Properties of non-dairy gluten-free millet-based fermented beverages developed with yoghurt cultures

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Summary
The aim of the study was to develop a recipe of fermented millet-based beverage with the selection of the allergen-free starter (such as milk proteins, lactose and gluten) and to determine fermentation conditions, followed with evaluation of the beverages (survivability of the starter microflora, pH, organoleptic evaluation) during 28 days of storage at 6 °C. Using a typical yoghurt starter for production, it was possible to obtain an attractive fermented millet-based beverage. It was necessary to use glucose addition to initiate the fermentation process. There was a need of using food additives to obtain a drinking fermented millet-based beverage with the appearance and consistency desired by the consumers. Two mixtures of pectin and native maize starch were selected as the best additives to improve texture. The final product contained living cells of the starter microorganisms at a level of $\geq 10^6$ CFU·g^{-1} for a period of 28 days at 6 °C. The fermented millet-based beverage was positively evaluated by sensory evaluation by consumers, including vegetarians and people suffering from lactose intolerance.

Keywords
fermented millet; beverage; yoghurt culture; lactose-free diet; gluten-free diet

Millet (*Panicum miliaceum* L.) is one of the oldest known crops used in the human diet. It was probably used as the first cereal grain for the production of bread. Today, millet is on the sixth place in the ranking of the most important cereals in the world. Nowadays, the most important feature of this cereal is that it does not contain gluten, so it can be successfully used by people suffering from intolerance or allergy to gluten [1–3]. Millet can be used on a large scale for the production of plant-based beverages, or for the production of cereals and groats [4, 5]. Millet beverage can be easily obtained from millet [1]. Before consumption, millet grains require proper processing [5], which aims to improve the nutritional and sensory properties, including increased bioavailability of micronutrients and reduced content of antinutritive substances, such as phytic acid [6]. An interest in the development of dairy-free probiotic products is increasing due to the large number of consumers who are looking for different exotic flavours, increasing number of vegetarians, and also because some consumers do not tolerate lactose or are allergic to milk proteins. Grain products subjected to fermentation have a longer shelf life and better nutritional properties compared to the starting material [1]. The production of milk-free fermented beverages involves fermentation of plant materials (in case of vegetable beverages) with the addition of sugar, e.g. glucose or saccharose, by yoghurt bacteria *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus thermophilus* and/or probiotic strains

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of species such as *Lactobacillus acidophilus* or *Bifidobacterium* sp. Lactic acid bacteria are increasingly being used for the fermentation of functional plant-based products declared to have several additional health benefits for the consumers who aim to follow a healthy diet. Lactic acid bacteria additionally profile the flavour characteristics of fermented products as a result of their small but significant proteolytic and lipolytic activities [7, 8].

The aim of the study was to develop a recipe of fermented millet-based beverage with the selection of the allergen-free starter (such as milk proteins, lactose and gluten) and to determine fermentation conditions, as well as to determine viability of starter bacteria during 28 days storage period of the fermented millet beverage at 6 °C.

MATERIALS AND METHODS

Materials

Industrially produced millet beverage (Natumi, Troisdorf, Germany), without preservatives and without added sugar, containing only naturally occurring sugars, was used for the study. The beverage is made from millet, sunflower oil and sea salt. Only raw materials of organic origin from the European Union were used for its production (certificate No. DE-ÖKO-013). The product composition was as follows: water, millet (150 $g \cdot l^{-1}$), sunflower oil, sea salt. The nutritional value of the product in 100 g was as follows: energy value 226 kJ (54 kcal); fat 1.10 g (including saturated fatty acids 0.10 g; monounsaturated fatty acids 0.30 g; polyunsaturated fatty acids 0.70 g); carbohydrates 10.00 g (including sugars 5.50 g; fibre < 0.50 g); protein 0.70 g; salt 0.08 g. The beverage was characterized by a delicate taste, with a palpable grain flavour and a light yellow colour.

