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Research Article

APPLICATION OF THE FERMENTED AND IMMOBILIZED *Cannabis sativa* PRODUCTS FOR THE BISCUITS PRODUCTION

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Abstract- The aim of this study was to increase the total phenolic compounds (TPC) content, antioxidant activity, and protein digestibility of *Cannabis sativa* protein and expeller by using fermentation with selected lactic acid bacteria (LAB) strains, isolated from spontaneous fermented cereal substrate, and to adapt the *Cannabis sativa* protein and expeller products by using immobilization for higher value biscuits production. Spontaneous sourdough is a good source for LAB isolation, and isolated strains (*Lactobacillus plantarum* and *Lactobacillus paracasei*) showed versatile carbohydrate metabolism and resistance to low pH conditions. *L. plantarum* and *L. paracasei* could be used for hemp seed protein and expeller fermentation in order to increase its antioxidant activity, and for hemp seed expeller protein digestibility increasing *L. plantarum* should be selected (digestibility increase 15.26%, compare to nonfermented expeller samples). Fermented hemp seed products have significant influence on most of the biscuits parameters, and for biscuits value increasing with *L. plantarum* fermented and immobilazed hemp seed protein and expeller could be recommended.

Keywords- Biscuits, *Cannabis sativa* products, Fermentation, Lactic acid bacteria

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Introduction

The main challenge of the food industry is to make food products more sustainable, competitive and healthier for consumers. In this case, different pseudo-cereal, having specific chemical composition, became very important subject of investigations. Hemp plant (*Cannabis sativa*) itself is easy to grow in temperate climates and requires good soil, fertilizer and water but no pesticides nor herbicides [1]. The whole seed contains roughly 25% protein, 30% carbohydrates, 15% insoluble fibre, carotene, phosphrous, potassium,magnesium, sulphur, calcium,iron and zinc as well as vitamin E, C, B1, B2, B3, B6. Hemp seed is good source of essential fatty acids, therefore, during the thermal treatment unsaturated fatty acids could be transformed to the *trans* fatty acids [2], and from this point of view, for thermal treated products (such as biscuits or/and bread) production defatted hemp seed products should be reccomended. In this case, hemp seed expeller, which is the by-product of the hemp seed oil production, could be used.

The use of pseudo-cereals for wheat products production is to fortify the deficiency of nutritional value in wheat flour. But, our previous studies showed, that rich in proteins plant additives could increase acrylamide content in baked products, and reduction of acrylamide content can be achieved by using fermentation with selected LAB [3]. Therefore, biscuits are a popular food, consumed by different population groups, and its safety parameters should be taken into account.

Also, our previous studies showed, that fermentation of proteinaceous plants could increase its protein digestibility [4], TPC content, and radical scavenging activity [5]. However, by using fermented raw material, to high moisture content in biscuits could be achieved, which could have negative impact on products texture and microbiological stability during the storage. To reduce water migration from fermented products to biscuits dough, immobilization technology could be adapted.

The aim of this study was to increase the TPC content, antioxidant activity, and protein digestibility of *Cannabis sativa* protein and expeller by using fermentation with selected LAB strains, isolated from spontaneous fermented cereal substrate, and to adapt the *Cannabis sativa* protein and expeller products by using immobilization for higher value biscuits production.

Materials and Methods

Spontaneous fermented rye preparation as a source for LAB isolation

Rye flour (type 1370, falling number > 130 s, ash 1.31%) obtained from Kauno Grudai Ltd. mill (Kaunas, Lithuania) were used for the preparation of spontaneous rye sourdough. Spontaneous rye sourdough was prepared by using the following scheme: mixing of 100 g rye flour with 1% of acetic acid, 1% of salt and 150 ml of water; fermentation for 48 h at 30 °C; addition of 50 g rye flour and 50 ml water; fermentation for 24 h at 30 °C.

Purification, isolation, and identification of *Lactobacillus plantarum* and *Lactobacillus paracasei* by analytical techniques based on polymerase chain reaction (PCR)

Purification of LAB cells was performed according to [6].

