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USE OF VEGETABLE FUNCTIONAL INGREDIENTS TO ACHIEVE HYPOGLYCEMIC BREAD WITH ANTIOXIDANT POTENTIAL, FOR DIABETICS

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Abstract

For diabetics, diet is a major therapeutic tool and a special nutrition form, in which food is adapted to metabolic disorders of disease. Bread is a key ingredient of the human diet and is consumed by billions of people worldwide. In this paper are presented results of the performed research to achieve a hypoglycemic bread assortment, with antioxidant potential, for diabetics. In the composition of bread, nutritionally valuable functional ingredients were used, but also with antioxidant potential: Jerusalem artichoke flour, hemp flour, apple waste flour, hemp husked seed and oat bran. The biphasic process was applied to obtain bread, and when the dough was fermented, the natural sourdough enriched with phenolic compounds and inulin was used. Bread for diabetics has superior sensory quality, high nutritional value and antioxidant potential. It has a low content of available carbohydrates (38.56-39.49%) and is noted for its content in protein (8.85-9.12%), total fiber (3.15-3.70%) and total polyphenols (100.85-115.12 mg GAE/100 g). At the same time, this product has an antioxidant capacity (117.45-125.15 mg TE/100 g). Shelf-life of this bread is 4 days.

Key words: *antioxidant, bread, diabetics, hypoglycemic, potential.*

INTRODUCTION

Diabetes is a metabolic disease, with an increased incidence both internationally and nationally. According to the International Diabetes Federation, in 2017, there were worldwide, 425 million people living with diabetes (largest age group: 40-59 years) and 352 and 352 million pre-stage of diabetes, called Impaired Glucose Tolerance (IGT). Also, by 2045, the number of people with diabetes is expected to rise by 48% worldwide (International Diabetes Federation, 2017).

Jerusalem artichoke tubers (*Helianthus tuberosus*) are characterized by their content in proteins, minerals potassium (K), calcium (Ca), magnesium (Mg), iron (Fe) and inulin. Inulin can be used in the diet of diabetics as a sugar substitute without having an impact on glycaemia (Meyer & Blaauwhoed, 2009; Long et al., 2016). Jerusalem artichoke tuber (*Helianthus tuberosus* L.) is considered a functional food (Radovanovic et al., 2015). Catană et al. (2018) achieved a functional ingredient (powder) with high nutritional value and antioxidant potential by processing of Jerusalem artichoke tubers (Red Jerusalem artichoke and White Jerusalem artichoke varieties). This powders achieved from Jerusalem artichoke tubers are important sources of minerals (K, Fe, Mg, Ca, phosphor - P) inulin and bioactive compounds. Thus, Jerusalem artichoke powders are characterized by a total polyphenol content of 18.51-44.03 mg GAE/g. Also, these powders have antioxidant potential being beneficial in a healthy diet for prevention of diseases caused by free radicals. On the other hand, powders achieved in this study are characterized by high inulin-type fructans content (51.60-57.45%), being beneficial for achieving of bakery and pastry products for diabetics. Hemp flour is a valuable component for enriching physicochemical and antioxidant properties of wheat bread (Mikulec et al., 2019). Flours from hemp are also a rich source of bioactive compounds from the polyphenols group, which have an anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, anti-viral, anticancer and cardioprotective effects (Callaway, 2004; Manach et al., 2005). Bread with hemp flour was characterized by significantly higher protein content (13.38–19.29 g/100 g DW), in comparison to wheat bread (11.02 g/100 g DW). The share of hemp flour influenced the polyphenols content by increasing from 256.43 (standard bread) to 673.59 mg GAE/kg (50% of the additive) (Mikulec et al., 2019). Hemp flour has been used for the production of gluten-free bread (Korus et al., 2017). Catană et al. (2018) achieved a functional ingredient from apple wastes resulting from the apple juice industry. Powders achieved from apple pomace are important sources of minerals (K: 450.12-508.45 mg/100 g; Fe 2.31-2.73 mg/100 g; Mg: 41.15-55.65 mg/100 g); Ca: 76.32-92.44 mg/100 g; Zn: 1.54-1.96 mg/100 g), dietary fibres (60.62-64.75%), polyphenols (17.83-38.83 mg GAE/g) and have antioxidant capacity (1.77-5.12 mg TroloxEquivalents/g). Due to the complex biochemical composition, the powders achieved from apple pomace can be used to obtain hypoglycemic bread with antioxidant potential, for diabetics. Also, for the achievement of hypoglycemic bread with antioxidant potential, for diabetics, an important role has sourdough. Lappi et al. (2010) showed that sourdough fermentation of wholemeal wheat bread increases solubility of arabinoxylan and protein and decreases postprandial glucose and insulin responses. Sourdough is considered the gold standard for bread-making. The biochemical changes deriving from the action of microbial enzymes along with indigenous flour ones greatly influence sourdough characteristics and ultimately the quality of bread. Effects of dough fermentation on texture, aroma, shelf life and nutritional value of the bakery products have been elucidated and highlight its positive role on bread production (Siepmann et al., 2018). Burnete et al. (2019) obtained sourdough enriched in phenolic compounds and inulin, using flour from Jerusalem artichoke tubers (*Helianthus tuberosus*).