Selected lyophilized industrial cultures of lactic acid bacteria were used as the research material. The following available products were selected for the experiments:

- Ceska-Star Y 820, Ceska-Star Y 107, and Ceska-Star Y 106 (CSK Food Enrichment, Wageningen, The Netherlands; consists of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*),
- FD-DVS YC-X16 Yo-Flex and FD-DVS YC-380 Yo-Flex (Chr. Hansen, Hørsholm, Denmark; consists of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*),
- FD-DVS ABY-3 Probio-Tec (Chr. Hansen; consists of *Streptococcus thermophilus, Lactobacillus delbrueckii* subsp. *bulgaricus, Lactobacillus acidophilus* La-5, *Bifidobacterium animalis* subsp. *lactis* BB-12),
- Yo-Mix 495 LYO, Yo-Mix 499 LYO, Yo-Mix 511 LYO, and Yo-Mix 883 LYO (DuPont Danisco, Copenhagen, Denmark; consists of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*),
- Yo-Mix 205 LYO and Yo-Mix 207 LYO (DuPont Danisco; consists of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus, Lactobacillus acidophilus*, *Bifidobacterium lactis*),
- YO-A, YO-B, and YO-S (Mediterranea Biotechnologie, Termoli, Italy; consists of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*).

The following food additives were also used in the experiments: glucose and saccharose with food grade, modified or native preparation of tapioca or maize, preparations of pectin, carrageenan, agar, xanthan gum, guar gum, fibre from sugar beet, as well as chicory inulin, obtained from Ingredion (Westchester, Illinois, USA), Fibrex (Lublin, Poland), Culinar (Warsaw, Poland), Agnex (Białystok, Poland) and Cargill (Minneapolis, Minnesota, USA).

Preparation of beverages

The preparation of the beverage for fermentation consisted in supplying appropriate amounts of selected additives (stabilizer or a mixture of stabilizers or sugar), homogenizing the mixture and subjecting it to pasteurization at 80 °C for 5 min. After pasteurization, the beverage was cooled to 45 °C and a pre-made starter batch was added (the measured weight of the starter lyophilizate was reconstituted in 10 ml of sterile beverage). The beverage was thoroughly mixed and then poured into sterile 170 ml jars, sealed, and placed in an oven at 45 °C. Fermentation was carried out for a controlled period until the final pH value was obtained, usually for 4.5 h. Next, the fermented millet-based beverage was transferred to a refrigerator and stored at 6 °C for 28 days or shorter, depending on the experiment. The beverages were examined regarding survivability of the starter microflora, pH and organoleptic properties before starting the fermentation (after adding the starter culture), immediately after the fermentation, and then every 7 days.

Microbiological analysis

Microbiological analyses were carried out for several independent replicates. Each jar was opened only once and only at the moment of

sample removal. The analyses were performed at weekly intervals. As part of the analysis, independent determination of the number of viable lactobacilli and the number of viable lactic streptococci cells were carried out. The *Lactobacillus* agar medium according to de Man, Rogosa and Sharpe (MRS) at pH 5.7 (Merck, Darmstadt, Germany) was used to determine the total cell numbers of lactobacilli and bifidobacteria. Due to the presence of polysorbate, acetate, magnesium and manganese, this medium induces the growth of *Lactobacillus* spp. MRS agar is not fully selective and bifidobacteria are able to grow in it under anaerobic conditions. The M17 agar medium according to Terzaghi (Merck) was used to determine the numbers of viable *Streptococcus thermophilus* cells. Due to the presence of sodium-glycerol phosphate, this medium is a kind of buffering medium allowing the growth of lactic acid streptococci. This medium is used for qualitative and quantitative determination of lactic acid streptococci.

The media were prepared and sterilized according to the manufacturer's instructions. The diluent, buffered peptone water (Merck), was prepared in a similar manner. Inoculations of samples were made by droplet surface method on Petri dishes with prepared dried media in duplicate. The plates were incubated at 37 °C for 72 h. The cultures on M17 agar medium were carried out under aerobic conditions, whereas cultures on MRS agar medium under anaerobic conditions. Anaerobic conditions were obtained by using anaerobic jars and Anaerocult A system (Merck). After incubation, the grown colonies were counted and the results were converted into colony forming units per 1 g of the original beverage sample, and then expressed as decadic logarithm.

pH measurement

The pH value of tested beverages was determined using the pH meter LPH 330T (Elmetron, Zabrze, Poland). The results were read with an accuracy of 0.01 pH unit. The examination was performed in triplicate.