The molecular fingerprinting of the final strains was done by rep-typing with the primer GTG5 (5'-GTG GTG GTG GTG GTG-3') [7]. PCR was carried out in a

Mastercycler apparatus (Eppendorf, Hamburg, Germany). The resulting (GTG)5-PCR fingerprints were analyzed using the BioNumerics v4.0 software package (Applied Maths, Sint-Martens-Latem, Belgium).

16S rDNA sequencing was conducted for selected strains by applying the primers (Bak4 (5'-AGG AGG TGA TCC ARC CGC A-3'); Bak11 (5'-AGT ATTG ATC MTG GCT CAG -3')) set, and PCR protocol published by [8]. The PCR products were purified by applying the peqGold-Cycle-Pure Kit (Peqlab Biotechnology GmbH, Erlangen, Germany) and sequenced (Eurofins MWG Operon, Ebersberg, Austria). The received sequences were analyzed with the BLASTn tool (http:// blast.ncbi.nlm.nih.gov), and a minimum sequence identity of 98% was selected as a criterion for species identification.

Colonies were identified as belonging to the genus *Lactobacillus* (Song *et al.*, 2000) [9] by means of PCR. The PCR-based identification of species followed the strategy reported by [10] for *Lactobacillus plantarum* and by [11] for *Lactobacillus paracasei.*

Evaluation of isolated *Lactobacillus plantarum* and *Lactobacillus paracasei* carbohydrate metabolism, gas production, tolerance to temperature and low pH conditions

Carbohydrate fermentation profiles of the strains were determined by using API 50 CH system (BioMerieux, Marcy-l'Etoile, France). Moreover, each pure culture was further characterized by the Durham tube method in MRS broth at 30 °C for 24 h for detecting gas evolution. The growth performance was monitored at 10 °C, 30 °C, 37 °C, and 45 °C for 24 h in MRS broth using a Thermo Bioscreen C automatic turbidometer (Labsystems, Helsinki, Finland). The ability of the strains to survive at low pH was evaluated in triplicate as described by [12], in acidified MRS broth (final pH 2.5).

***Cannabis sativa* protein and expeller fermentation and immobilisation in agar**

Cannabis sativa protein and expeller (*Cannabis sativa* L., Finola cultivar) were purchased at a JSC "Agropro" (Vilnius, Lithuania) and used as the starting material for fermentation and biscuits production (hemp seed protein nutritional value: protein content 49.60 g/100 g; fat content 10.50 g/100 g; carbohydrates content 29.90 g/100 g; hemp seed expeller nutritional value: protein content 24.60 g/100 g; fat content 6.30 g/100 g; carbohydrates content 31.47 g/100 g).

The *Lactobacillus plantarum* and *Lactobacillus paracasei* strains, previously isolated from spontaneous rye sourdough, were cultured at 30 °C temperature for 48 h in MRS broth (CM0359, Oxoid Ltd, Hampshire, UK) with the addition of 40 mmol/L fructose and 20 mmol/L maltose prior to be used.

Hemp seed products, tap water, and LAB cell suspension (5 mL), containing of 8.9 $\log_{10}$ colony-forming units (CFU) per mL of the above individual LAB strains were used to prepare fermented products following the fermentation at 30 °C temperature for 24 h. Water content was calculated with reference to moisture content of the raw materials, water absorption capacity and required humidity of the end product. Final moisture of products was 47 g/100 g. In addition, immobilization of fermented hemp seed products was performed by using agar ("Radix – Bis", Poland). 25 g of agar powder were dissolved in 200 ml of water and boiled, in case to increase viscosity. After, agar/water mass was cooled till 30 °C temperature and mixed with fermented hemp seed products (1:10; agar/hemp seed product). Prepared fermented immobilized and nonimmobilazed products were used for biscuits production. Ten different hemp seed products for biscuits production were used: nonfermented hemp seed protein (NFP) and expeller (NFE), fermented with *L. plantarum* hemp seed protein and expeller (LplP and LplE, respectively), fermented with *L. paracasei* hemp seed protein and expeller (LpaP and LpaE, respectively), fermented with *L. plantarum* and immobilised in agar hemp seed protein and expeller (LplPIm and LplEIm, respectively), fermented with *L. paracasei* and immobilised in agar hemp seed protein and expeller (LpaPIm and LpaEIm, respectively). In addition, control biscuits without hemp seed products were produced.

