Preparation and characterization of novel flaxseed oil cake yogurt-like plant milk fortified with inulin

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Summary
This study aimed at investigating the suitability of flaxseed oil cake plant milk to produce an innovative highly bioactive yogurt-like beverage by using yogurt lactic acid bacteria (LAB). Three variants of beverage were prepared, namely, without inulin and supplemented with 5 $g \cdot l^{-1}$ and 10 $g \cdot l^{-1}$ of inulin. Viability of LAB, levels of polyphenolics, flavonoids, reducing sugars, lactic acid and radical-scavening activity were estimated. Also colour, rheological and particle size distribution measurements were performed. During storage at 6 °C for 21 days, viability of LAB was better than the recommended minimum level for yogurt (> 10^6 CFU·ml^{-1}). Fermentation improved the antioxidant activity, polyphenolics and flavonoids concentrations, whereas viscosity of the samples decreased. Inulin, added as a prebiotic, increased acidity and total polyphenolics concentration as well as enhanced survival of LAB. Besides the well-known positive properties of the raw matrix, fermentation allowed to obtain beverages with different features. Due to the functional and biochemical characteristics conferred to the yogurt-like flaxseed oil cake milk beverages, the use of yogurt cultures showed a potential for industrial application. These beverages could be used as a new, non-dairy vehicle for LAB consumption, in particular by vegetarians and lactose-intolerant consumers.

Keywords
fermentation; yogurt; plant milk; beverage; flaxseed oil cake

Increased consumption of fermented foods is a global trend [1–7]. Yogurt is one of the most popular fermented cows' milk-based products worldwide and has gained a widespread consumer acceptance as a health-promoting food. This is mainly due to a high content of live and active microorganisms, which are believed to play an important role in promoting health, with the counts of viable lactic acid bacteria (LAB) ranging between 10^6 CFU·ml^{-1} and 10^8 CFU·ml^{-1} [6–8]. However, consumer demand for cows' milk alternatives increased as a result of the increase in the diagnosis of lactose intolerance, allergies, cholesterol issues as well as following vegetarian or vegan diets or, among others, concerns about growth hormone or antibiotic residues in cows' milk, which has created a dramatic and fast-growing demand for plant-based foods [7, 9]. Plant-based products are perceived as health-promoting because they are rich in bioactive compounds and do not contain any dairy allergens or cholesterol that might prevent their use by certain segments of the population [10]. Plant „milks" are colloidal suspensions or emulsions consisting of the dissolved and disintegrated plant material that resemble cows' milk in appearance [3, 9]. Currently, consumers are interested in novel plant "milk"-based products including „organic", „biodynamic", „personalized" food, allergen-free, ready-to-eat products etc. [11]. Thus, food industry is stimulated to explore non-conventional raw materials or to use new technologies or processes with the aim to develop new „functional" products. Additionally, increased utilization of plant proteins in human diet will be necessary to meet nutritional requirements of the growing world population. Plant-based dairy analogues are one approach and the development of such products has attracted rising interest [7].

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In the literature, there are many reports about plant-based fermented beverages that can be obtained from plant non-conventional sources such as millet [12], white kidney beans [13], cashew [2], quinoa [3, 7], hazelnut [14], dandelion [8], ginger [6], tiger nut tubers [4], fruits [1] and vegetables [10]. Moreover, some of these plant products contain prebiotics (or can be easily fortified by them), which also provides the fermentation process rendering the raw material into a more palatable form with technological benefits, such as viscosity increase, and might have a synergistic effect on beneficial microflora survival during processing and storage [4, 9, 13, 14]. One of the most important prebiotics used in food industry is inulin. Inulin is a water-soluble storage polysaccharide belonging to a group of non-digestible carbohydrates called fructans [15]. Inulin has attained the Generally Recognized as Safe status (GRAS) and is extensively available in approximately 36 000 species of plants, among which chicory roots are considered the richest source of inulin [16]. The wide use of inulin in the food sector is based on its techno-functional attributes and because it meets a range of consumer requirements [15, 16]. It provides numerous nutritional and health benefits to humans including low caloric value, immunomodulatory activity, acting as dietary fibre, lowering the pH of intestine, providing assistance in relieving constipation and increasing stool load or rate. Inulin also enhances absorption of calcium, magnesium and iron. Moreover, it is known that inulin stimulates the growth of beneficial microflora, which helps in increasing the population of "good" bacteria in the colon, thus reducing the risk of gastrointestinal diseases [15–18].