Organoleptic analysis

Organoleptic evaluation was carried out by a panel of potential consumers (32 women and 28 men, aged 18–60). Among consumers participating, 18 women declared a standard diet, 6 a vegetarian diet, 8 a lactose-free diet, while all men declared a standard diet. The appearance, consistency, taste and smell of fermented millet-based beverage samples were evaluated using quantitative descriptive method on a 5-point scale for each indicator (Tab. 1).

Nutritional and calorific values of beverages

The nutritional and calorific value of the final fermented millet-based beverages was calculated based on the selected recipe composition of fermented millet-based beverages and the chemical composition of the raw materials. According to the Regulation (EU) No 1169/2011 [9], the energy value to be declared shall be calculated using the following conversion factors: carbohydrate (except polyols) 17 $kJ \cdot g^{-1}$ (4 $kcal \cdot g^{-1}$), polyols 10 $kJ \cdot g^{-1}$ (2.4 $kcal \cdot g^{-1}$), protein 17 $kJ \cdot g^{-1}$ (4 $kcal \cdot g^{-1}$), fat 37 $kJ \cdot g^{-1}$ (9 $kcal \cdot g^{-1}$), salatrims 25 $kJ \cdot g^{-1}$ (6 $kcal \cdot g^{-1}$), alcohol (ethanol) 29 $kJ \cdot g^{-1}$ (7 $kcal \cdot g^{-1}$), organic acid 13 $kJ \cdot g^{-1}$ (3 $kcal \cdot g^{-1}$), fibre 8 $kJ \cdot g^{-1}$ (2 $kcal \cdot g^{-1}$), and erythritol 0 $kJ \cdot g^{-1}$ (0 $kcal \cdot g^{-1}$). Based on the recipe composition of fermented millet-based beverages, the content of the following nutrients was calculated: energy value (expressed as kilojoules per 100 g and/or kilocalories per 100 g), fat content, includ-

Tab. 1. Quality criteria of fermented millet beverages.

Score	Appearance	Consistency	Taste	Smell
1	water separation, curd precipitation	thin or ductile, not homogeneous	vegetable, gentian, off-taste	strange flavour, vegetable, gentian
2	water separation no more than 40 %, no curd precipitation	thin or ductile, homogeneous	moderately vegetable, slightly gentian, without foreign taste	slightly vegetable, tart, no off-flavour
3	water separation no more than 25 %, no curd precipitation	thin or thick, homogeneous, not ductile	moderately fermented, delicate taste, pure, without foreign taste	moderately sweet, without foreign flavour
4	water separation no more than 5 %, no curd precipitation	dense, insufficiently viscous, homogeneous, not ductile	insufficiently fermented, pure, without foreign taste	moderately fermented flavour, no off-flavour
5	homogeneous, without water separation, no curd precipitation	dense, viscous, homogeneous, not ductile	fermented, pure, without foreign taste	sufficiently fermented flavour, no off-flavour

ing saturated fatty acids, carbohydrates, including sugars, protein and salt (expressed as grams per 100 g).

Statistical evaluation

The results obtained by the analyses were statistically evaluated by one-way analysis of variance (ANOVA) in order to observe the effect of different formulations on quality parameters of fermented millet-based beverages. For comparison of parameters, Tukey's comparison test was used ($p < 0.05$).

RESULTS AND DISCUSSION

The food industry is increasingly developing towards the production of fermented foods. In the case of products for consumers suffering from food intolerance or allergies, it is essential that each component of the product is free of key allergens. Especially interesting are fermented plant beverages used as a substituents for fermented milk products. They represent a good source of beneficial microflora for lactose-intolerant people and for those suffering from allergy to milk proteins. We observed that in the case of fermented products, numerous bacterial starters are prepared using milk ingredients and finally may contain traces of them.