In this paper are presented results of the performed research to achieve a hypoglycemic bread assortment, with antioxidant potential, for diabetics. Composition of the products and the proposed technological solutions considered the decrease of glycemic index, on the one hand and the increase of antioxidant capacity, on the other hand.

MATERIALS AND METHODS

Materials

In order to obtain the product "Hypoglycemic bread with antioxidant potential" the following raw materials and auxiliary materials were used: white wheat flour type 650, whole wheat flour, hemp flour, Jerusalem artichoke flour, apple waste flour, hemp husked seeds, flax seeds, oat bran and sea salt. For the fermentation and final proofing processes, natural sourdough enriched in phenolic compounds was used, using Jerusalem artichoke flour.

Bread-making

In order to achieve the product "Hypoglycemic bread with antioxidant potential", there were performed two experimental variants (PDV1 and PDV2), togheter with the control sample (C:"White bread with natural sourdough") (Figure 1).

Figure 1. Product "Hypoglycemic bread with antioxidant potential" (PDV1 and PDV2) and control sample (C)

Reduction of the glycemic index was achieved by introducing in the composition of products of some natural ingredients, in well defined proportions, scientifically based and by applying the biphasic technology, which allows the preservation, in the highest concentrations, of the bioactive compounds from these.

Methods

Sensory analysis

Sensory evaluation of the product "Hypoglycemic bread with antioxidant potential" was performed 12 hours afterbaking, using descriptive method and *"Comparison method with unitary score scales"*. Sensory quality of the product was established based on the total average score by comparison with a scale from 0 to 20 points (18.1 ... 20 - qualyfing "very good"; 15.1 ... 18 - qualyfing "good"; 11.1 ... 15 - "satisfactory"; 7.1 ... 11 - "unsatisfactory"; 0 ... 7 - "inadequate").

Measurement of the colour parameters of samples was performed at room temperature, using a CM-5 colorimeter (Konica Minolta, Japan), equipped with SpectraMagic NX software, to register CIELab parameters (the Commission Internationale de l'Eclairage - CIE), *L**, *a** and *b**: *L** - colour luminance (0 = black, 100 = white); *a** - red-green coordinate (-a = green, +a = red); *b** - yellow-blue coordinate (-b = blue, +b = yellow).

The texture properties of the product were measured through a compression test using an Instron Texture Analyzer (model 5944, Illinois Tool Works Inc., USA).