Oil cakes or oil meals are by-products obtained after extraction of oil from seeds, which have a high nutritional value as their protein content ranges from 15 % to 50 %. Due to their high protein content, they are used as animal feed supplement, in particular for ruminants and fish [19]. Flaxseed oil cake (FOC) is a cheap by-product of flaxseed (*Linum usitatissimum* L.) oil pressing. It is a source of many bioactive substances such as proteins, fibre and lignans. Many studies reported about positive influence of flaxseed consumption regarding e.g. colon cancer prevention and reduction of the risk of cardiovascular diseases [20–23]. It is considered a "superfood" and GRAS and is a plant food that meets the needs of the 21st century consumers in terms of being rich in nutrients as well as in „bioactive" and „functional" ingredients [20]. In our previous work [24] it was proven that thermal processing led to significant reduction of cyanogenic compounds in FOC to a level that is safe for consumers. Moreover, FOC was found to be a suitable material to develop a non-dairy kefir-like fermented beverage characterized by high „bioactivity" and microorganisms viability during refrigerated storage for 21 days [24].

To the best of our knowledge, there has been no report about utilization of flaxseed oil cake obtained via cold pressing technique to produce milk-like extract used in the development of a fermented yogurt-like beverage. Thus, the aim of the present study was to produce a fermented beverage based on flaxseed oil cake „milk" (FOCM) with various contents of inulin, intended to serve as a prebiotic, by commercial yogurt starter cultures and to evaluate microbiological, rheological and physico-chemical properties of the product during refrigerated storage for 21 days.

MATERIALS AND METHODS

Materials and chemicals

Flaxseed oil cake (FOC) obtained via cold pressing technique was kindly donated by ACS (Bydgoszcz, Poland). According to manufacturer's information, the proximate composition of FOC was: solids content 80.5 %, protein content 42 %, carbohydrates 28 %, fibre 6.3 %, fat content 6.1 %, ash content 4.5 %.

Commercial yogurt starter culture "Zakwaska" consisting of *Streptococcus thermophilus*, *Lactobacillus delbruecki* ssp. *bulgaricus, Lactobacillus acidophilus* and *Bifidobacterium lactis* was obtained from VIVO-AKTIV (Browary, Ukraine).

All reagents (analytical grade) were purchased from Sigma Aldrich (St. Louis, Missouri, USA). Inulin (Beneo GR, from chicory roots) was obtained from Orafti (Oreye, Belgium). Buffered peptone water and de Man, Rogosa and Sharpe (MRS) agar were obtained from Merck (Darmstadt, Germany).

Preparation and fermentation of flaxseed oil cake "milk"

The preparation of FOCM consisted of the following steps. Firstly, FOC was mixed with distilled water in a ratio of 1:10 (w/w). Then, the mixture was heated at 90 °C for 1 h with constant stirring (4.2 Hz) and cooled down to room temperature. The extract was centrifuged (560 ×*g*) at 20 °C for 30 min (MPW-352R; MED Instruments, Warsaw, Poland). The supernatant was filtered (Whatman No 1 filter paper, Whatman, Maidstone, United Kingdom) under vacuum to obtain a clear, milky fluid (FOCM). FOCM was homogenized for 5 min with SilentCrusherM homoge-

nizer (Heidolph, Schwabach, Germany) at 200 Hz. After preparation, FOCM was dispensed into containers, pasteurized by heating at 60 °C for 30 min and cooled down in a refrigerator (6 °C) for one day before fermentation. Commercial yogurt starter culture "Zakwaska" was activated from lyophilized form (stored in refrigerated conditions at 6 °C) by mixing 0.5 g of the lyophilizate with 3 ml of buffered peptone water by vigorous vortexing and then was incubated at 37 °C for 30 min.

Yogurt-like beverages were produced in three variants, namely, without inulin (Y0), with 5 $g \cdot l^{-1}$ inulin (Y5) and with 10 $g \cdot l^{-1}$ inulin (Y10). A volume of 500 ml of FOCM was pre-heated to 42 °C, mixed with 1 ml of starter culture (1.3×10^8 $CFU \cdot ml^{-1}$) and fermented at 42 °C in 100 ml closed sterile plastic cups until pH was reduced to pH 4.8 (approximately 8 h). After fermentation, beverages were cooled down and stored at 6 °C for 21 days. Analyses were performed after 1, 3, 7, 14 and 21 days of storage. FOCM incubated at 42 °C and FOCM stored at 6 °C for 1 day served for comparison.

Determination of total solids content, pH and titratable acidity

Total solids content (*TSC*) was determined by drying the samples at 105 °C for 24 h.

The pH values of non-fermented and fermented samples was measured directly at 25 °C using a pH-meter CP-411 (Elmetron, Zabrze, Poland).

Titratable acidity (*TA*) determination consisted of mixing 5 g of a sample with 20 ml of distilled water and titration with 0.01 $mol \cdot l^{-1}$ NaOH solution, using phenolphthalein (1 $g \cdot l^{-1}$ in 95% ethanol) as an indicator [25].

Determination of viable lactic acid bacteria

During entire storage, samples of 1 ml were collected and diluted with 9 ml of sterile buffered peptone water, and serial dilutions were prepared [24]. Microbial counts were determined on MRS agar after incubation at 37 °C under anaerobic conditions for 72 h. The enumeration of microorganisms was performed in triplicate (by counting plates with 30–300 colonies) and the viable cell counts was expressed as logarithm of colony forming units per millilitre of the samples.