The first experiments consisted of carrying out the test fermentation of the millet-based beverage using starters. Highly promising results were obtained using starter cultures FD-DVS ABY-3 Probio-Tec, the FD-DVS YC-X16 Yo-Flex, the YO-S and the Yo-Mix 495 LYO. However, since the most important criterion of starter cultures selection was absence of the most important allergens (milk proteins, lactose and gluten), declared by the starter producer based on the available starter specifications, the DuPont starter Yo-Mix 495 LYO, with declared absence of the mentioned allergens, was selected for the study. This starter contains a typical microflora to produce yoghurts, i.e. two species of lactic acid bacteria, namely, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. This is a yoghurt culture that gives a very creamy, full-bodied yoghurt with high viscosity. The culture forms a moderate to strong aroma. This product contains saccharose and maltodextrin and it does not contain any of the 14 allergens requiring declaration (cereals containing gluten, crustaceans, eggs, fish, peanuts, soybeans, milk, nuts, celery, mustard, sesame, sulphur dioxide/sulphites, lupin, molluscs). The culture is marketed in the form of a lyophilized powder with the recommended time and storage temperature of up to 18 months at +4 °C. The recommended optimal fermentation temperature for this culture is in the range from 42 °C to 43 °C for less than 6.5 h, usually 3.5–4.5 h. As the culture is characterized by fairly broad technological parameters, we decided to determine the basic parameters of the technological cycle (final pH value, starter culture dose, fermentation time) based on the developed recipe.

Further experiments aimed to determine the dose and kind of sugar (glucose, saccharose)

Tab. 2. Changes in pH value of the fermented millet-based beverage during fermentation process with addition of glucose at a starter culture dose of 0.6 g·l^{-1}.

Incubation time [h]	pH			
	Glucose (20 g·l^{-1})	Glucose (30 g·l^{-1})	Glucose (40 g·l^{-1})	Glucose (50 g·l^{-1})
0.0	5.7 ± 0.1^{a}	5.7 ± 0.1^{a}	5.6 ± 0.1^{a}	5.6 ± 0.1^{a}
0.5	5.5 ± 0.1^{a}	5.6 ± 0.1^{a}	5.4 ± 0.1^{a}	5.5 ± 0.1^{a}
1.0	5.3 ± 0.1^{a}	5.3 ± 0.1^{a}	5.2 ± 0.2^{ab}	5.2 ± 0.2^{b}
1.5	5.0 ± 0.1^{b}	4.9 ± 0.1^{b}	4.9 ± 0.1^{bc}	4.9 ± 0.1^{c}
2.0	4.7 ± 0.2^{bc}	4.7 ± 0.1^{bc}	4.7 ± 0.1^{cd}	4.7 ± 0.1^{cd}
2.5	4.7 ± 0.1^{bc}	4.6 ± 0.1^{bcd}	4.7 ± 0.1^{cd}	4.6 ± 0.1^{cde}
3.0	4.6 ± 0.1^{c}	4.6 ± 0.1^{cd}	4.5 ± 0.1^{cd}	4.5 ± 0.1^{de}
3.5	4.5 ± 0.2^{c}	4.5 ± 0.2^{cd}	4.5 ± 0.1^{d}	4.4 ± 0.1^{de}
4.0	4.5 ± 0.1^{c}	4.4 ± 0.1^{cd}	4.4 ± 0.1^{d}	4.4 ± 0.1^{de}
4.5	4.5 ± 0.1^{c}	4.4 ± 0.2^{cd}	4.4 ± 0.1^{d}	4.3 ± 0.1^{e}
5.0	4.4 ± 0.1^{c}	4.4 ± 0.1^{d}	4.3 ± 0.1^{d}	4.3 ± 0.1^{e}

Values are expressed as mean ± standard deviation ($n = 3$). Values in the same column with the same letters in superscript are not significantly different at the p-value of 0.05.

Tab. 3. Changes in pH value of the fermented millet-based beverage during fermentation process with addition of saccharose at a starter culture dose of 0.6 g·l^{-1}.