pH and total titratable acidity (TTA) of the fermented hemp seed products evaluation

The pH value of fermented hemp seed was measured and recorded by a pH electrode (*PP–15, Sartorius,* Goettingen, Germany). TTA was determined on 10 g of sample homogenized with 90 mL of distilled water and expressed as the amount (mL) of 0.1M NaOH to get a pH of 8.2.

Microbiological analysis of the fermented hemp seed products

The viable LAB cells in fermented hemp seed products were evaluated under standard serial dilution method on MRS agar medium and expressed in $\log_{10}$ CFU/g. The plates were incubated at 30 °C temperature for 4 days under anaerobic conditions in a jar (Sigmae Aldrich, Broendby, Denmark) with anaerobic atmosphere generation bags (Sigmae Aldrich, Broendby, Denmark). The number of microorganisms was calculated and expressed as $\log_{10}$ CFU/g. All of the results were expressed as the mean of three determinations.

Determination of TPC, antioxidant activity, and *in vitro* protein digestibility of hemp seed products

The content of TPC in fermented hemp seed products was determined by spectrophotometric method, as reported elsewhere [13]. The absorbance of samples was measured at 765 nm using spectrophotometer "J.P. SELECTA S.A. V-1100D" (Barcelona, Spain).

Antioxidant activity of samples was evaluated according to the method reported by [14].

Determination of protein digestibility was carried out according to [15]. Samples containing 62.5 mg of protein were suspended in 10 mL of water and the pH was adjusted to 8 with 0.1 mol/L NaOH. An enzymatic solution containing 1.6 mg of trypsin (18 U/mg), 3.1 mg of α-chymotrypsin (40 U/mg) and 1.3 mg of protease (15 U/mg) per millilitre was added to the protein suspension in a 1:10 (v/v) ratio. The pH of the mixture was measured exactly after 10 min and the *in vitro* digestibility calculated as a percentage of digestible protein (DP) using the equation DP = 210.464 – 18.103 × pH.

Biscuits formula and preparation

Main formula for biscuits preparation: wheat flour 300 g, margarine 150 g, saccharose 80 g, eggs 40 g, salt 1 g, baking powder 1.5 g. Test samples were prepared by addition of hemp seed products to the main biscuit dough by replacing the same amount of wheat flour. Control samples were prepared without hemp seed products (BC), and with nonfermented hemp seed protein and expeller (BNP and BNE, respectively). Test samples were prepared by addition of fermented with *L. plantarum* hemp seed protein and expeller (BLplP and BLplE, respectively), fermented with *L. paracasei* hemp seed protein and expeller (BLpaP and BLpaE, respectively), fermented with *L. plantarum* and immobilized in agar hemp seed protein and expeller (BLplPIm and BLplEIm, respectively), fermented with *L. paracasei* and immobilized in agar hemp seed protein and expeller (BLpaPIm and BLpaEIm, respectively) to the main formula.

Sugar and fat were creamed in a mixer (Guangzhou R & M Machinery, Guangdong, China). Eggs were added to this cream and mixed for 0.5 min. to obtain a homogeneous cream. Finally, flour was added and mixed for 1 min. to obtain a homogeneous dough. Biscuits were formed manually by rolling with rolling machine "Roll S5B" (Vicenza, Italy), the thickness of the dough was 2.5 mm, and stamping was carried out by hands. Biscuits were baked in a deck oven (MIWE, Michael Wenz, Germany) at 220°C for 10 min.

