of zinc and chromium and complex of bilberry polyphenols, sorbed on brown buckwheat flour.

2. Materials and methods

Objects and research methods. Soy protein isolates «Supro Plus 221 D IP» (80% of protein, «Solae», USA), zinc chloride (analytic grade) and chromium (III) chloride (analytic grade) were used.

The SPI enzymatic hydrolysate preparation. The enzymatic hydrolysate of SPI (EHSPI) was obtained with the use of apparatus, containing dispenser Ya9-ORP with built-in rotor pump, reservoir Ya1-OSV (1000L) with mixer and heat insulation layer. Pancreatin (150g) was added to a 5% aqueous solution of SPI at constant mixing at ratio protein/enzyme 50/1. The hydrolysis was conducted at 50-52 °C for 3 hours at constant mixing after the product was pasteurized at 75 °C for 20 min. The EHSPI was dried on the spray drier Niro Atomizer (Denmark). The productivity of the apparatus was 20kg of evaporated moisture per hour at entrance temperature 160-165°C and at exit temperature 80-85°C.

The preparation of zinc and chromium organic forms. Aqueous EHSPI solution (10%) was mixed with 25% aqueous solution of zinc chloride in the ratio 10/1. The pH of the mixture was lead to 7.0-7.1 with NaOH. The mixture was then incubated for 60 min at constant mixing and 25°C. After the end of the process, the obtained microelement complex was exposed to microfiltration (tangential flow, hole diameter 5.0 µm) to remove residue and mechanical admixtures. The filtered solution was then exposed to nanofiltration to remove possible zinc ions non-bound to peptide matrix, after that the mixture was pasteurized at 75°C for 30 sec. For control the fullness of non-organic admixtures removal, a refractometer was used. The content of dry substances should not exceed 0.5%.

The prepared solution, containing Zn-EHSPI complex, was lyophilized, powdered using knife grinder GRINDOMIX GM200 (Retsch, Germany) and sifted through the 1.0mm sieve. The main differences of Cr-EHSPI complex preparation were the following: 10% aqueous chromium chloride solution was mixed with EHSPI solution in the ratio 100 protein weight/ 1 chromium salt weight, and nanofiltration stage was excluded from the process.
The preparation of the food matrix, enriched with bilberry polyphenols. The sample of buckwheat flour (HlebZernoProdukt, Russia) was powdered using knife grinder GRINDOMIX GM200 (Retsch, Germany) at 8000 rpm for 10 min. The sample contained 9.7% of protein, 70.0% of carbohydrates, 1.6% of ash, moisture – 5.5%. The granulometric parameters of the brown buckwheat sample were determined by electron-scanning microscopy using scanning electron microscope AIS 1800C (Seron Technologies Inc., Korea) [12].

Aqueous alcoholic extract of bilberry fruits was obtained as follows. Bilberry berries (Vologodskaya Yagoda, Russia) were freeze-dried on the LS-500 apparatus (PROINTEH, Russia). The extract from dried berries was obtained by mixing them with 990 mL of 70% ethanol and blending for 15 min in laboratory combine Bosch MUMXL 40G (Robert Bosch Hausgerate GmbH, Germany). The obtained mixture was then centrifuged at 3000 rpm for 10 min using Beckman J6B centrifuge (AL-TAR, USA). The supernatant was moved to glass flask and ethanol was removed on the rotary evaporator IR1M3 (Himlaborpribor, Russia).

The sorption process was conducted at constant mixing of bilberry berries extract with buckwheat flour in the ratio 10/1 on the orbital shaker Loip LS 110 (RNPO RusPribor, Russia) for 45 min. After that, the mixture was centrifuged at 4000 rpm for 20 min. The supernatant was decanted, and the precipitate was lyophilized and powdered.

Analytic methods. Chromatographic analysis of EHSPI and its complexes with chromium (Cr-EHSPI) and zinc (Zn-EHSPI) was conducted by high-pressure exclusion chromatography (Superosa 12 column, 1.6*50cm).