Colour analysis

The samples were measured for colour by a Konica Minolta CR-5 colorimeter with the Hunter Lab colour system (Konica Minolta, Osaka, Japan). Colour coordinates were expressed as: lightness (L^*), redness/greenness (+/– a^*) and yellowness/blueness (+/– b^*). Analyses were carried out at three independent times and presented as mean ± standard deviation.

Total polyphenolics, total flavonoids and reducing sugars concentration

To obtain clear fluids for analyses, the samples were transferred into 1.5 ml microtubes and centrifuged (1 683 ×*g*) at 20 °C for 10 min (Centrifuge 5418; Eppendorf, Hamburg, Germany). The supernatants of individual sample types were mixed and filtered through nylon membrane filters (pore size 0.22 μm, Merck). The obtained clear fluids were used for further analyses.

Total polyphenolics concentration (*TPC*) was determined by the Folin-Ciocalteu method, total flavonoids concentration (*TFC*) was determined by the aluminium chloride colorimetric assay and reducing sugars concentration (*RSC*) was determined by the colorimetric assay with 3,5-dinitrosalicylic acid as described in our previous study [24].

Radical-scavenging activity

The radical-scavenging activity (*RSA*; analysed as DPPH radical-scavenging activity) was determined spectrophotometrically at 517 nm against a blank [24, 26]. *RSA* was determined as a percentage of the DPPH radical inhibition with respect to the decrease in absorption of control using the formula:

$$RSA = \frac{(A_0 - A_x)}{A_0} \times 100 \quad (1)$$

where A_x is the absorbance of the solution containing the sample and A_0 is the absorbance of the control sample [26].

Rheological measurements and particle size distribution analysis

Rheological measurements were performed in a rheometer (AR G2; TA Instruments, New Castle, Delaware, USA). Flow properties of the samples at 20 °C were analysed using a stainless steel cone plate with a diameter of 62 mm. Steady-state flow measurements were carried out in the range of 0.1 s^{-1} to 100 s^{-1} and the rheological parameters were obtained by the TA Rheology Advantage Data Analysis equipment software V5.4.7.

The particles size distribution of the beverages was analysed by the use of the particle size analyser Mastersizer 2000 (Malvern Panalytical, Malvern, United Kingdom). The non-fermented and fermented samples (approximately 1 ml each) were dispersed in 800 ml of distilled water using a stirrer at 33.3 Hz at room temperature (20 °C) to

reach obscurance of 10.0 %. Optical properties of the samples were as follows: refractive index 1.500 and absorption 1.00. Droplet size measurements were reported as the volume-weighted mean diameter. Each sample was measured in triplicate.

Statistical analysis

All data were expressed as mean ± standard deviation (*SD*). Statistical significance was determined using analysis of variance (one-way ANOVA) followed by NIR Fisher test [27]. The values were considered as significantly different when $P < 0.05$. All statistical analyses were performed with Statistica version 10 (StatSoft, Tulsa, Oklahoma, USA).

RESULTS AND DISCUSSION

Changes in total solids content, pH and titratable acidity

Average values of *TSC*, pH and *TA* are summarized in Tab. 1.

After 1 day, *TSC* was found to be the highest in Y10 (26.8 ± 0.1 g·kg^{-1}) in comparison to *TSC* of FOCM (21.1 ± 0.4 g·kg^{-1}). During storage, the highest *TSC* value (32.7 ± 2.0 g·kg^{-1}) was noted after 7 days for Y5.

Fermentation significantly reduced pH of FOCM (5.95 ± 0.01) to the range from 4.38 ± 0.01 (Y10) to 4.44 ± 0.01 (Y0). Similar reduction in pH was reported for kefir containing flaxseed soluble fibre [22] as well as for kefir-like beverage produced from FOC [24]. Also, reduction in pH was reported for other fermented plant products [13, 25, 28]. The pH values of all samples decreased over the storage period and were statistically different ($P < 0.05$).

After 1 day of storage, *TA* values were determined to be 2.18 ± 0.01 g·l^{-1}, 2.33 ± 0.02 g·l^{-1} and 2.50 ± 0.01 g·l^{-1} for samples Y0, Y5 and Y10, respectively (statistically different at $P < 0.05$). Those values were much lower than in case of a standard yogurt (8–10 g·l^{-1} [14]). It was noticed that, during storage, the lactic acid concentration of all samples significantly increased. After 21 days of storage, lactic acid level reached 3.22 ± 0.05 g·l^{-1}, 3.34 ± 0.01 g·l^{-1} and 3.43 ± 0.03 g·l^{-1}, for samples Y0, Y5 and Y10, respectively (statistically different at $P < 0.05$). Our observations are comparable to the results of Bernat et al. [14] who used *Lactobacillus rhamnosus* GG to obtain fermented hazelnut "milk" fortified with inulin. The observed changes were expected to be due to the high viability of bacteria over storage time, which might still generate organic acids thus maintaining or increasing the acidity of fermented products [5, 6, 24]. Fodje et al. [21] demonstrated that high amounts of short-chain fatty acids (such as acetate and pro-

Tab. 1. Total solids content, pH and titratable acidity of flaxseed oil cake „milk" beverages.