Biscuits quality parameters evaluation

The color characteristics of the biscuits were evaluated using CIEL*a*b* system (CromaMeter CR-400, Conica Minolta, Japan). L* is a measure of lightness from completely opaque (0) to completely white (100), a* is a measure of redness (or –a* of greenness), and b* is a measure of yellowness (or –b* of blueness). The moisture content was determined according to the ICC Standard Method 110/1. Biscuits firmness was determined as the maximum compression force using the Texture Profile Analysis (TPA) (Stevens-LFRA Texture Analyzer, Poland). The overall acceptability of biscuits was carried out according to the ISO 8586-1 method for preliminary sensory acceptability using a 140 mm hedonic line scale ranging from 140 (extremely like) to 0 (extremely dislike).

Statistical analysis

In order to evaluate the influence of fermentation and different LAB on fermented hemp seed products parameters, and the effects of different hemp seed products (fermented and nonfermented, immobilized and nonimmobilized) on biscuits quality parameters, data were analyzed by the one-way and two-way ANOVA (statistical program R 3.2.1, R Core Team 2015). The results were recognized as statistically significant at $p \leq 0.05$.

Results and Discussion

The properties of isolated LAB strains

Identification of the isolated *L. plantarum* and *L. paracasei* strains by using the BioNumerics v4.0 software package, as well as carbohydrate metabolism, gas production, tolerance to temperature and low pH conditions (pH 2.5 for 2 h of incubation) are shown in [Table-1].

Table-1 *Bands of the isolated LAB genus (analyzed by the BioNumerics v4.0 software package), carbohydrate metabolism of LAB tested by API 50 CH system, gas production, tolerance to temperature and low pH conditions (pH 2.5 for 2 h of incubation).*

		Lactobacillus plantarum JCM 1149 (135)	Lactobacillus paracasei NBRC 15889 (244)	Bands of isolated LAB genus: 100bp DNA-leiter extended	Lactobacillus plantarum JCM 1149 (135)	Lactobacillus paracasei NBRC 15889 (244)
Glicerol		-	-			
Erythritol		-	-			
D-arabinose		-	-			
L-arabinose		+++	-			
D-ribose		+++	+++			
D-xylose		-	-			
L-xylose		-	+++			
D-adonitol		-	+			
Methyl-ßd-xYlopiranoside		-	-			
D-galactose		+++	+++			
D-glucose		+++	+++			
D-fructose		+++	+++			
D-mannose		+++	+++			
L-sorbose		-	-			
L-rhamnose		+	+++			
Dulcitol		-	+++			
Inositol		-	-			
D-mannitol		+++	+++			
D-sorbitol		+++	+++			
Methyl-αD-mannopyranoside		+++	-			
Methyl-αD-glucopyranoside		+	+++			
N-acetylglucosamine		+++	+++			
Amigdalin		+++	+++			
Arbutin		+++	+++			
Esculin		+++	+++			
Salicin		+++	+++			
D-cellobiose		+++	+++			
D-maltose		+++	+++			
D-lactose		+++	+++			
D-melibiose		+++	-			
D-saccharose		+++	+++			
D-trehalose		+++	+++			
Inulin		-	+++			
D-melezitose		+++	+++			
D-raffinose		-	-			
Amidon		-	-			
Glycogen		-	-			
Xylitol		-	-			
Gentiobiose		++	+++			
D-turanose		+++	+++			
D-lyxose		-	-			
D-tagatose		+++	+++			
D-fucose		-	-			
L-fucose		-	-			
D-arabitol		-	-			
L-arabitol		-	-			
Potassium gluconate		++	++			
Potassium 2-ketogluconate		-	-			
Potassium 5-ketogluconate		-	-			
Gas production (+/-)		-	-			
Tolerance to temperature	10 °C	-	-			
	30 °C	+++	++			
	37 °C	++	++			
	45 °C	+	-			
pH	0 h log10 CFU/mL	8.08±0.2	9.41±0.2			
	2 h log10 CFU/mL	7.69±0.1	9.29±0.1			

Interpretation of LAB growth in API 50 CH system +++= high growth (yellow); ++= quite growth (green); += little growth (dark green); -= not growth (blue).