NaCl 0.2M with the addition of sodium azide was used as eluent at elution speed 2.0 mL/min. The optical density of the solution was determined at \( \lambda = 280 \text{nm} \) using ultraviolet streaming current detector UV-1 (Pharmacia, Switzerland). The column was preliminary calibrated using standard globular proteins (SERVA, Germany). Standard curves of time dependence against standards` molecular weights were built using the cubic regression method (selection of coefficients of the third-degree polynomial using the least-squares method) with Microsoft Excel 2007 software. The peptide fraction content (different ranges of molecular weights) in the composition of enzymatic hydrolysate was determined by chromatogram integration by means of weight method in the range of free to the full volume of the chromatographic column. The osmolality of 1% aqueous solution of enzymatic hydrolysate equal to 32 mmol/kg was determined by the cryoscopic method on thermoelectric milliosmometer-cryoscope MT-5-01 (Russia).

Determination of total polyphenols. The content of total polyphenols was determined in the bilberry berries extract and in the composition of FFI through Folin-Ciocalteu [11] spectrophotometric method as mg-eq of gallic acid (97.5%, Sigma, USA). The content of total polyphenols in the composition of FFI was determined as follows. A 0.5g portion of the FFI was placed in a 20mL vial. The adsorbed polyphenols were eluted with 8mL of a 1% solution of glacial acetic acid in 80% ethanol in an ultrasonic bath “Sapphire 2.8 TTC” (Sapphire, Russia) for 5 minutes at 55°C. The mixture was centrifuged at 4000 rpm for 10 min, and the supernatant was transferred to a pear-shaped flask. The elution in the same mode was repeated twice more. The eluates were combined, and the alcohol was evaporated on a rotary evaporator IR1M3 at 60°C to achieve 15% of the initial volume of the combined eluents. The resulting concentrate was transferred to a graduated cylinder. The volume was adjusted to 50 cm\(^2\) with distilled water. In the obtained solution, the content of total polyphenols was determined by the Folin-Ciocaltelte method.

Determination of total anthocyanins. The content of total anthocyanins as cyaniding-3-glycoside equivalents was determined using pH-differential spectroscopy using Shimadzu UV-1800 spectrophotometer (Shimadzu Corporation, Japan) with a wavelength range from 190 to 1100 nm. The profile of individual anthocyanins was determined by HPLC using Agilent 1100 chromatograph (Agilent Technologies, USA), equipped with degasser, binary pump, column thermostat, autosampler, photodiode array spectrophotometric detector. The data were analyzed with ChemStation for LC 3D Systems v B.04.03.
Determination of simple carbohydrates. The content of simple carbohydrates (glucose, fructose and sucrose) was determined by reverse phase HPLC with refractometry detection, and column Separon SGX NH2 was used as stationary phase, the mixture of bidistilled water (23%) and acetonitrile (73%) as mobile phase.

There is the determination of zinc and chromium content. The content of zinc and chromium was determined through the atomic adsorption method with the use of Z 5300 spectrophotometer (Hitachi, Japan).

3. Results
The obtained organic sources of zinc and chromium represent hygroscopic fine powder cream-coloured (Zn-EHSPI) and stone-coloured (Cr-EHSPI) with a specific flavour and bitter-salt taste. Physical and chemical properties of microelements organic forms are presented in Table 1.

| Parameter                          | Value         | Zn-EHSPI | Cr-EHSPI |
|------------------------------------|---------------|----------|----------|
| Moisture, %, no more               |               | 5.0      | 5.0      |
| Ash, %, no more                    |               | 10.0     | 10.0     |
| Dilution time, min, no more        |               | 2.0      | 2.0      |
| Solubility till silt, g/L          |               | 150.0    | 150.0    |
| Protein mass fraction (N*6.25), %, no more |       | 79.0     | 75.0     |
| - including, amine nitrogen, %, no more |           | 2.0      | 2.0      |
| Bulk density, g/cm³                |               | 0.65     | 0.65     |
| Zinc mass fraction, mg/g, no more  |               | 45.0     | -        |
| Chromium mass fraction, mg/g, no more |            | -        | 4.5      |

The organic sources of zinc and chromium were included as FFI into the composition of SFP, developed for MS patients. Clinical studies of the developed product in the composition of a low-calorie diet for patients with MS were conducted in the Department of Metabolic Diseases of the Therapeutical Nutrition Clinic of the Federal Research Center of Nutrition and Biotechnology. The obtained data shows that the inclusion of the developed product into the diet of these patients leads to improvement of their lipid metabolism (lower total cholesterol, decrease in low-density lipoproteins, triglycerides, atherogenic coefficient).

