FUNCTIONAL BIOPRODUCT "FucoSTEM" - EVALUATION OF THE PRODUCT ON ORGANOLEPTIC, PHYSICOCHEMICAL, BIOCHEMICAL AND MICROBIOLOGICAL INDICATORS

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Abstract

The authors present the results of organoleptic, physicochemical, biochemical and microbiological studies of a new lyophilized functional bioproduct "FucoSTEM". The product is a nutritious concentrate developed on the basis of buffalo colostrum. The inclusion in the composition of fruit (chokeberry) leads to an increase in the content of total phenolic substances, anthocyanins and high antioxidant activity of the product. The obtained results prove that the new product "FucoSTEM" has a balanced composition, preserved quality characteristics, high biological and energy value. It is microbiologically pure and safe to use. These features define it as suitable for use by a wide range of users.

Key words: lyophilized bioproduct, colostrum, chokeberry, antioxidant activity, phenolic substances.

INTRODUCTION

The increased interest in healthy and environmentally friendly nutrition stimulates the production and regular inclusion in the daily menu of functional products. The production of foods with a favorable impact on health human status implies the use of biologically active ingredients in the basic formulations for these foods. Extensive scientific and clinical research has highlighted the role of colostrum from large horned cattle as a functional ingredient due to its health benefits. Interest in colostrum is growing due to the high content of biologically active peptides, immunological components and growth factors. Scientists predict that colostrum-based products may play a significant role in the functional products market in the future (Mehra et al., 2021; Bartkiene et al., 2020; Dzik et al., 2017; Altomare et al., 2016).

The chokeberry (Aronia melanocarpa) is one of the richest sources of dietary polyphenols with healthy effects. Anthocyanins, proanthocyanidins, flavan-3-ol and flavonol glycosides and phenolic acids are the main polyphenols present in chokeberry. Chokeberry extracts and fruits show various positive health properties in animal and human studies, such as antihyperlipidemic, hypoglycemic, hepatoprotective, antiproliferative, anti-inflammatory, antimicrobial and others (Čujić et al., 2018; Sosnowska et al., 2018; Denev et al., 2014; Hawkins et al., 2020). In the last few years, research interest in the chemical composition and healing potential of chokeberry fruits, their potential health benefits and their clinical efficacy has increased (Kulling and Rawel, 2008; Valcheva-Kuzmanova and Belcheva, 2006; Chrubasik et al., 2010). Chokeberry fruits are used in the food industry for the production of juices, nectars, various food products and food supplements, and in pharmacy as traditional medicines based on healthy actions against influenza and immune enhancer (Sidor et al., 2019; Kitryte et al., 2017). Sidor et al. (2019) show that a diet enriched with black chokeberry juice or extract improves the lipid balance of people with metabolic syndrome. Yamane et al. (2017) in an experiment with feeding hypertensive rats on a diet with 10% lyophilized black chokeberry fruit, found that it causes a decrease in their blood pressure.

Fucose (L-Fucose), as a rare monosaccharide, finds applications in cosmetics, pharmaceuticals and as a dietary supplement. Fucose acts as an immunomodulator, increases cellular communication, suppresses tumor growth, protects against respiratory infections, suppresses allergic skin reactions, kills bacteria and helps the body in the fight against
infections (Roca, 2005; Garcia-Vaquero et al., 2018; Vanhooren, 1999).

Xylitol represents a natural birch sugar, which is applied as a substitute for ordinary sugar (sucrose) in various products such as food supplements, medicines, toothpaste and more. Xylitol is metabolized insignificantly by insulin, so it has little effect on blood sugar. It is absorbed more slowly than sucrose, has a low glycemic index and is suitable for diabetics. It has been found to be effective in improving the symptoms of chronic rhinosinusitis (Riley et al., 2015; Weissman et al., 2011).

Mannitol is used as a sweetener and medicine. Characteristically, it raises blood glucose to a lesser extent than sucrose and is poorly absorbed by the intestine. It has anti-edema effect, stimulates the functions of the urinary system and intestinal peristalsis. It is suitable for diabetics (Grenby, 2011; Lawson, 2007).