Physic-chemical analysis

The moisture content was determined according to the AACC 44-15A method. Protein content was determined by the Kjeldahl method with a conversion factor of nitrogen to protein of 6.25 (AOAC Method 979.09, 2005). Fat content was determined according toAOAC Method 963.15, and ash content according to AOAC Method 923.03 (AOAC, 2005).Total dietary fiber (TDF) was determined by enzymatic method using the assay kits: KTDFR "Total dietary fiber" (AOAC Method 991.43).

Calorie contents were calculated using the following conversion factors: 9 for fat, 4 for carbohydrates, 4 for protein and 2 for fibre, according to the Commission Regulation no. 1169/2011 (European Commission, 2011). Joule contents were calculated using thefollowing conversion factors: 37 for fat, 17 for carbohydrates, 17 for protein and 8 for fibre,according to the Commission Regulation no. 1169/2011 (European Commission, 2011).

Total polyphenol content

Total polyphenol content was conducted according to Horszwald & Andlauer (2011) with some modifications (concerning extract volumes of the used sample and reagents, using UV-VIS Jasco V 550 spectrophotometer), based on calibration curve of gallic acid achieved in the concentration range 0 to 0.20 mg/mL. The extraction of phenolic compounds was performed in methanol: water = 50:50 and the absorbance of the extracts were determined at a wavelenght λ = 755 nm. Results were expressed as mg of Gallic Acid Equivalents(GAE) per g product.

Antioxidant capacity

The DPPH scavenging radical assay was conducted according to Horszwald & Andlauer (2011) with some modifications (concerning extract volumes of the used sample and reagents, using UV-VIS Jasco V 550 spectrophotometer). The reaction was performed in dark for 30 min (at ambient temperature) and after this time the absorbance was read at 517 nm. It was achieved the calibration curve Absorbance = f (Trolox concentration), in the concentration range 0-0.4375 mmol/L and the results were expressed as mg Trolox Equivalents per g product.

Microbiological analysis

Yeasts and molds were determined by the method SR ISO 21527-1:2009. *Enterobacteriaceae* were determined according to the SR EN ISO 21528-1:2017 method.

RESULTS AND DISCUSSIONS

Sensory analysis

A complex mixture of dietary fibers, slow carbohydrates (mainly sourced from whole wheat flour, Jerusalem artichoke flour, hemp flour, apple waste flour, oat bran) and vegetable proteins (derived from hemp flour and hemp husked seeds) and dough fermentation, using natural sourdough, enriched in phenolic compounds and inulin, lead to the production of some hypoglycemic products and will decrease the inulin resistance (and, consequently, the inulin secretion), when there are consumed.

Sensory analysis plays an important role in characterizing the quality of food products.

As a result of the sensory analysis it was found that the product „Hypoglycemic bread with antioxidant potential", achieved in two experimental variants (PDV1 and PDV2) is well developed, presents an elastic, dense core, with uniform pores and pleasant taste and aroma, specific to the product. So, the analyzed products were tested by an expert panel receiving qualifying „very good",with next scores: Control Sample - 19.76; PDV1-19.44; PDV2-19.76 (Figure 2).

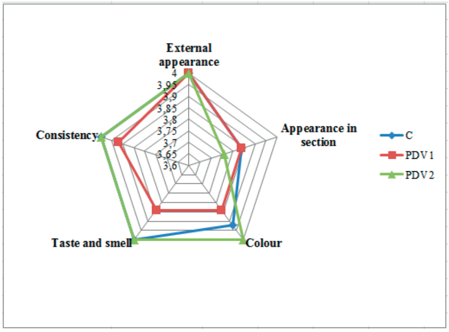

Figure 2. Sensory evaluation of the product “Hypoglycemic bread with antioxidant potential” (PDV1 and PDV2) and of the control sample (C)