	Time of storage				
	1 day	3 days	7 days	14 days	21 days
Total solids content [g·kg^{-1}]					
FOCM	21.1 ± 0.4^{b}	–	–	–	–
Y0	24.4 ± 0.8^{a}	22.4 ± 0.5^{a}	30.7 ± 1.0^{a}	22.4 ± 1.0^{a}	22.6 ± 1.0^{a}
Y5	24.2 ± 0.1^{a}	23.8 ± 0.3^{a}	32.7 ± 2.0^{b}	21.9 ± 0.1^{a}	24.7 ± 0.6^{a}
Y10	26.8 ± 0.1^{a}	20.3 ± 0.1^{b}	28.9 ± 1.0^{a}	25.8 ± 0.2^{b}	26.7 ± 1.0^{a}
pH					
FOCM	5.95 ± 0.01^{c}	–	–	–	–
Y0	4.44 ± 0.01^{a}	4.23 ± 0.00^{a}	4.31 ± 0.00^{a}	4.33 ± 0.01^{a}	4.41 ± 0.01^{a}
Y5	4.39 ± 0.01^{b}	4.16 ± 0.01^{b}	4.27 ± 0.01^{a}	4.20 ± 0.01^{b}	4.23 ± 0.01^{b}
Y10	4.38 ± 0.01^{b}	4.15 ± 0.01^{b}	4.22 ± 0.00^{b}	4.18 ± 0.01^{b}	4.21 ± 0.01^{b}
Titratable acidity [g·l^{-1}]					
FOCM	0.73 ± 0.01^{d}	–	–	–	–
Y0	2.18 ± 0.01^{a}	2.78 ± 0.05^{a}	2.97 ± 0.01^{a}	3.08 ± 0.02^{a}	3.22 ± 0.05^{a}
Y5	2.33 ± 0.02^{b}	2.99 ± 0.07^{b}	3.09 ± 0.06^{b}	3.20 ± 0.05^{b}	3.34 ± 0.01^{b}
Y10	2.50 ± 0.01^{c}	3.01 ± 0.01^{b}	3.11 ± 0.01^{b}	3.29 ± 0.06^{c}	3.43 ± 0.03^{c}

Values are mean ± standard deviation of triplicate determinations. Means with different letters in superscript in the same column are significantly different at $P < 0.05$.
FOCM – non-fermented flaxseed oil cake „milk" (control sample), Y0 – FOCM fermented without inulin, Y5 – FOCM fermented with 5 g·l^{-1} inulin, Y10 – FOCM fermented with 10 g·l^{-1} inulin.

Tab. 2. Counts of lactic acid bacteria during storage of flaxseed oil cake „milk“ beverages.

	Time of storage				
	1 day	3 days	7 days	14 days	21 days
Bacterial counts [log CFU·ml^{-1}]					
Y0	7.74 ± 0.00^{a}	7.74 ± 0.00^{a}	7.68 ± 0.00^{a}	7.21 ± 0.00^{a}	6.87 ± 0.04^{a}
Y5	7.88 ± 0.00^{b}	7.79 ± 0.00^{a}	7.93 ± 0.00^{b}	7.35 ± 0.01^{b}	6.83 ± 0.05^{a}
Y10	7.88 ± 0.00^{b}	7.93 ± 0.00^{b}	7.75 ± 0.00^{a}	7.59 ± 0.01^{c}	6.98 ± 0.01^{b}

Values are mean ± standard deviation of triplicate determinations. Means with different letters in superscript in the same column are significantly different at $P < 0.05$.
Y0 – flaxseed oil cake „milk“ fermented without inulin, Y5 – flaxseed oil cake „milk“ fermented with 5 g·l^{-1} inulin, Y10 – flaxseed oil cake „milk“ fermented with 10 g·l^{-1} inulin.

pionate) resulted from flaxseed fermentation. The inulin treatment resulted in a more acidified ($P < 0.05$) beverage than the control treatment because of saccharide metabolism by microorganisms. This behaviour had been reported previously by DOS SANTOS et al. [5], who evaluated a fermented soya “milk” with kefir cultures in the presence of inulin.