Beta-glucans represent complex soluble fibers derived from the cell wall of bacteria, fungi, yeast, seaweed and the aleurone layer of cereals. β-glucans are well-known immune modulators that activate the immune system. They increase the body's resistance to bacterial and fungal pathogens. Beta-glucans possess antioxidant, antidiabetic, hypocholesterolemic activity and probiotic function (Wani et al., 2021; Colosimo et al., 2021).

Fructooligosaccharides (FOS) are low-calorie, indigestible carbohydrates, prebiotics, which encourage the growth of beneficial bacteria in the host's intestinal tract while suppressing the spread of potentially pathogenic bacteria. FOS stimulate the health of the host by intensifying peristalsis and digestion, increase the absorption of essential minerals and ions (Mg$^{2+}$ and Ca$^{2+}$) in the small intestine, regulate blood glucose metabolism, lower the levels of serum triglycerides, phospholipids and cholesterol, etc. Fructooligosaccharides are present in various inulin-rich raw materials such as chicory, Jerusalem artichoke and others (Singh et al., 2020; Ojwach et al., 2020; Han et al., 2021).

In response to the increased consumer interest in functional foods with additional positive effects for prophylactic and therapeutic purposes the lyophilized product "FucoSTEM" has been developed and adapted for use by a wide range of users. The main goal of the present study is its analytical determination by organoleptic, physicochemical, biochemical and microbiological indicators.

MATERIALS AND METHODS

1. Organoleptic evaluation

The evaluation of the organoleptic qualities of the new lyophilized product "FucoSTEM" was performed on the indicators: appearance, consistency, aroma and taste.

2. Physicochemical methods

- **water content (%)** - on a hygrometer “Sartorius”;
- **dry matter (%)** - calculated as the difference from the water content;
- **total protein content (%)** - (BDS 6231);
- **content of total lipids (%)** - (BDS EN 16932-3: 2018);
- **active acidity (pH)** - with pH-meter "Hanna";
- **total ash (%)** - (BDS 9373-80);
- **reducing sugars (%)** - by Schoorl (Razola-Díaz et al., 2020).

The energy value per 100 g of product (kcal/kJ) was calculated based on the chemical composition.

3. Biochemical methods

**Determination of antioxidant activity of functional bioproduct "FucoSTEM"** - The method is spectrophotometric and is based on analysis of radical scavenging activity with DPPH to Trolox. Toward 0.3 ml solution on 0.2 mM DPPH is added 0.5 ml of MeOH (HPLC 99.5%) 0.5 ml of the test samples. The solution thus obtained is homogenized and allowed to stand in the dark at room temperature for 120 minutes. Absorption (A) is measured at 517 nm (UV-VIS Spectrophotometer, Biochrom). The total antioxidant activity was calculated from a calibration curve (R = 0.9906) using Trolox as a standard. The results were expressed relative to Trolox concentration (mg/g).

**Determination of total content of phenolic substances in functional bioproduct "FucoSTEM"** - A modified spectrophotometric method by Singleton and Rossi was applied to quantitative assessment the total content of phenols (Alhakmani et al., 2013; Valyova et al., 2012). The total phenolic content (TPC) was determined with Folin-Ciocalteu reagent against a gallic acid standard.
TPC is expressed as Gallic Acid (GAE) equivalents in mg/g or mg/ml. The results of the studies were expressed in GAE mg/g. **Determination of anthocyanins in a functional bioproduct "FucoSTEM"** - The content of anthocyanins in the product was quantified by a differential method (Lee et al., 2005). The amount of anthocyanins is presented as cyanidin-3-glucoside equivalents, expressed in cyd eq mg/l and calculated by the following formula:

\[
\text{Anthocyanin pigment (cyd eq mg/l)} = \frac{A \times Mw \times DF \times 10^3}{\varepsilon \times L},
\]

where:
- \(A = (A_{520 \text{ nm}} - A_{700 \text{ nm}}) \text{ pH 1.0} - (A_{520 \text{ nm}} - A_{700 \text{ nm}}) \text{ pH 4.5} \)
- \(Mw \) (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside;
- \(DF \) = dilution factor;
- \(L \) = thickness of the cuvette layer in cm;
- \(\varepsilon = 26900 \) molar extinction coefficient for cyanidin-3-glucoside;
- \(10^3 \) = conversion factor from g to mg.