The tested strains were able to ferment D-ribose, D-galactose, D-glucose, D-fructose, D-mannose, D-mannitol, D-sorbitol, N-acetylglucosamine, amigdalin, arbutin, esculin, salicin, D-cellobiose, D-maltose, D-lactose, D-saccharose, D-trehalose, D-melezitose, gentiobiose, potassium gluconate, D-turanose, and D-tagatose. The tested strains were grown at 30 °C and 37 °C temperatures [Table-1]. After 2 h of incubation at 2.5 pH, the viable count of *L. plantarum and L. paracasei* was 7.69 ± 0.1 log_{10} CFU/mL and 9.29 ± 0.1 log_{10} CFU/mL, respectively. The acid resistance mechanisms used by LAB to survive the by-products of their own metabolism and the responses available to spoilage and pathogenic organisms in low-pH substrate is thus of great importance [16], it helps to ensure the stability of the fermented products in industrial conditions. Gas production of tested strains was not observed.

Parameters of fermented hemp seed products

Parameters of the fermented with *L. plantarum* and *L. paracasei* hemp seed protein and expeller are shown in [Table-2]. After 24 hours of fermentation lower pH and higher TTA in hemp seed protein samples was observed (LPlP pH 4.30 ± 0.01, LpaP pH 4.30 ± 0.02, and TTA 3.50 ± 0.04 °N and 3.40 ± 0.05 °N, respectively), compare with hemp seed expeller samples. Between the pH and TTA of samples strong negative correlation was found (r = -0.8910). LAB produce lactic acid as the major metabolite during the fermentation process and initiate rapid and adequate acidification in the raw materials through the production of various organic acids from carbohydrates, for this reason decrease the pH and increase the TTA of the fermentable substrate [17] , [18].

LAB count in fermented hemp seed protein and expeller samples was ranged from 8.28 ± 0.03 log_{10} CFU/g till 8.11 ± 0.04 log_{10} CFU/g (in LplE and LpaP samples, respectively). Between the LAB count and acidity parameters of the products a weak correlations were found (between the LAB count and pH r=0.2713, between the LAB count and TTA r=-0.3140). Plant material could be good source of carbohydrate for growing of high count of viable LAB populations [19], which is necessary to get the desired reduction in the pH, which not only affects the product organoleptic properties but also prolongs shelf-life and prevents product contamination [20].

Table-2 *Parameters of fermented with L. plantarum and L. paracasei hemp seed protein and expeller.*

Samples	pH	TTA, °N	LAB, log_{10} CFU/g	TPC	RSA	Protein digestibility, %
NFP	-	-	-	107.02±3.41	81.59±4.01	72.88±1.09
NFE	-	-	-	76.42±5.16	71.04±5.44	74.69±1.58
LplP	4.30±0.01	3.50±0.04	8.27±0.04	132.39±9.32	93.17±3.56	67.45±1.35
LplE	4.50±0.01	2.60±0.06	8.28±0.03	84.52±4.98	96.77±4.21	86.09±1.57
LpaP	4.30±0.02	3.40±0.05	8.11±0.04	129.29±5.69	86.89±2.98	75.95±1.63
LpaE	4.70±0.01	2.60±0.02	8.22±0.02	90.15±10.23	97.42±3.54	65.27±1.42

NFP – nonfermented hemp seed protein; NFE – nonfermented hemp seed expeller; LplP - hemp seed protein fermented with *L. plantarum*; LplE - hemp seed expeller fermented with *L. plantarum*; LpaP - hemp seed protein fermented with *L. paracasei*; LpaE -hemp seed expeller fermented with *L. paracasei.*
TTA – total titratable acidity; LAB – lactic acid bacteria count; TPC – total phenolic compounds content; RSA – radical scavenging activity.
Data expressed as a mean values (n = 6) ± SD; SD – standard deviation.
[a-d] Mean values within a column with different letters are significantly different ($p \leq 0.05$).