Survival of lactic acid bacteria during cold storage

As regards the survival of LAB, food matrix is considered one of the major factors regulating colonization, since it might help to buffer the microorganisms through the stomach or might contain other „functional“ ingredients (such as prebiotics) that could interact with them [14]. As can be seen in Tab. 2, the flaxseed „milk“ formulations, after thermal treatment, are an appropriate matrix to develop „functional“ non-dairy products, since LAB concentrations were meaningfully stable over the storage period. The bacterial counts were maintained in the product at the recommended level >10^6 CFU·ml^{-1}. These results are comparable with those of other authors using plant products to produce fermented beverages [1, 7, 8, 10]. The bacterial counts in variants supplemented with inulin (Y5 and Y10) were statistically different from the non-supplemented variant (Y0; $P < 0.05$). It is noteworthy that LAB were able to utilize FOCM in cell synthesis and lactic acid production without external nutrient supplementation in variant Y0. The fact that LAB in the fermented FOCM remained highly concentrated might be due to the prebiotic effect of flaxseed fibre and the added inulin [5, 14, 25]. Indeed, HADINEZHAD et al. [22] demonstrated that flaxseed soluble dietary fibre acts as a good prebiotic, enhancing LAB growth in a kefir model. High LAB viability was observed in hazelnut and almond “milks” [14, 25] fermented by yogurt cultures as well in soya milk fermented by kefir grains when inulin was added [5]. Another important factor determining microorganisms viability and their metabolic activity is the product storage temperature [24, 29]. Refrigerated storage generally increases the shelf life of fermented products and the survival of microorganisms, maintaining their viability [24, 29]. In fact, as the beverages were stored at a low temperature (6 °C), the refrigerated conditions were presumably one of the main factors providing high LAB counts over a 21-day storage. However, no differentiation of bacterial genera (lactobacilli, streptococci, bifidobacteria) and changes in their concentration during refrigerated storage was carried out. Therefore, no clear conclusion can be done in this concern.

Colour changes

Tab. 3 shows the colour parameters of non-fermented and fermented products stored in cold for different times. In comparison to FOCM ($L^* = 53.34 \pm 0.01$; $a^* = 2.12 \pm 0.00$; $b^* = 36.01 \pm 0.01$), colour values significantly changed after the fermentation process in all product variants ($P < 0.05$). This observation is partially in accordance with the results of previous study on kefir-like fermented beverage from FOC, where increase in lightness was observed, though decrease in a^* and b^* values was noticed [24]. These changes in colour coordinates can be attributed to a different level of opacity, which is related to the aggregation degree of particles [25]. It was noted that colour parameters were slightly affected during storage. Luminosity of Y0 and Y5 decreased after 21 days, whereas luminosity of Y10 increased. The results are partially in agreement with the study of LORUSSO et al. [3] who noted that the colour after fermentation (20 h) and cold storage (20 days) of quinoa flour yogurt-like beverages was dependent on the bacterial strains used for fermentation. The colour of fermented beverages is often related to the presence of pigments in the raw material, variations in storage time and pH that may affect the colour of fermented foods [5].

Tab. 3. Colour changes in flaxseed oil cake „milk“ beverages.

	Time of storage				
	1 day	3 days	7 days	14 days	21 days
L*					
FOCM	53.34 ± 0.01 c	–	–	–	–
Y0	72.02 ± 0.26 a	72.09 ± 0.03 a	71.68 ± 0.03 a	72.42 ± 0.03 a	71.60 ± 0.03 ab
Y5	72.04 ± 0.07 a	71.95 ± 0.03 b	70.30 ± 0.04 b	71.45 ± 0.04 b	70.99 ± 0.03 a
Y10	71.83 ± 0.05 b	72.17 ± 0.03 c	71.51 ± 0.05 c	71.77 ± 0.02 c	72.26 ± 0.03 b
a*					
FOCM	2.12 ± 0.00 b	–	–	–	–
Y0	1.24 ± 0.14 a	1.37 ± 0.01 a	1.33 ± 0.01 a	1.25 ± 0.02 a	1.29 ± 0.02 a
Y5	1.39 ± 0.04 a	1.39 ± 0.02 a	2.13 ± 0.02 b	1.39 ± 0.03 b	1.82 ± 0.04 b
Y10	1.34 ± 0.03 a	1.26 ± 0.04 b	1.46 ± 0.03 c	1.19 ± 0.03 c	1.34 ± 0.04 c
b*					
FOCM	36.01 ± 0.01 c	–	–	–	–
Y0	20.47 ± 0.06 a	21.22 ± 0.02 a	20.57 ± 0.02 a	20.27 ± 0.01 a	19.97 ± 0.01 a
Y5	20.97 ± 0.03 b	20.82 ± 0.03 b	21.26 ± 0.06 b	20.41 ± 0.03 b	20.59 ± 0.05 b
Y10	20.52 ± 0.03 a	20.79 ± 0.07 b	20.82 ± 0.01 c	20.30 ± 0.01 c	20.03 ± 0.01 c

Values are mean ± standard deviation of triplicate determinations. Means with different letters in superscript in the same column are significantly different at $P < 0.05$.
FOCM – non-fermented flaxseed oil cake „milk“ (control sample), Y0 – FOCM fermented without inulin, Y5 – FOCM fermented with 5 g·l^{-1} inulin, Y10 – FOCM fermented with 10 g·l^{-1} inulin.

Tab. 4. Radical-scavenging activity and „bioactive“ compounds in flaxseed oil cake „milk“ beverages.