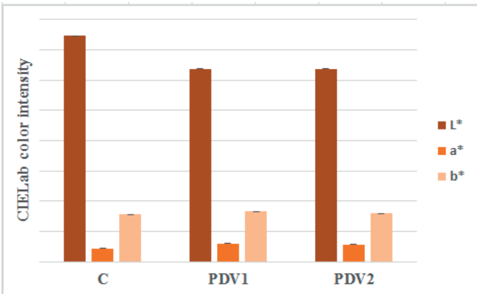

Figure 3. Color parameters of the product “Hypoglycemic bread with antioxidant potential” (PDV1 and PDV2) and of the control sample (C)

As a result of the ingredients used to decrease the glycemic index and to increase the antioxidant potential, the product “Hypoglycemic bread with antioxidant potential”, intended for diabetics, has a darker color, compared to the control sample, recording lower values of luminance (PDV1 - L^* = 63.68; PDV2 - L^* = 63.69) and higher values of the parameters a^* and b^* (Figure 3).

Also, the texture of the product “Hypoglycemic bread with antioxidant potential” was analyzed, compared to the control bread sample (C), for a period of 6 days (Table 1). The products were packed in polypropylene foil (oxygen permeability 1336.47 $cm^3/m^2 \cdot 24$ h·bar; water vapor permeability 1.284 $g/m^2 \cdot 24$ h·bar). Using the Instron texture analyzer (model 5944), the following texture parameters were calculated: firmness (hardness), elasticity, cohesiveness, and guminess. The test parameters were: compression speed: 100 mm/min; deformation of the sample: at a distance of 10 mm in height; load cell: 50 N. Figures 4-7 show the compression curves obtained for the product “Hypoglycemic bread with antioxidant potential” (experimental variants PDV1 and PDV2) on day 1 and day 3, respectively, on the date of manufacture.

Table 1. The textural properties of the product “Hypoglycemic bread with antioxidant potential”, compared to the control sample C

Product	Perioad (days)	Firmness (N)	Elasticity	Cohesiveness	Gumminess (N)
C	1	7.23 ± 0.26	1.00 ± 0.00	0.22 ± 0.18	2.11 ± 1.33
	2	9.74 ± 0.66	0.98 ± 0.00	0.30 ± 0.08	2.86 ± 0.61
	3	11.62 ± 1.87	1.04 ± 0.09	0.22 ± 0.11	2.70 ± 1.48
	6	14.57 ± 2.59	0.98 ± 0.00	0.28 ± 0.09	4.19 ± 1.95
PDV1	1	7.18±1.73	0.99±0.01	0.33±0.03	2.33±0.66
	2	8.71±0.51	0.99±0.00	0.35±0.03	2.98±0.09
	3	8.98±2.01	0.98±0.00	0.44±0.02	3.82±0.73
	6	11.94±3.79	0.98±0.01	0.28± 0.02	3.32±1.23
	7	13.67±3.74	1.14±0.19	0.23±0.02	3.62±1.33
PDV2	1	5.46±1.47	0.98±0.00	0.31±0.07	1.71±0.69
	2	5.78±1.12	0.98±0.00	0.32±0.09	1.82±0.49
	3	7.16±1.72	0.98±0.00	0.46±0.00	3.26±0.77
	6	8.97±2.90	1.03±0.04	0.36±0.04	3.35±1.11
	7	9.98±2.38	1.01±0.03	0.27±0.11	2.88±1.56

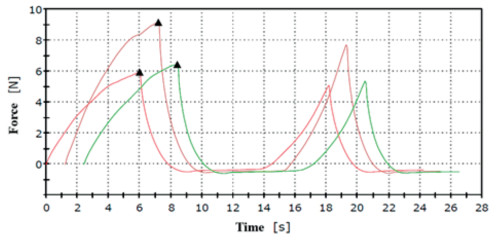

Figure 4. Compression curves for the product “Hypoglycemic bread with antioxidant potential” (PDV1) - day 1

