The production of food-grade protein matrix enriched with polyphenolic compounds from cranberry

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Abstract: The technology of innovative functional foodstuffs (FFSs) for the prophylaxis of type 2 diabetes involves supplementation with polyphenols (PPs) featuring hypoglycemic and/or hypolipidemic properties. The aim of the study presented was to develop an approach for the enrichment of food-grade protein matrix (PM) – coagulated egg albumen (CEA) – with PPs absorbed from cranberry juice (CJ). Concentration of total polyphenols (TPP) in gallic acid equivalents was determined using the Folin-Ciocalteu method. Concentration of total anthocyanins (A) in cyanidin-3-glucoside equivalents was determined using pH-differential spectrophotometry; profile of individual anthocyanins was determined according to State Standard GOST 32709-2014. Concentrations of simple saccharides (SS) were determined by the reverse-phase HPLC. The PPs from 100 ml portions of CJ with different juice concentration were sorbed by 10 g portions of CEA. After preliminary incubation and centrifugation the precipitates were lyophilized. The sorption rates of TPP and A were determined as a difference between the initial concentration in cranberry juice and in corresponding supernatants after centrifugation. The quantification of TPP and A in the lyophilized precipitates involved triple elution by 1% solution of HCl in 80% ethanol at 55°C and subsequent ultrasound treatment. Maximal specific sorption for TPP and A from 100% CJ was 14.0 mg-eq. of gallic acid/g and 1.48 mg/g, respectively. Sorption rate for SS on the CEA was 30.3±3.1% of their initial concentration in CJ. The TPP/SS ratio in the enriched PM was 2.0 times higher as compared to CJ. The results of the study can be used in the development of new FFSs with antidiabetic properties.

1. Introduction
High incidences of metabolic syndrome, type 2 diabetes, and associated complications dictate the necessity of the development of a wide range of innovative functional foodstuffs (FFSs) for the nutrition of patients with these disturbances of carbohydrate and/or fat metabolism. The results of clinical and experimental research by the nutritionists worldwide evidenced the hypolipidemic and hypocholesterolemic effects of a wide range of polyphenolic compounds [1].
The aim of the study presented was to develop a technology for the production of food-grade protein matrix (based on coagulated egg albumen) enriched with polyphenols from cranberry. The choice of coagulated egg albumen (CEA) as a base for the protein matrix was related to its high nutritional and biological values found in earlier in vivo experiments [2].

Cranberry (Oxycoccus palustris Pers.) is growing in abundance in tundra and forest-steppe zones of European Russia, Siberia, and Russian Far East; it contains a large set of polyphenolic compounds, organic and phenolic acids, terpenes, vitamins, and minerals [3-5]. Polyphenols from cranberry include anthocyanins, flavonols, catechols, oxycinnamic acids, proanthocyanidins, etc. Basic anthocyanins in cranberry are 3-galactosides and 3-arabinosides of cyanidin and peonidin. According to authors’ earlier unpublished data, flavonols in cranberry are also presenting mainly as glycosides, derivatives of myricetin, quercetin, and isorhamnetin. B-type proanthocyanidins are presenting together with A-type; the content of the latter in cranberry is substantially higher in compare to blueberry, lingonberry, cinnamon, cocoa. Oligomeric A-type proanthocyanidins are significantly more effective compared to B-type in the prevention of the adhesion of uropathogenic P-fimbrial strains of Escherichia coli [3,6,7]. Triterpenes from the cranberry playing an important role in the formation of its taste and aroma are primarily ursolic acid and iridoid glycosides. The content of simple carbohydrates (monosaccharides) in cranberry (ca. 2.8%) is significantly lower compared to that of blueberry and lingonberry [8]. Antioxidative, hypocholesterolemic, hypoglycemic, antimicrobial properties were found in cranberry. The most active free radical scavengers in the polyphenolic compounds from cranberry are anthocyanins [9-12].

2. Materials and Methods

CEA was produced by separation of egg albumen from the yolk; stirring of resulted liquid albumen; acidification by citric acid with sodium chloride; incubation for 15 min at room temperature; one- or two-stage thermal treatment until 86±2°С at constant stirring. The shift in pH (by no more than 2 points) was made by the addition of organic acid. The detached liquid phase was separated, the coagulate was cooled and lyophilized [13,14].

Granulometric parameters in the samples of dried CEA were determined using electron scanning microscope VEGA II LMU (TESCAN, Czech Republic) with microarray system INCA Energy (Oxford, Great Britain) in the regime of extra vacuum, the backscattered electrons were detected at accelerating voltage 20 KV. For adequate estimation of particle sizes the equivalent diameters (μm) were calculated as an arithmetic average of the length and the width of a particle [15].