In all the cases, higher TPC content and RSA in fermented with *L. plantarum* and *L. paracasei* hemp seed samples was found, compare to nonfermented [Table 2], and fermentation have significant influence on TPC and RSA changes ($p = 0.001$ and $p = 0.0001$, respectively) in samples [Table-3]. The type of product have significant influence on the TPC content ($p = 0.0001$), and interaction of analysed factors (type of product and fermentation) was significant ($p = 0.002$) on RSA of hemp seed protein and expeller samples [Table-3]. It was found a strong negative correlation between the TPC and pH (r=-0.8083), and a moderate positive correlation between the RSA and pH of samples (r=0.6772). Also, RSA was influenced by LAB count in samples (r=0.8151), however, between the TPC content and the LAB count in samples a weak negative correlation (r=-0.2854) was found.

Fermentation has a positive influence on the TPC and antioxidant activity of grain [21]. During LAB fermentation, the grain constituents are modified by the action of both endogenous and bacterial enzymes, including esterases, xylanases, and phenoloxidases, thereby affecting their structure, bioactivity, and bioavailability: increase the levels of folates, soluble dietary fiber, TPC, improve the protein digestibility and RSA [22-24]. *L. plantarum* is the species most frequently used to ferment food products of plant origin [25].

Different tendencies of digestibility of samples were found [Table-2]. The highest digestibility of LplE was established (86.09 ± 1.57%), and it was 15.26% higher, than of nonfermented samples. However, hemp seed expeller samples fermented with *L. paracasei* showed 12.61% lower digestibility, compare to nonfermented samples [Table-2]. Opposite tendencies of hemp seed protein digestibility were found, and the following results were obtained: NFP digestibility 72.88 ± 1.09%, LplP digestibility 67.45 ± 1.35%, and LpaP digestibility 75.95 ± 1.63%. It was found significant influence of type of product and fermentation on digestibility of samples (p= 0.0001; p=0.0001), and interation of analysed factors was significant ($p = 0.0001$) [Table-3].

Results of the ANOVA test indicated that there is a significant effect of the type of product on fermented samples pH, LAB count, and digestibility ($p < 0.0001$, respectively), of bacteria used for the fermentation on samples pH ($p < 0.0001$), TTA ($p < 0.0001$), LAB count ($p < 0.015$), TPC content ($p < 0.0001$), RSA ($p < 0.009$), and digestibility ($p < 0.002$), and interaction of these factors was significant on pH ($p < 0.0001$), LAB count ($p < 0.033$), and digestibility ($p < 0.0001$) of samples [Table-4].

The effects of different hemp seed products on biscuits quality parameters

Quality parameters of biscuits are shown in [Table-5]. In all the cases, hemp seed products decrease biscuits lightness (L*), redness (a*), and yellowness (b*), from 16.92% till 45.01%, from 2.16% till 3.66%, and from 0.40% till 19.36%, respectively.

Immobilisation of fermented hemp seed products allow to reduce biscuits moisture content (50.00% lower in BLplPIm, 39.62% lower in BLplEIm, 27.66% lower in BLpaPIm, and 58.49% lower in BLpaEIm), in compare with biscuits, produced with nonimmobilised hemp seed products. However, in most of the cases, immobilized products reduce firmness of biscuits, compare to biscuits produced with nonimmobilized hemp seed products (except BLplPIm biscuits), but negative impact on biscuits shape was not observed.

Different tendencies of biscuits overall acceptability were estimated, and the highest overall acceptability of biscuits, produced by using hemp seed products fermented with *L. plantarum* was found (overall acceptability 125 and 132 of BLplPIm and BLplEIm biscuits, respectively).

Results of the ANOVA test indicated that there is a significant effect of analysed factors (the type of product, the LAB used for hemp seed products fermentation, and immobilization) on biscuits quality parameters ($p < 0.05$) [Table-6]. Interaction of factors was significant on most of the analysed biscuits parameters (except Product × Bacteria was not significant on overall acceptability of biscuits, Product

× Immobilization was not significant on overall acceptability of biscuits, Bacteria × Immobilization was not significant on moisture content, and Product × Bacteria × Immobilization was not significant on firmness and overall acceptability of biscuits).