**Determination of reducing sugars (glucose and fructose) of product "FucoSTEM"** - The determination of reducing sugars (glucose and fructose) is performed by the method of Luff Schoorl (Taufik and Guntarti, 2016). The total amount of sugars is determined by hydrolyzing sucrose to glucose and fructose in the sugar extract with acid. The results were expressed in % content by weight of the sample.

4. Microbiological assessment
A general analysis of microbiological insemination of the lyophilized product was performed according by main microbiological indicators:
- Total number of mesophilic aerobic and facultative anaerobic microorganisms - CFU/g (BDS EN ISO 4833-2013);
- *Escherichia coli* in 0.1 g of the product (ISO 16649 - 1,2,3);
- Pathogenic microorganisms, incl. *Salmonella* (BDS EN ISO 6579: 2003) in 25.0 g of the product;
- Coagulase-positive staphylococci in 1.0 g of the product (BDS EN ISO 6888-1-2005);
- Sulphite-reducing clostridia in 0.1 g of the product (ISO 15213-2003);
- Spores of microscopic molds and yeasts, CFU/g (BDS ISO 6611-2006).

5. Statistical processing of results
The statistical processing of the results and their graphic image was performed using the software product MS Office Excel 2007.

RESULTS AND DISCUSSIONS
The composition of the bioproduct "FucoSTEM" includes sources of constructive and energy elements, as well as physiologically active substances of different origin: buffalo colostrum; chokeberry; fucose; natural birch sugar (xylitol); mannitol; β-glucans; FOS (fructooligosaccharides); sodium citrate. The data on the organoleptic characteristics of the new lyophilised product are presented in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Organoleptic indicators of functional bioproduct &quot;FucoSTEM&quot;</th>
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<tbody>
<tr>
<td><strong>Product</strong></td>
</tr>
<tr>
<td>Lyophilic product with pale purple color</td>
</tr>
<tr>
<td><strong>Appearance</strong></td>
</tr>
<tr>
<td>Fine, quickly soluble</td>
</tr>
<tr>
<td><strong>Consistency</strong></td>
</tr>
<tr>
<td>Pleasant, composition-specific</td>
</tr>
<tr>
<td><strong>Aroma</strong></td>
</tr>
<tr>
<td>Pleasant, lactic sour with chokeberry aroma</td>
</tr>
<tr>
<td><strong>Taste</strong></td>
</tr>
</tbody>
</table>

The lyophilized bioproduct "FucoSTEM" has a fine texture, with a pleasant, composition-specific aroma and color, quickly soluble, prepared for direct consumption in the form of a sachet. Table 2 presents the main physicochemical parameters of the lyophilized product.

<table>
<thead>
<tr>
<th>Table 2. Physicochemical indicators of the lyophilized bioproduct (n = 3)</th>
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<tbody>
<tr>
<td><strong>Indicators</strong></td>
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<tr>
<td><strong>Values for analysis</strong></td>
</tr>
<tr>
<td>Water content, %</td>
</tr>
<tr>
<td>Dry matter, %</td>
</tr>
<tr>
<td>Total protein, %</td>
</tr>
<tr>
<td>Total lipids, %</td>
</tr>
<tr>
<td>Total ash, %</td>
</tr>
<tr>
<td>Reducing sugars, %</td>
</tr>
<tr>
<td>Active acidity - pH</td>
</tr>
<tr>
<td>Energy value / 100 g product, kcal/kJ</td>
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</tbody>
</table>

The obtained data show that the lyophilized product represents a food concentrate with a residual moisture content of 2.72% after lyophilization within limits of the norm (2-5%), which is proof of an optimally conducted technological process. The active acidity is 6.05, which largely predetermines the preservation of enzymatic and biological...
activity in the product. The content of total protein is mainly due to the main raw material in the product - colostrum. Relatively low for concentrated foods is the quantitative value of total fat in the product - 4.38%.