	Time of storage				
	1 day	3 days	7 days	14 days	21 days
Reducing sugars concentration [mg·ml^{-1}]					
FOCM	5.17 ± 0.03 d	–	–	–	–
Y0	7.70 ± 0.00 a	8.26 ± 0.02 a	9.11 ± 0.02 a	10.07 ± 0.01 a	10.67 ± 0.01 a
Y5	8.43 ± 0.01 b	9.60 ± 0.00 b	10.62 ± 0.00 b	11.33 ± 0.00 b	12.28 ± 0.02 b
Y10	11.12 ± 0.01 c	11.23 ± 0.01 c	12.61 ± 0.00 c	13.06 ± 0.01 c	15.14 ± 0.01 c
Total polyphenolics concentration [mg·ml^{-1}]					
FOCM	9.23 ± 2.48 d	–	–	–	–
Y0	14.41 ± 0.11 a	10.83 ± 0.05 a	11.04 ± 0.05 a	10.93 ± 0.00 a	11.12 ± 0.05 a
Y5	11.60 ± 0.10 b	11.53 ± 0.21 b	12.82 ± 0.05 b	12.23 ± 0.05 b	13.41 ± 0.16 b
Y10	13.19 ± 0.05 c	10.86 ± 0.11 a	10.84 ± 0.11 a	10.42 ± 0.00 c	12.34 ± 0.11 c
Total flavonoids concentration [mg·ml^{-1}]					
FOCM	0.76 ± 0.01 d	–	–	–	–
Y0	0.82 ± 0.00 a	0.77 ± 0.01 a	0.79 ± 0.01 c	0.72 ± 0.01 c	1.23 ± 0.02 a
Y5	0.97 ± 0.01 b	0.80 ± 0.01 b	0.84 ± 0.01 b	0.74 ± 0.01 b	0.81 ± 0.01 b
Y10	1.40 ± 0.01 c	1.00 ± 0.00 c	0.78 ± 0.01 c	0.73 ± 0.00 c	0.82 ± 0.01 b
Radical-scavenging activity [%]					
FOCM	35.50 ± 0.10 c	–	–	–	–
Y0	96.92 ± 0.87 a	69.02 ± 1.34 a	79.04 ± 1.19 a	66.01 ± 0.88 a	61.05 ± 1.48 a
Y5	67.65 ± 0.75 b	80.03 ± 0.22 b	62.38 ± 0.45 b	63.03 ± 1.41 a	89.95 ± 0.68 b
Y10	60.19 ± 3.96 b	67.54 ± 5.07 a	38.40 ± 1.31 c	59.74 ± 0.56 a	60.30 ± 0.46 a

Values are mean ± standard deviation of triplicate determinations. Means with different letters in superscript in the same column are significantly different at $P < 0.05$.
Radical-scavenging activity is expressed as percentage of DPPH radical-scavenging effect. Total polyphenolics concentration is expressed as milligrams of gallic acid equivalents.
FOCM – non-fermented flaxseed oil cake „milk“ (control sample), Y0 – FOCM fermented without inulin, Y5 – FOCM fermented with 5 g·l^{-1} inulin, Y10 – FOCM fermented with 10 g·l^{-1} inulin.

Changes in reducing sugars concentration

As stated in Tab. 4, there was a significant increase in *RSC* after fermentation in comparison to *RSC* of FOCM (5.17 ± 0.01 mg·ml^{-1}). This observation is in line with our previous study [24]. It was noted that with increasing inulin concentration, *RSC* increased ($P < 0.05$). Throughout storage, the increasing tendency in *RSC* was noted for all product variants, being the highest on day 21 in Y10 (15.14 ± 0.01 mg·ml^{-1}). The increase in *RSC* may be linked to enzymatic hydrolysis of polysaccharides such as inulin and flaxseed fibre by LAB, to obtain energy required for growth, thus generating higher amounts of polysaccharide derivatives [13, 14]. This assumption is consistent with high bacterial survival and high acidity until the last controlled day. Similar observations were reported for fermented almond and hazelnut "milks" supplemented with inulin [14, 25]. On the contrary, in kefir-like beverages from FOC, the decreasing tendency in *RSC* was observed from day 4 of cold storage, which was linked to the consumption of saccharides by microorganisms. The differences may be linked to the different species composition of the microflora used for fermentation, because yogurt starter cultures were used in this study while kefir grains (containing LAB and yeasts) were used in the previous study [24].