Table-3 *The influence of fermentation on TPC, RSA and digestibility of hemp seed protein and expeller.*

Source	Dependent Variable	Mean Square	F	*p*
Different treatment (fermented and non)	TPC	687.209	14.768	0.001
	RSA	606.839	37.350	0.0001
	Digestibility	56.936	27.016	0.0001
Different products (protein and expeller)	TPC	6651.273	142.939	0.0001
	RSA	6.408	0.394	0.542
	Digestibility	47.726	22.646	0.0001
Treatment * Product	TPC	143.771	3.090	0.083
	RSA	173.153	10.657	0.002
	Digestibility	324.728	154.080	0.0001

TPC – total content of phenolic compounds; RSA – radical scavenging activity.

Table-4 *The influence of different factors (different LAB and different hemp seed products) on parameters of fermented hemp seed protein and expeller.*

Source	Dependent Variable	Mean Square	F	*p*
Different products (protein and expeller)	pH	0.030	171.429	0.0001
	TTA	0.008	3.704	0.090
	LAB	0.036	32.267	0.0001
	TPC	4.801	0.077	0.788
	RSA	23.773	1.835	0.213
	Digestability	113.837	50.816	0.0001
Different bacteria (*L. plantarum* and *L. paracasei*)	ph	0.270	1542.857	0.0001
	tta	2.168	1070.370	0.0001
	lab	0.011	9.600	0.015
	tpc	5678.055	91.327	0.0001
	rsa	149.743	11.561	0.009
	digestability	47.521	21.213	0.002
Product * Bacteria	pH	0.030	171.429	0.0001
	TTA	0.007	3.704	0.090
	LAB	0.007	6.667	0.033
	TPC	57.160	0.919	0.366
	RSA	36.019	2.781	0.134
	Digestability	644.747	287.811	0.0001

TTA – total titratable acidity; LAB – lactic acid bacteria count; TPC – total phenolic compounds content; RSA – radical scavenging activity.

Table-5 *Parameters of biscuits, produced by using hemp seed products.*

Samples	Color characteristics			Moisture content, %	Firmness, mJ	Overall acceptability
	L*	a*	b*			
BC	86.90±0.23	3.28±0.09	34.92±0.09	3.0±0.11	1.5±0.04	110±12
BNP	51.40±0.14	1.91±0.07	28.16±0.06	5.8±0.03	3.2±0.06	118±11
BNE	57.30±0.11	0.29±0.04	30.32±0.11	4.2±0.09	1.2±0.07	120±14
BLplP	47.79±0.20	2.77±0.10	31.46±0.21	10.4±0.06	1.8±0.03	70±10
BLplE	58.86±0.17	1.25±0.06	31.27±0.14	10.6±0.12	3.2±0.09	100±9
BLpaP	57.81±0.09	0.23±0.07	32.15±0.17	9.4±0.10	2.9±0.01	86±5
BLpaE	57.26±0.16	3.08±0.11	32.92±0.13	10.6±0.04	3.8±0.08	107±9
BLplPlm	72.20±0.12	2.40±0.12	23.27±0.09	5.2±0.06	1.8±0.07	125±11
BLplElm	69.47±0.10	0.01±0.01	34.71±0.25	6.4±0.10	1.4±0.04	132±6
BLpaPlm	57.46±0.13	3.16±0.02	32.98±0.18	6.8±0.12	2.0±0.05	80±5
BLpaElm	60.30±0.15	2.59±0.08	34.78±0.09	4.4±0.05	1.1±0.11	99±8

BC - control biscuit samples produced without hemp seed products; BNP and BNE - biscuit samples produced with nonfermented hemp seed protein and expeller, respectively; BLplP and BLplE - biscuit samples produced with fermented with *L. plantarum* hemp seed protein and expeller, respectively; BLpaP and BLpaE - biscuit samples produced with fermented with *L. paracasei* hemp seed protein and expeller, respectively; BLplPlm and BLplElm - biscuit samples produced with fermented with *L. plantarum* and immobilized in agar hemp seed protein and expeller, respectively; BLpaPlm and BLpaElm - biscuit samples produced with fermented with *L. paracasei* and immobilized in agar hemp seed protein and expeller, respectively.
L* - lightness; a* - redness; b* - yellowness.
Data expressed as a mean values (n = 6) ± SD; SD – standard deviation.
[a-d] Mean values within a column with different letters are significantly different ($p \leq 0.05$).