The high content of reducing sugars is due to the imported additional nutrients of polysaccharide type. The high water-binding ability of the fructooligosaccharide allows interaction in a characteristic way with the protein of the product. This interaction brings mainly electrostatic in nature, as a result of which a strong bond is formed between the individual particles of the product and after rehydration, the lyophilized product receives the required density.

Based on the chemical composition, the energy value of the obtained lyophilisate was calculated (for 100 g: 29.6 kcal/125.8 kJ and for 2 g: 0.6 kcal/2.55 kJ), as the main energy carrier is the carbohydrate-protein complex.

The antioxidant activity of the product was determined spectrophotometrically on the basis of an analysis of the radical scavenging activity with DPPH relative to Trolox. Trolox Equivalent Antioxidant Capacity (TEAC) is a measure of the antioxidant power based on Trolox, measured in units called Trolox equivalents (TE). The antioxidant activity of chokeberry juice was also determined experimentally, as it is assumed that it is he who imports in the final product the antioxidant potential. According to the prescription composition of the product, chokeberry juice is 15% of the total amount, which fully corresponds as a ratio between the two tested samples. The results are pronounced relative to Trolox concentration (mg/g) and are presented in Table 3.

As with antioxidant activity, so in the results for the content of total phenols in the product, the ratio between the indications of pure lyophilized chokeberry juice and its percentage content in the finished product is preserved. The assumption that the main carrier of phenolic substances is the added amount of fruit (chokeberry) is confirmed.

Quantitative analysis of the content of anthocyanins in the product and in chokeberry juice was performed spectrophotometrically. The results of the studies performed are presented in Table 5 in Anthocyanin pigment, cyd eq mg/l.

The high content of anthocyanins in chokeberry juice also determines the content available in the product, which provides additional health benefits.

The results of the tests performed to determine the reducing sugars (glucose and fructose) of the obtained functional product, expressed as a percentage content of the sample weight, are presented in Table 6.

Table 7 presents the data from the performed microbiological tests of the lyophilized product. The results of the conducted microbiological analyzes prove that the indicators of the studied lyophilized bioproduct “FucoSTEM” are in the admissible norms for microbial contamination and meet the requirements and criteria of BDS ISO.
Table 7. Values of microbiological contamination of functional bioproduct "FucoSTEM"

<table>
<thead>
<tr>
<th>Indicators</th>
<th>Functional bioproduct &quot;FucoSTEM&quot;</th>
<th>CFU/g</th>
<th>Log</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of mesophilic aerobic and facultative anaerobic microorganisms - CFU/g</td>
<td>3.1 x 10^2</td>
<td>2.49</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli in 0.1 g of product</td>
<td>Not established</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Pathogenic microorganisms, including Salmonella in 2.0 g of product</td>
<td>Not established</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Coagulase-positive staphylococci in 1.0 g of product</td>
<td>Not established</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Sulphite-reducing clostridia in 0.1 g of product</td>
<td>Not established</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Spores of microscopic molds, CFU/g</td>
<td>1.2 x 10^1</td>
<td>1.07</td>
<td></td>
</tr>
</tbody>
</table>

CONCLUSIONS

The composition of the bioproduct "FucoSTEM" includes sources of constructive and energy elements, as well as physiologically active substances of different origin - colostrum, mono, polysaccharides, beta-glucans, natural antioxidants and others. High levels of content of total phenolic substances, anthocyanins and high antioxidant activity of the product have been established. The assumption that the main carrier of these properties is the added amount of fruit (chokeberry) is confirmed.

Summarizing the results of the analytical studies, it is established that the new product "FucoSTEM" is a lyophilized concentrate with preserved quality characteristics, high biological and energy value, microbiologically pure and safe for use. These characteristics define it as suitable for use for prophylactic and curative purposes by a wide range of users.

REFERENCES


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