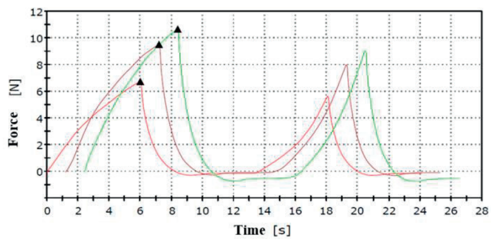

Figure 5. Compression curves for the product “Hypoglycemic bread with antioxidant potential” (PDV1) - day 3

According to the obtained results, during the 7 days from the date of manufacture, the firmness of the product “Hypoglycemic bread with antioxidant potential”, achieved in two experimental variants PDV1 and PDV2, varied in the range 5.46 N-13.67 N (the minimum value was registered in the case of the experimental variant PDV2, one day from the date of manufacture, and the maximum one, in the case of the experimental variant PDV1, 7 days from the date of manufacture).

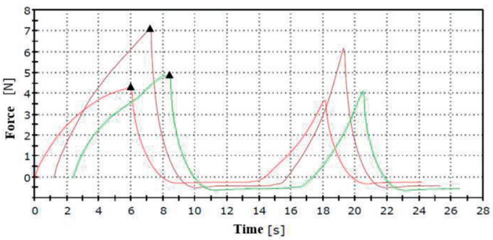

Figure 6. Compression curves for the product “Hypoglycemic bread with antioxidant potential” (PDV2) - day 1

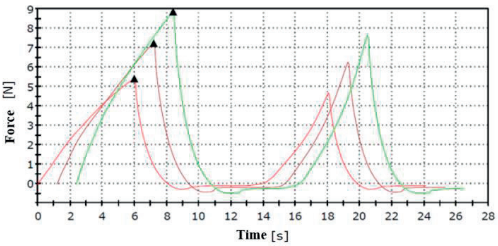

Figure 7. Compression curves for the product “Hypoglycemic bread with antioxidant potential” (PDV2) - day 3

It is noteworthy that in the case of the experimental variant PDV2, there were registered lower values of firmness, during the 7 days from the date of manufacture, compared with the experimental variant PDV1 and the control sample (C). Thus, the bread corresponding to the experimental variant PDV2 has a less dense, less hard core, compared to the experimental variant PDV1 and the control sample (C).

The elasticity of the product “Hypoglycemic bread with antioxidant potential” (experimental variants PDV1 and PDV2) and of the control sample (C) recorded close values, during the 7 days from the date of manufacture.

The product “Hypoglycemic bread with antioxidant potential” (experimental variants PDV1 and PDV2) had higher values of cohesiveness compared to the product “White bread with natural sourdough” (control sample - C). According to the values recorded for this parameter of texture, the product “Hypoglycemic bread with antioxidant potential” has a higher compressive strength compared to the control bread sample (C), which leads us to the conclusion that it has stronger internal bonds, which maintain its structure. The highest value of cohesiveness was recorded after 3 days from the date of manufacture, in the case of the experimental variant PDV2 (0.46).

The product “Hypoglycemic bread with antioxidant potential” (experimental variants PDV1 and PDV2) had lower of guminess, compared to the product “White bread with natural sourdough” (control sample - C).

Physic-chemical analysis

As a result of the physic-chemical analysis it was found that the product “Hypoglycemic bread with antioxidant potential”, achieved in two experimental variants (PDV1 and PDV2) for diabetics, has a low carbohydrate content and is noted for its protein, ash and total fiber content (Table 2).

Table 2. Physic-chemical analysis of the product “Hypoglycemic bread with antioxidant potential” and of the control sample C

Component	**C**	**PDV1**	**PDV2**
Nominal mass (kg)	0.404	0.412	0.416
Volume (cm³/100 g)	272	233	236
Porosity (%)	71.30	69.53	69.04
Elasticity (%)	95	95	95
Acidity (degrees)	3.2	4.2	4.4
Moisture (%)	41.25	45.68	45.41
Ash (%)	0.50	1.12	1.27
Protein (%)	7.58	8.85	9.12
Fat (%)	0.88	1.71	1.94
Carbohydrates (%)	49.79	42.64	42.26
Available carbohydrates (%)	49.21	39.49	38.56
Total dietary fiber (%)	0.58	3.15	3.70
Energy value (kcal/100g)	236.24	215.05	215.58
Energy value (kJ/100g)	1002.63	910.25	911.94