Table-6 *The influence of different factors (different LAB, different hemp seed products, and immobilization) on parameters of biscuits.*

Source	Dependent Variable	Mean Square	F	p
Different products (protein and expeller)	L*	1092.396	47873.938	0.0001
	a*	9.887	1635.493	0.0001
	b*	77.579	3491.700	0.0001
	Moisture content	2.455	8.680	0.002
	Firmness	1.317	417.258	0.0001
	Overall acceptability	750.964	8.310	0.002
Different bacteria (*L. plantarum* and *L. paracasei*)	L*	47.322	2073.854	0.0001
	a*	5.884	973.257	0.0001
	b*	37.873	1704.613	0.0001
	Moisture content	66.378	234.731	0.0001
	Firmness	1.531	485.117	0.0001
	Overall acceptability	2163.313	23,940	0.0001
Immobilization (immobilised or non)	L*	533.267	23370.247	0.0001
	a*	3.534	584.627	0.0001
	b*	0.421	18.964	0.0001
	Moisture content	124.215	439.261	0.0001
	Firmness	10.935	3464,330	0.0001
	Overall acceptability	1998.375	22.115	0.0001
Product * Bacteria	L*	7.068	309.743	0.0001
	a*	19.852	3283.728	0.0001
	b*	19.318	869.452	0.0001
	Moisture content	2.126	7.517	0.003
	Firmness	5.149	1631.181	0.0001
	Overall acceptability	277.813	3.074	0.066
Product * Immobilization	L*	40.638	1780.950	0.0001
	a*	1.799	297.502	0.0001
	b*	69.973	3149.373	0.0001
	Moisture content	2.535	8.965	0.007
	Firmness	4.860	1539.702	0.0001
	Overall acceptability	234.375	2.594	0.122
Bacteria * Immobilization	L*	391.961	17177.567	0.0001
	a*	14.183	2346.133	0.0001
	b*	15.553	699.994	0.0001
	Moisture content	0.135	0.477	0.497
	Firmness	1.215	384.926	0.0001
	Overall acceptability	3825.375	42.333	0.0001
Product * Bacteria * Immobilization	L*	110.811	4856.261	0.0001
	a*	15.601	2580.606	0.0001
	b*	50,460	2271.113	0.0001
	Moisture content	7.935	28.061	0.0001
	Firmness	0.0001	0.0001	1.000
	Overall acceptability	165.375	1.830	0.190
L* - lightness; a* - redness; b* - yellowness.				

Conclusions

Spontaneous rye sourdough is good source for LAB isolation, and isolated strains (*Lactobacillus plantarum* and *Lactobacillus paracasei*) showed versatile carbohydrate metabolism and resistance to low pH conditions. *Lactobacillus plantarum* and *Lactobacillus paracasei* could be used for hemp seed protein and expeller fermentation in order to increase their antioxidant activity. For hemp seed expeller protein digestibility increasing *L. plantarum* could be reccomended (digestibility increase 15.26%, in compare with nonfermented expeller samples). Fermented hemp seed products have significant influence on most of the biscuits parameters, and for biscuits value increasing with *L. plantarum* fermented and immobilazed hemp seed protein and expeller could be used.

Conflicts of Interest: None declared

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Author Contributions

V. Krungleviciute: lactic acid bacteria isolation and characterisation of ; P. Zavistanaviciute: cells immobilisation; E. Mozuriene: fermented products parameters evaluation; D. Baguckaite, V. Bauzyte, D. Budreckis, I. Kriksciunas, E. Cimbalaru, M. Domskis, E. Gudaityte: baking experiment; A. Santini: statistical analysis; E. Bartkiene: experimental design.

Abbreviations: TPC – Total phenolic compound; LAB – Lactic acid bacteria; PCR – Polymerase chain reaction; TTA – Total titratable acidity.

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