The average particle size of powdered buckwheat flour was 135±4 µm. Table 2 shows the concentration of polyphenolic compounds in the composition of bilberry berries, their extract and FFI.

| Sample            | Total polyphenols | Anthocyanins |
|-------------------|-------------------|--------------|
| Fresh berries     | (6.0±0.1) mg-eq g.a./g | (3.4±0.1) mg/g |
| Bilberry berries extract | (1.68±0.04) mg-eq g.a./ml | (0.95±0.01) mg/ml |
| FFI               | (7.6±0.3) mg-eq g.a./g | (4.6±0.1) mg/g |

The percentage of sorption on buckwheat flour was 45% of the total polyphenols and 48% of anthocyanins from their content in the first extract of bilberry fruits. The content of polyphenolic compounds in the FFI increased 1.3 times against the content in the equivalent quantity of fresh berries. It was found, that simple carbohydrate did not sorb on the flour.

The hypoglycemic properties of the obtained FFI were established in a preclinical in vivo experimental study. Mice, consuming FFI in the composition of high lipid high carbohydrate (HLHC)
diet had significantly lower glucose and glycated haemoglobin levels and relative liver weight in comparison with control animals, consuming just HLHC diet.

4. Discussion
One of the main effects of chromium is that it can indirectly stimulate the uptake of glucose by muscles and adipose tissue through insulin; it regulates the functions of the genes of some intracellular signalling systems, including GLUT4 molecules. Chromium enhances the effects of insulin concerning glucose metabolism. With insufficient Cr intake, metabolic disorders occur in the body, with the symptoms similar to those observed in diabetes and cardiovascular diseases. The biochemical and physiological effects of zinc in mammals include a decrease in the effects of oxidative stress, participation in lipid and carbohydrate metabolism. Accordingly, the use of zinc and chromium for the prevention of MS is promising. The developed complexes are the organic forms of these essential microelements, which leads to their increased digestibility and effectiveness.

Our in vivo experiment is consistent with the data presented in numerous studies, which indicate a pronounced hypoglycemic and hypocholesterolemic effect of polyphenols, both individual compounds and in plant extracts. It is proved that plant polyphenols have pronounced antioxidant properties, which are primarily associated with their potential effects. Bilberry fruits, due to the full range of polyphenols contained in them, are of particular interest as a natural food source, primarily of anthocyanins, since it is known that their content in blueberries is significantly higher than in strawberries, cranberries, elderberries, cherries, raspberries. The choice of brown buckwheat flour as a matrix that sorbs bilberry polyphenols are due to its widespread use in modern medical practice – inclusion into the diet in obesity, diabetes, kidney disease, to reduce cholesterol and total lipids, strengthen the walls of blood vessels [13]. We also previously determined the optimal conditions for the sorption of polyphenols from an aqueous solution of blueberry leaves extract on brown buckwheat flour, and the obtained product was found to be highly stable when stored under light, temperature and humidity [11].

The claimed approaches can contribute to the development of the technologies and production of new FFI and dietary supplements for food, aimed at ensuring effective dietary prevention of disorders of carbohydrate metabolism.

5. Conclusion
In the result of the study, organic forms of chromium and zinc were obtained and physically-chemically characterized. The high content of microelements in the complexes with EHSPHI has determined the prospects of their use as FFI in the composition of SFP for the dietary prevention of metabolic disorders and microelement deficiency. A complex of bilberry polyphenols, concentrated by sorption on a natural plant matrix – brown buckwheat flour, had a protective effect in cases of carbohydrate metabolism disorders during in vivo experiments.

6. Acknowledgments
The reported study was funded by the subsidies for the fulfilment of a state task within the framework of the Fundamental Research Program (project № 0529-2019-0055).

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