Product “Hypoglycemic bread with antioxidant potential”, achieved in two experimental variants (PDV1 and PDV2) has higher moisture compared to that of bread with *Aronia*, achieved by Catană et al. (2018), but also the content in protein, lipids, ash and total fiber, lower than that of this bread. Chemical characteristics of the bread achieved within this study determine a fast and lasting satiety when consumed and a low glycemic impact, being

beneficial in the diet of diabetics and obese people. Due to the ingredients used to decrease the glycemic index and to increase the antioxidant potential, the product “Hypoglycemic bread with antioxidant potential” has a smaller volume (PDV1 - V = 233 cm^3/100 g; PDV2 - V = 236 cm^3/100 g), compared to the control sample (C - V = 272 cm^3/100 g). Similar results were obtained by Šporin et al. (2017) in the case of the bread fortified with grape pomace flour, levels of fortification of 6, 10 and 15%. Reduction of the volume of the bread fortified with grape pomace flour could be explained by the activities of enzymes and yeasts. Amylases in the enriched dough could be inhibited by the phenolic compounds of the grape pomace flour, resulting inadequate concentrations of maltose. At the same time, in the case of bread fortified with grape pomace flour, a smaller quantity of gas is produced by the activity of yeasts, which results in a smaller volume of bread and a relatively compact texture.
Elasticity of the product “Hypoglycemic bread with antioxidant potential” (experimental variants PDV1 and PDV2) is similar to the control sample one, instead, the porosity recorded slightly lower values (C - 71.03%, PDV1 - 69.53% and PDV2 - 69.04%). Both the porosity and the elasticity are expressed as a percentage, the higher their value, the more bakery products are considered to be better qualitative and more appreciated by consumers.

Total polyphenol content

Total polyphenol content of the product “Hypoglycemic bread with antioxidant potential” recorded the following values: 100.85 mg GAE/100 g experimental variant PDV1 and 115.12 mg GAE/100 g experimental variant PDV2 (Figure 8).
Due to the valuable ingredients used in the composition (Jerusalem artichoke flour, apple waste flour, hemp flour, hemp husked seeds, flax seeds, oat bran etc.), the product “Hypoglycemic bread with antioxidant potential” has the total polyphenol content 1.53-1.75 times higher compared to the control sample, “White bread with natural sourdough”.

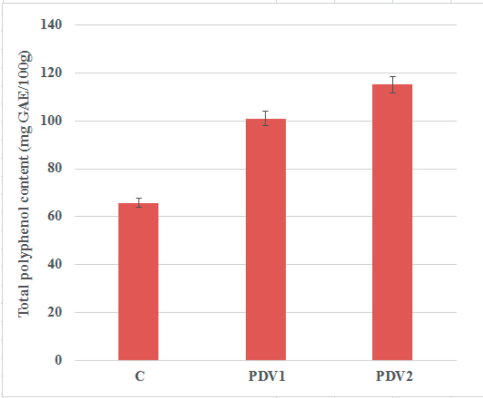

Figure 8.Total polyphenol content of the product “Hypoglycemic bread with antioxidant potential” (PDV1 and PDV2) and of the control sample (C)

Total polyphenol content of product “Hypoglycemic bread with antioxidant potential”, is superior to those recorded in case of bread prepared with 10% of grape pomace powder (89.43 mg GAE/100 g; Hayta et al., 2014).
According to the research performed at international level (Lutz et al., 2019), phenolic compounds can be considered as natural inhibitors of platelet aggregation, helping to reduce the risk of cardiovascular diseases, caused by thrombosis. Also, performed studies have shown that a diet rich in phenolic compounds is associated with anti-inflammatory effects in the human body (Cassidy et al., 2015).