Changes in total phenolics concentration, total flavonoids concentration and radical-scavenging activity

TPC, *TFC* and *RSA* values of the samples are presented in Tab. 4. All samples of fermented products had significantly higher *TPC*, *TFC* and *RSA* than non-fermented flaxseed oil cake "milk", which is in line with the results of our previous study [24]. This is comparable with results of other authors who reported high antioxidant activity of fermented plant beverages [8, 10]. In this study after day 1 of storage, the highest *TPC* and *RSA* values were recorded for Y0 (14.41 ± 0.11 mg·ml^{-1} (expressed as gallic acid equivalents) and 96.92 ± 0.87 %, respectively). It was observed that during cold storage there were fluctuations in *TPC*, *TFC* and *RSA* values in all samples. After 21 days, significant differences ($P < 0.05$) between samples and the highest values were observed for Y5. *TPC* and *RSA* of flaxseed oil cake are comparable to those of some medicinal plants, spice extracts and synthetic antioxidants commonly used in food products, such as butylated hydroxytoluene or butylated hydroxyanisole [22, 23].

Particle size distribution, particle diameter and rheological behaviour

The results of particle size distribution measurements (mean particle diameters $D_{3,2}$ and $D_{4,3}$) are presented in Tab. 5. It was found that the particles size distribution of fermented samples shifted to bigger sizes, reaching maximum values of $D_{3,2}$ and $D_{4,3}$ on day 7 of storage for Y0 and Y10, and on day 21 for Y5. It was observed that up to day 14 of storage, $D_{3,2}$ and $D_{4,3}$ values of Y0 showed a tendency to grow, whereas on day 21 a drastic decrease was observed. On the contrary, Y5 and Y10 showed tendency to increase $D_{3,2}$ and $D_{4,3}$, thus, the stability of the samples was significantly ($P < 0.05$) affected by the addition of

Tab. 5. Mean particle size of flaxseed oil cake „milk" beverages.

	Time of storage				
	1 day	3 days	7 days	14 days	21 days
Surface area mean $D_{3.2}$ [μm]					
FOCM	4.30 ± 0.04^{c}	–	–	–	–
Y0	4.71 ± 0.05^{a}	5.48 ± 0.07^{a}	5.82 ± 0.07^{a}	5.12 ± 0.08^{a}	4.11 ± 0.03^{a}
Y5	5.40 ± 0.11^{b}	5.78 ± 0.12^{b}	5.26 ± 0.09^{b}	5.44 ± 0.07^{a}	5.55 ± 0.34^{b}
Y10	4.91 ± 0.08^{a}	5.09 ± 0.08^{c}	5.31 ± 0.06^{b}	5.44 ± 0.36^{a}	5.20 ± 0.24^{b}
Volume moment mean $D_{4.3}$ [μm]					
FOCM	32.40 ± 0.05^{c}	–	–	–	–
Y0	35.52 ± 3.03^{a}	58.77 ± 4.22^{a}	64.64 ± 4.22^{a}	61.75 ± 2.69^{a}	22.64 ± 0.22^{a}
Y5	55.56 ± 5.06^{b}	58.40 ± 4.89^{a}	55.41 ± 5.58^{a}	55.99 ± 5.22^{a}	58.46 ± 6.78^{b}
Y10	38.25 ± 4.46^{a}	55.13 ± 6.23^{a}	62.90 ± 5.47^{a}	57.50 ± 5.01^{a}	53.43 ± 2.69^{b}

Values are mean ± standard deviation of triplicate determinations. Means with different letters in superscript in the same column are significantly different at $P < 0.05$.
FOCM – non-fermented flaxseed oil cake „milk" (control sample), Y0 – FOCM fermented without inulin, Y5 – FOCM fermented with 5 g·l^{-1} inulin, Y10 – FOCM fermented with 10 g·l^{-1} inulin.

Tab. 6. Rheological properties of flaxseed oil cake „milk“ beverages.

	Time of storage				
	1 day	3 days	7 days	14 days	21 days
Yield stress τ_y [10^{-4} Pa]					
FOCM	10.0 ± 0.1^{f}	–	–	–	–
Y0	35.6 ± 0.5^{a}	50.4 ± 0.1^{b}	21.6 ± 0.0^{c}	29.8 ± 0.3^{d}	9.3 ± 0.0^{e}
Y5	20.0 ± 0.4^{a}	20.3 ± 0.1^{b}	31.0 ± 0.3^{c}	2.1 ± 0.0^{d}	2.4 ± 0.1^{a}
Y10	14.7 ± 0.0^{a}	17.3 ± 0.1^{b}	5.9 ± 0.1^{c}	36.7 ± 0.1^{d}	5.1 ± 0.0^{e}
Apparent viscosity at shear rate 50 s^{-1} η_{50} [Pa·s]					
FOCM	0.05 ± 0.05^{f}	–	–	–	–
Y0	0.05 ± 0.01^{af}	0.04 ± 0.02^{b}	0.03 ± 0.05^{b}	0.02 ± 0.01^{c}	0.02 ± 0.01^{c}
Y5	0.04 ± 0.01^{c}	0.03 ± 0.02^{b}	0.03 ± 0.02^{b}	0.02 ± 0.02^{cf}	0.02 ± 0.02^{cf}
Y10	0.03 ± 0.01^{a}	0.03 ± 0.01^{a}	0.03 ± 0.02^{b}	0.03 ± 0.01^{a}	0.02 ± 0.02^{c}
Consistency index K [Pa·s^n]					
FOCM	0.08 ± 0.01^{f}	–	–	–	–
Y0	0.09 ± 0.00^{af}	0.07 ± 0.01^{bf}	0.05 ± 0.02^{c}	0.03 ± 0.02^{d}	0.03 ± 0.01^{d}
Y5	0.08 ± 0.01^{af}	0.05 ± 0.02^{b}	0.05 ± 0.01^{b}	0.04 ± 0.02^{c}	0.03 ± 0.01^{c}
Y10	0.06 ± 0.00^{a}	0.06 ± 0.02^{b}	0.04 ± 0.05^{c}	0.05 ± 0.01^{b}	0.03 ± 0.00^{d}
Flow behaviour index n					
FOCM	0.88 ± 0.01^{f}	–	–	–	–
Y0	0.78 ± 0.02^{a}	0.82 ± 0.01^{b}	0.86 ± 0.00^{cf}	0.91 ± 0.01^{d}	0.91 ± 0.01^{d}
Y5	0.79 ± 0.01^{a}	0.85 ± 0.02^{b}	0.86 ± 0.02^{cf}	0.89 ± 0.00^{df}	0.90 ± 0.04^{e}
Y10	0.81 ± 0.02^{a}	0.83 ± 0.03^{b}	0.87 ± 0.01^{cf}	0.84 ± 0.03^{bf}	0.90 ± 0.06^{d}

