Elderberry oil-cake „*Sambucus Ebulus*“ – natural immune stimulant

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Abstract. In the production of elderberry tincture “*Sambucus ebulus*”, parts of the berries – oil-cake, which have already passed alcohol fermentation, remain as a waste product. The received results from the conducted analyses on the nutrient profile and energy value, the antioxidant activity and microbiological contamination of the remaining oil-cake, are described in the present research. The nutrient profile and energy value of the elderberry oil-cake (of a 100 g product) contain fibre – 46.25 g, total carbohydrates - 10.90 g, fats - 18.71 g, protein quantity - 10.20 g, total content of ash - 3.18 g and energy value - 345.45 g. The antioxidant activity was determined with the four different in mechanism and conditions of passing methods – DPPH 32.04±4.23 mMTE/g extract, ABTS 5.97±0.10 mMTE/g extract, FRAP 3.13±0.17 mMTE/g extract and CUPRAC 2.23±0.15 mMTE/g extract. The microbiological indicators – total number of mesophilic aerobic and facultative anaerobic microorganisms are in the permissible limits 2.102 CFU/g. The spores of the microscopic mold fungi, as well as the presence of *Enterobacteriaceae, Escherihia coli* and *Staphylococcus aureus* is below the permissible limit, *Salmonella* sp. is not discovered. The examined product is not contaminated with the spore-developing and pathogen for people *Bacillus cereus*. The received results prove the statement that the berries of the elderberry and partially the examined waste product, have all the qualities of a natural immune stimulant.

1 Introduction

In the last years more and more researches are oriented in searching of alternative variants of usage of waste products rich in natural nutrients of different productions [1]. The therapeutic herbs are an integral part of almost each traditional and untraditional medicine schools from thousands of years. In the long story of the herb treatment, the effect and the clinical applications of the therapeutic plants have been proved a lot of times [2]. One of the plants which are very valuable and in great demand for its numerous benefits and qualities, is the elderberry. The shrubby elderberry, which Latin name is “*Sambucus ebulus*”, is famous as one of the most therapeutic herbs since ancient times, well-known in the herbal medicine as “the black pearl of the forest” [3].

It is used for treatment of a number of illnesses till nowadays. It can be found in different parts of the world but it originates from Southern and Central Europe and South-western Asia. The elderberry is a plant which also grows on the territory of Bulgaria and it grows as well as a weed in fields, forests and bushes. It usually can be seen along by rivers and roads and in plains in altitude above the sea level of 1900 m. The berry is spherical and its diameter is 5-6 mm. It is distinguished by its dark blue to dark violet color. 100 gram fresh berries contain – 73 calories, 18.4 carbohydrates and less than 1 gram of fats and proteins. The elderberry is with high content of vitamin C (approximately 60% of the recommended daily amount), dietetic fibre (7 g fibre to 100 g fresh berries), phenolic acids (strong antioxidants which can help for lowering the damages of the antioxidant stress in the body), it is rich in flavonols. The antioxidant activity of the elderberry is 3.5 times higher than the one in vitamin E [4-6].

The therapeutic ingredient of the elderberry is not in the rich vitamin and microelement content which is met in almost every fruit. It is concentrated mostly in its specific paint substances – anthocyanins because of which it has its intensive dark color [7-8].

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The berries of the elderberry have the strongest therapeutic influence on the human organism unlike the other parts of the plant. They possess a strong anticancer activity, strengthen the organism and increase its resistance force against influenza and other diseases. The elderberry eliminates the toxins in the organism through the urine, and also through sweat by increasing the excretory activity of the sweat glands. It also increases the secretion of the lactiferous glands of women who breast-feed [4-6, 9].

The berries of the plant (fresh or dry) deal excellently in the fight against anaemia. They have a positive influence on the heart function by helping to lower the blood pressure [9].

The consumption can be in the form of ripen fresh and dry berries, and in the form of extract (juice) as well. The tincture of the berries of the elderberry helps to activate the immune system. In the production of the therapeutic beverage, parts of the berries – oil-cake, which have already passed alcohol fermentation, remain as a waste product [5-7, 9]. It is not known a production for their usage till the moment, which is an object of research to the National Science Fund of the University of Food Technologies - Plovdiv.