The cranberries (purchased from a retail company, gathered in Pskov Province, meet the requirements of Technical Regulations of the Customs Union, 2011) were grinded by “Blikser-3” mixer to a squash and frozen. Prior to analyses the squash was defrozen, centrifugated, and decanted; the cranberry juice (CJ) obtained was used as the source of polyphenols for sorption. Concentration of total polyphenols (in gallic acid equivalents) was determined by spectrophotometry using the Folin-Ciocalteu method [16].

Concentrations and profile of individual anthocyanins were determined according to State Standard GOST 32709-2014. Total anthocyanins (in cyanidin-3-glucoside equivalents) were determined using pH-differential spectrophotometer Shimadzu “UV-1800” (Shimadzu Corp., Japan) at wavelength range 190-1100 nm. The profiles of individual anthocyanins were determined using high-performance gas-liquid chromatography (HPLC) on chromatograph Agilent 1100 (Agilent Technologies, USA) with degasser, duplex pump, thermostat for the columns, autosampler, and diode array spectrophotometric detector. The data obtained was processed using ChemStation for LC 3D Systems (v. B.04.03) software.

Concentrations of simple saccharides (glucose and fructose) were determined by the reverse-phase HPLC with refractometric detection, using Separon SGX NH2 column as a stationary phase and the mixture of redistilled water (23%) with acetonitrile (77%) [16].

The production protein matrix enriched with cranberry polyphenols. The sorption of CJ polyphenols on CEA was performed via constant stirring of 100 ml portions of CJ of different
concentration with 10 g of CEA on a magnetic mixer, centrifugation of the suspension obtained on centrifuge Beckman J6B (Beckman Coulter, USA) at 4,000 rpm for 20 min at 25°C, with subsequent decantation of the supernatant. The precipitate was lyophilized using drier LS-500 (PROINTECH, Russia).

The sorption rates for total polyphenols (in gallic acid equivalents) and anthocyanins (mg/g) were determined as a difference between the initial concentration in cranberry juice and in corresponding supernatants after centrifugation. The quantification of total polyphenols and anthocyanins in the lyophilized precipitates involved triple elution by 1% solution of HCl in 80% ethanol at 55°C and subsequent ultrasound treatment for 5 min according to [17].

3. Results and Discussion

The granulometric analysis of grinded and sieved samples of CEA revealed the decrease in median particle size from 337.5 to 149.3 μm, respectively (Figure 1, Figure 2). The samples of CEA for subsequent sorption of polyphenols were sieved with screen size of 0.18 mm. The median average particle size was 149.0±2.6 μm.

Figure 1. a – digital photograph of sample of coagulated egg albumen (CEA) before sorption of polyphenols from cranberry juice (CJ); b – electron scanning microphotograph of particles of coagulated egg albumen (CEA) before sorption of polyphenols from cranberry juice (CJ)

Figure 2. a – digital photograph of sample of coagulated egg albumen (CEA) after the sorption; b – electron scanning microphotograph of particles of coagulated egg albumen (CEA) after the sorption (with evener particle surfaces).
With the increase in CJ concentration in its aqueous solution from 10 to 100% the specific sorption of total polyphenols and anthocyanins grew almost linearly. Maximal specific sorption for TPP and A from 100% CJ was 14.0 mg-equiv. of gallic acid/g and 1.48 mg/g, respectively (see Figures 3, 4).

**Figure 3.** The influence of cranberry juice concentration (%) on the specific sorption of polyphenols (mg of gallic acid equivalents per 1 g of coagulated egg albumen).

**Figure 4.** The influence of cranberry juice concentration (%) on the specific sorption of anthocyanins (mg/g of CEA).

The typical absorbance spectra for CJ in the visible region in buffer solutions with pH 1.0 and 4.5 are presented in Figure 5. The wavelength of maximal absorbance of the anthocyanins within the CJ at pH=1.0 in all samples was 512±2 nm.
Figure 5. Absorbance spectra of cranberry juice (CJ) in buffer solutions with pH=1 and pH=4.5

Typical anthocyan profile in cranberry is represented by 3-galactosides, 3-glucosides and 3-arabinosides of cyanidin and peonidin, 3-arabinoside of malvidin; 3-galactosides and 3-arabinosides of cyanidin and peonidin being the prevalent anthocyanins in cranberry (Figure 6, Table 1).

Figure 6. The chromatogram of cranberry juice (CJ) sample at $\lambda$=520 nm. 1 - cyanidin-3-galactoside (Cyd-3-gal), 2 - cyanidin-3-glucoside (Cd-3-glu), 3 - cyanidin-3-arabinoside (Cyd-3-ara), 4 - peonidin-3-galactoside (Pnd-3-gal), 5 - peonidin-3-glucoside (Pnd-3-glu), 6 - peonidin-3-arabinoside (Pnd-3-ara), 7 - malvidin-3-arabinoside (Mvd-3-ara).