Values are mean ± standard deviation of triplicate determinations. Means with different letters in superscript in the same row are significantly different at $P < 0.05$.
FOCM – non-fermented flaxseed oil cake „milk“ (control sample), Y0 – FOCM fermented without inulin, Y5 – FOCM fermented with 5 g·l^{-1} inulin, Y10 – FOCM fermented with 10 g·l^{-1} inulin.

inulin. This can be probably assigned to a particle flocculation phenomenon associated with the system acidification, but also might be linked to formation of inulin microcrystals [5]. Similar findings were observed previously by Bernat et al. [25].

Rheological parameters of the samples are summarized in Tab. 6. The apparent viscosities at a shear rate of 50 s^{-1} are also shown. The shear-rated flow behaviour of the samples could be described with a Herschel-Bulkey model, which revealed a shear-thinning behaviour of both fermented and non-fermented product variants ($n < 1$) [14, 25]. As can be seen, the fermentation process modified the rheological behaviour of FOCM, significantly decreasing the apparent viscosity ($P < 0.05$) during storage. Although after day 1 the consistency index and viscosity increased in Y0 and Y5, a decreasing tendency was observed throughout cold storage. These observations are contradictory to results of Bernat et al. [14] who observed that viscosity of the fermented hazelnut „milk“ reached a maximum on day 21 of storage, although using xanthan gum as a stabilizer in their study. On the other hand, no changes in the apparent viscosity of fermented almond “milk” supplemented with inulin were observed in another study of Bernat et al. [25]. However, in the study of Lorusso et al. [3] apparent viscosity of quinoa yogurt-like beverage decreased after fermentation by *Lb. rhamnosus* and *Lb. plantarum*, whereas increased after fermentation by exopolysaccharide-producing *Weissella confusa*. The higher viscosity and consistency index after day 1 of storage may be due to the production of bacterial exopolysaccharides (EPS) [7]. Overall, the texture of food products is dependent on both the bacteria used for fermentation and process parameters, as some LAB can improve texture through production of metabolites to the matrix or through hydrolysis of the added fibres in foods during processing [30]. Thus, the breakdown of EPS as well as enzymatic hydrolysis of inulin and flaxseed fibre could be reasons of the reduction in viscosity of the samples [31]. Inulin is a water-structuring agent, hence acts as a thickener [17]. When inulin is added to food in low concentrations, the rheological properties of the product will not be affected strongly due to the limited effect on viscosity of

this ingredient [18]. However, inulin shows gelling properties at high levels (for standard chicory inulin >25 % and for long-chain inulin >15 %) and makes a gelling structure after shearing [16].

CONCLUSIONS

Taking into account the increasing complexity of the needs of different types of consumers, including vegan or vegetarian and subjects with intolerance or allergy to dairy products, an approach in this work was applied to obtain yogurt-like beverages from flaxseed oil cake „milk“, using commercial yogurt starter cultures containing LAB. LAB viability and antioxidant activity of fermented products were relatively high during the storage period, making the beverages of high added value which can be characterized as “functional food”. Also, the use of flaxseed oil cake offers a good alternative to traditional plant-based “milks”. It is also noteworthy that inulin, added as a prebiotic, increased acidity, total polyphenolics concentration as well as enhanced survival of LAB. The beverages produced in this work may help to link the gap between the actual and an ideal consumption of innovative plant products as well as beneficial microflora recommended in human diet. Another key point to be noted is that the bioprocess utilizing oil cakes is attractive due to their relatively cheaper availability throughout the year, making it even more favourable when economic issues are considered.

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