2 Materials and methods

2.1 Raw Materials

Bulgarian herbaceous elder "*Sambucus ebulus*" after alcohol fermentation – waste product in tincture production. The fruit are delivered from the area of the village of Zlatocel, Brezovo municipality, Plovdiv area, a Southern Bulgaria. Semi-mountainous village situated in the southern slopes of Sarnena Sredna Gora.

2.2 Methods

- Moisture content [%] – by express method through drying of 5g flour for 24 hours 105 ºC according to AOAC [10].
- Fibre - BSS 11374:1986 [11];
- Fat content, (%) – Soxhlet method via solvent extraction with petroleum ether. BSS 6997:1984 [12];
- Carbohydrate - BSS 7169:1989 [13];
- Protein – Direct Kjeldahl method analysis (determination of nitrogen content/ nitrogen determination method) – Regulation (EC) №152/2009 [14];
- The antioxidant activity of "*Sambucus ebulus*" was evaluated using several methods: DPPH (1,1-diphenyl-2-picrylhydrazyl radical), ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)), FRAP (Ferric Reducing Antioxidant Power Assay), and CUPRAC (CUPric Reducing Antioxidant Capacity). This evaluation involved a two-step triple extraction process. Initially, approximately 1 gram of the sample was combined with 10 milliliters of 70% ethanol and heated in a water bath at 80°C. An ultrasonic bath was also used for ultrasonic extraction at 50°C, which was performed three times in 20-minute intervals. Following the extraction, the sample was centrifuged at 6000 rpm for 15 minutes. The resulting supernatant was transferred to a new tube, and another 10 milliliters of ethanol was added to the precipitate for the second extraction. After completing the third extraction, the supernatants were combined and refrigerated. This protocol closely followed the methods described in detail by Ivanov et al. (2014) and Bogoeva et al. (2017) [15-16].
- Microbiological contamination

BSS EN ISO 6579 – 1: 2017
Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of *Salmonella* spp. [17].
BSS EN ISO 6888 – 1:2005
Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of coagulase-positive staphylococci (*Staphylococcus aureus* and other species) [18].
BSS EN ISO 16649-2:2014
Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of beta-glucuronidase-positive *Escherichia coli* [19].
BSS EN ISO 21527 – 2:2011
Microbiology of food and animal feeding stuffs – Horizontal method for the determination of yeasts and moulds [20].
BSS EN ISO 21528-+12:2017
Microbiology of the food chain - Horizontal method for the detection and enumeration of *Enterobacteriaceae* [21].
BSS EN ISO 7932:2004/A1:2020
Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of presumptive *Bacillus cereus* - Colony-count technique at 30 degrees C [22].
BSS EN ISO 4833-1:2013
Microbiology of the food chain - Horizontal method for the enumeration of microorganisms - Part 1: Colony count at 30 degrees C by the pour plate technique (ISO 4833-1:2013) [23].

3 Results and discussion

The carbohydrates, including fibre are the main components of the fruit base product [24]. The examination of the nutrient profile of oil-cake elderberry after alcohol fermentation, received from tincture production, is presented in table 1.

Table 1. Nutrient profile and energy value of elderberry pressing (100 g product)

Indicators	Values
Carbohydrates, g/100 g	10.90±0.59
Ash, g/100 g	3.18±0.07
Nutrient fibre, g/100 g	46.25±0.04
Proteins, g/100 g	10.26±0.08
Fats, g/100 g	18.71±0.43
Energy value, kcal/100 g	345.45±6.63

The fibre content is 46.25g/100 g, which indicates that the pressed elderberry is a considerable source of fibre. Fibre help for a better digestion and their intake can reduce the risk from some chronical diseases. People with higher intake of fibre usually have a lower risk of heart diseases, obesity, stroke, high blood pressure and digestion problems [25]. The content of total carbohydrates is e 10.90 g/100 g, fats - 18.71g/100g, and protein quantity - 10.20g/100g. The energy value of the elderberry oil-cake is 345.45g/100 g.