Table 1. The anthocyan profile in the samples

| CJ concentration in solution, % | Sample     | Concentration, % of total anthocyanins | Cyd-3-gal | Cyd-3-glu | Cyd-3-ara | Pnd-3-gal | Pnd-3-glu | Pnd-3-ara | Mvd-3-ara |
|-------------------------------|------------|----------------------------------------|----------|-----------|-----------|-----------|-----------|-----------|-----------|
| 100                           | CJ         |                                        | 20.9     | 2.9       | 19.4      | 30.6      | 8.9       | 16.6      | 0.7       |
|                               | Supernatant|                                        | 22.7     | 3.0       | 14.7      | 36.9      | 9.2       | 13.4      | 0.1       |
|                               | Eluate     |                                        | 20.7     | 3.1       | 21.7      | 28.1      | 7.9       | 18.0      | 0.5       |
| 50                            | CJ         |                                        | 20.9     | 2.8       | 19.4      | 30.6      | 8.7       | 16.8      | 0.8       |
|                               | Supernatant|                                        | 22.0     | 3.4       | 14.3      | 37.3      | 9.9       | 13.1      | -         |
|                               | Eluate     |                                        | 20.8     | 3.2       | 21.4      | 28.6      | 7.9       | 17.4      | 0.7       |
| 25                            | CJ         |                                        | 21.2     | 3.1       | 19.1      | 30.9      | 8.8       | 16.5      | 0.4       |
|                               | Supernatant|                                        | 20.0     | 3.0       | 14.6      | 39.0      | 9.1       | 14.3      | -         |
|                               | Eluate     |                                        | 20.7     | 2.9       | 20.1      | 30.0      | 8.0       | 18.3      | -         |
The sorption rates of 3-arabinosides of cyanidin and peonidin on CEA were substantially higher in compare to 3-galactosides and 3-glucoside (Table 1).

The results obtained in our study are in principal accordance with the data of earlier research where the sorption of total polyphenols and anthocyanins from CJ on different protein sorbents (isolates of soybean and pea protein, defatted soybean and peanut floor) was comparatively studied [17].

(FFSs) for the nutrition of patients with metabolic disorders providing the dietetic prophylaxis, improving public health and life quality, decreasing the expenses for the suitable medical service. FFSs of new generation with necessary parameters of quality and safety can be produced using adequate raw materials with high biological and nutritive values and targeted supplementation with functional food ingredients (FFIs) with the effectiveness in the reduction of risks of certain alimentary dependent diseases established by the evidentiary medicine. However, the effectiveness of dietary polyphenols as disease-preventing agents is limited by their low bioavailability; this fact is inspiring the search for the technologies of FFIs with maximal possible concentration of polyphenols for subsequent inclusion into the special foodstuffs. The sorption on protein matrix is known as an effective method for the concentrating of the polyphenols [18] and for the improving of their stability at high temperatures and low pH values [19].

Interaction of polyphenols with a protein matrix involves the formation of hydrogen bonds between the peptide carbonyl groups within the protein and hydroxyl groups within the polyphenols [20]. Hydrophobic interactions of aliphatic and aromatic amino acid residues within the protein (primarily proline, histidine, arginine, phenylalanine, tryptophan, lysine, cysteine, and methionine) with aromatic structures of the polyphenols are also possible [21].

As it was mentioned in a review [22], phenolic compounds from juices of berries and fruits are featuring high bioavailability for human and high conservation rates. However, high concentration of simple saccharides with the juices prevents the latter from the inclusion into the diets for patients with the disturbances of carbohydrate metabolism. The concentrations of simple saccharides (glucose + fructose) in CJ and protein matrix were 18.33 mg/ml and 55.5 mg/g of CEA, respectively. Sorption rate for simple saccharides on the CEA was 30.3±3.1% of their initial concentration in CJ. The resulting total polyphenols/simple saccharides ratio in the enriched protein matrix was 2.7 times higher in compare to CJ. Earlier US researchers suggested that electrostatic interactions between defatted soybean powder and moderately charged polyphenols allow for the non-covalent binding of the latter without binding of highly polar saccharides [2]. This approach allows for the production of protein matrix with relatively low glycemic index which is important in the nutrition of patients with the risks of type 2 diabetes.

It should be also noted that our study is related to the emerging trend in food technology - development and advancement of the technologies of isolation, concentration, and conservation of useful biologically active compounds as minor components of natural edible matrices. CEA enriched with polyphenols from cranberry can be used as FFI for the inclusion into health-promoting foodstuffs.

4. Conclusions
The influence of CJ concentration on the specific sorption rates of polyphenols was studied. Maximal specific sorption for TPP and A from 100% CJ was 14.0 mg-eq. of gallic acid/g and 1.48 mg/g, respectively.

Sorption rate for simple saccharides (glucose + fructose) on the CEA was 30.3±3.1% of their initial concentration in CJ; total polyphenols/simple saccharides ratio in the enriched protein matrix was 2.7 times higher in compare to CJ.

The results of the study can be used by nutritionists in the development of new functional foodstuffs and food ingredients with antidiabetic properties.

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