Antioxidant capacity

Due to its content in phenolic compounds, the product “Hypoglycemic bread with antioxidant potential” has antioxidant capacity (Figure 9). The highest values of antioxidant capacity were registered in the case of experimental variant PDV2: 125.15 mg Trolox Equivalents/100 g. Due to the ingredients with antioxidant potential, used in the composition of product “Hypoglycemic bread with antioxidant potential” for diabetics, it has an antioxidant capacity of 1.7, respectively 1.8 times higher, compared to the control sample (C).

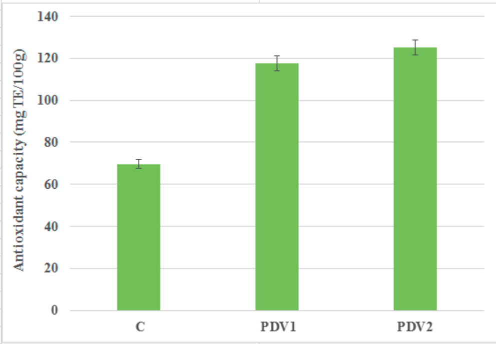

Figure 9. Antioxidant capacity of the product "Hypoglycemic bread with antioxidant potential" (PDV1 and PDV2) and of the control sample (C)

Corroborating the results of the sensory analysis with those of the physico-chemical analysis, respectively with those obtained for the content in polyphenols and for the antioxidant capacity, for the product „Hypoglycemic bread with antioxidant potential", the experimental variant PDV2 was selected as the optimal variant.

Microbiological analysis
Following the microbiological analysis of the products "Hypoglycemic bread with antioxidant potential" and "White bread with natural sourdough" (Control) packed in polypropylene bags, it was found that they comply with the provisions of the legislation into force and at 7 days from the date of manufacture (Table 3).

Table 3. Microbiological analysis of the product "Hypoglycemic bread with antioxidant potential" and of the control sample (C)

Product	Microbiological indicator					
	Yeasts and molds (CFU/g)			*Enterobacteriaceae* (CFU/g)		
	24h	5 days	7 days	24h	5 days	7 days
C	< 10	< 10	< 10	< 10	< 10	< 10
PDV1	< 10	< 10	< 10	< 10	< 10	< 10
PDV2	< 10	< 10	< 10	< 10	< 10	< 10

Corroborating the results of the microbiological analysis with those of the sensory analysis, the minimum durability of the product "Hypoglycemic bread with antioxidant potential", corresponding to the experimental variant PDV2, was established at 4 days. Shelf-life relative high of the product "Hypoglycemic bread with antioxidant potential" can be explained mainly by antioxidant and antibacterial potential of Jerusalem artichoke flour, hemp flour, apple waste flour, used in the composition of the product, due to their total polyphenol content. Flours from hemp are a rich source of polyphenols, which have an anti-microbial and anti-viral effects (Callaway, 2004; Manach et al., 2005, Mikulec et al., 2019). Also, Zhang et al. (2016) demonstrated antioxidant and antibacterial activities of phenolics from *Golden Delicious* apple pomace (phloridzin and phloretin are the most important phenolic compounds that have antioxidant and antibacterial activities).
However, lactic acid bacteria from sourdough enriched in phenolic compounds, used for fermentation and final proofing, act as a natural antibiotic, thereby increasing the shelf-life of product "Hypoglycemic bread with antioxidant potential" (Catană et al., 2018).

CONCLUSIONS

Bread for diabetics has superior sensory quality, high nutritional value and antioxidant potential. It has a low content of available carbohydrates (38.56-39.49%) and is noted for its content in protein (8.85-9.12%), total fiber (3.15-3.70%) and total polyphenols (100.85-115.12 mg GAE/100 g). At the same time, this product has an antioxidant capacity (117.45-125.15 mg TE/100 g). Shelf-life of this bread is 4 days.

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