The evaluation of the ash content in the fruit oil-cake is considerably important because it indicates the mineral content in the evaluated sample. The total ash content in the oil-cake is 3.18 g/100 g.

In table 2 are represented the received results for antioxidant activity of elderberry oil-cake after alcohol fermentation. The analysis is conducted with 72.45% extract from a 1 g sample.

Table 2. Antioxidant activity of the elderberry

Method	mMTE/g extract	mMTE/g dry weight
DPPH	32.04±4.23	23.21±3.07
ABTS	5.97±0.10	4.33±0.07
FRAP	3.13±0.17	2.27±0.12
CUPRAC	2.23±0.15	1.62±0.11

The multiple examinations confirm the antioxidant activity of the extracts of S. *ebulus* L. and it is influenced by the method of preparation and the polarity of the used solvent, as well as by the total content of polyphenol components [7-8]. From all the methods, the results for DPPH are the highest (23.21±3.07 mMTE/g dry weight). The lowest value of antioxidant activity was registered with the method CUPRAC. According to a literature consult, the flavonols and anthocyanins presence is mainly responsible for the antioxidant activity of the berries of the herbaceous elder *Sambucus ebulus* [7, 26]. There are multiple proofs for the high antioxidant activity of the elder berries. The published results are in a wide range depending on the different samples – whole berries or oil-cake/waste product of different productions of elderberry extract. We consider the differences are based on geographical – regional special features, as well as on the different technologies in the production treatment of the berries [27].

It was made a microbiological analysis of elderberry oil-cake – a waste product in the production of a tincture, by indicators - *Enterobacteriaceae, Escherichia coli, Salmonella* sp., *Staphylococcus aureus, Bacillus cereus*, spores of microscopic mould fungi and total number of mesophilic aerobic and facultative anaerobic microorganisms.

- *Enterobacteriaceae*, CFU/g <100
- *Escherichia coli*, CFU/g <10
- *Staphylococcus aureus*, CFU/g <10
- *Salmonella* sp. / 25 g - Not detected
- *Bacillus cereus*, CFU/g <100
- Fungi, CFU/g <10
- Total numbers of mesophilic aerobic and facultative anaerobic bacteria, CFU/g - 2.10^2

The data indicate that the elderberry corresponds to the standard requirements of the microbiological indicators for this type of products [17-23]. The total number of mesophilic aerobic and facultative anaerobic microorganisms is in the permissible limits 2.10^2 CFU/g. The spores of the microscopic mould fungi, as well as the presence of *Enterobacteriaceae*, *Escherihia coli* and *Staphylococcus aureus* is below the permissible value, and *Salmonella* sp. is not discovered. The examined product is not contaminated with the spore-developing and pathogen for people *Bacillus cereus*, which spores can survive years and can cause spoiling of the ready products or nutrient diseases through the formed toxins [22].

4. Conclusions

There are results for the data received for the nutrient profile and energy value of elderberry oil-cake after alcohol fermentation (of a 100 g product) – fibre – 46.25 g/100 g, total carbohydrates - 10.90 g/100 g, fats - 18.71 g/100 g, protein quantity - 10.20 g/100 g., total content of ash - 3.18 g/100 g. and energy value - 345.45 g/100 g.

There are results for the data received for the antioxidant activity of the examined product with the four different in mechanism and conditions of passing methods – DPPH - 32.04±4.23 mMTE/g extract, ABTS -5.97±0.10 mMTE/g extract, FRAP - 3.13±0.17 mMTE/g extract and CUPRAC - 2.23±0.15 mMTE/g extract.

There are results for the data received from the microbiological analysis of elderberry oil-cake after alcohol fermentation - total number of mesophilic aerobic and facultative anaerobic microorganisms are in the permissible values 2.102 CFU/g. The spores of the microscopic mould fungi, as well as the presence of *Enterobacteriaceae*, *Escherihia coli* and *Staphylococcus* aureus is below the permissible value, and *Salmonella* sp. is not discovered. The examined product is not contaminated with the spore-developing and pathogen for people *Bacillus cereus*.

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