Incubation time [h]	pH			
	Saccharose 20 g·l^{-1}	Saccharose 30 g·l^{-1}	Saccharose 40 g·l^{-1}	Saccharose 50 g·l^{-1}
0.0	5.7 ± 0.1^{a}	5.7 ± 0.1^{a}	5.7 ± 0.1^{a}	5.6 ± 0.1^{a}
0.5	5.6 ± 0.2^{a}	5.6 ± 0.1^{a}	5.6 ± 0.1^{a}	5.6 ± 0.1^{ab}
1.0	5.4 ± 0.1^{a}	5.4 ± 0.1^{a}	5.3 ± 0.3^{a}	5.3 ± 0.3^{b}
1.5	4.9 ± 0.1^{b}	5.0 ± 0.1^{b}	4.9 ± 0.1^{bc}	4.8 ± 0.1^{c}
2.0	4.6 ± 0.1^{bc}	4.8 ± 0.2^{bc}	4.7 ± 0.1^{cd}	4.6 ± 0.1^{cd}
2.5	4.5 ± 0.1^{cd}	4.7 ± 0.1^{bcd}	4.6 ± 0.1^{cd}	4.5 ± 0.1^{cde}
3.0	4.4 ± 0.1^{cd}	4.5 ± 0.1^{cde}	4.5 ± 0.1^{cd}	4.4 ± 0.1^{de}
3.5	4.4 ± 0.1^{cd}	4.4 ± 0.1^{cde}	4.4 ± 0.2^{cd}	4.4 ± 0.1^{de}
4.0	4.3 ± 0.1^{d}	4.4 ± 0.1^{de}	4.4 ± 0.2^{cd}	4.3 ± 0.1^{de}
4.5	4.3 ± 0.1^{d}	4.3 ± 0.1^{e}	4.3 ± 0.2^{d}	4.4 ± 0.1^{de}
5.0	4.4 ± 0.1^{cd}	4.3 ± 0.1^{e}	4.3 ± 0.2^{d}	4.2 ± 0.1^{e}

Values are expressed as mean ± standard deviation (n = 3). Values in the same column with the same letters in superscript are not significantly different at the p-value of 0.05.

added to the beverage to initiate the fermentation process, as well as to conduct sensory evaluation and measure the final pH value after fermentation under laboratory conditions. The beverages were enriched with saccharose or glucose at a level in the range of 20–50 g·l^{-1}. Addition of the starter at a level of 0.6 g·l^{-1} and fermentation at 43 °C for 5 h allowed to choose the level of sugar (saccharose or glucose) addition, which was very positively perceived in the sensory evaluation of the products. Data on the dynamics of acidification of millet-based beverage with the addition of glucose or saccharose are presented in Tab. 2 and Tab. 3, respectively. The experiments proved that the most beneficial taste and smell were obtained with the use of glucose rather than saccharose, and that a level of 30 g·l^{-1} glucose allowed to quickly, i.e. within a few hours, as is the case with the production of cows' milk yoghurts, obtain the final pH value of 4.6–4.7.

Further experiments aimed to determine the minimum starter level required to obtain an established pH value during fermentation, which is similar to that for the production of fermented milk beverages, i.e. up to 5 h. Based on the starting level of the Yo-Mix 495 LYO starter culture

Tab. 4. Changes in pH value of the fermented millet-based beverage during fermentation process with different starter culture doses at a glucose dose of 30 g·l^{-1}.

Incubation time [h]	pH				
	Starter culture 0.6 g·l^{-1}	Starter culture 0.2 g·l^{-1}	Starter culture 0.1 g·l^{-1}	Starter culture 0.05 g·l^{-1}	Starter culture 0.025 g·l^{-1}
0.0	5.7 ± 0.1^{a}	5.6 ± 0.1^{a}	5.6 ± 0.1^{a}	5.6 ± 0.1^{a}	5.6 ± 0.1^{a}
0.5	5.6 ± 0.1^{a}	5.6 ± 0.0^{ab}	5.6 ± 0.0^{ab}	5.6 ± 0.1^{ab}	5.6 ± 0.1^{ab}
1.0	5.3 ± 0.1^{a}	5.4 ± 0.2^{abc}	5.5 ± 0.0^{bc}	5.5 ± 0.1^{ab}	5.5 ± 0.0^{abc}
1.5	4.9 ± 0.1^{b}	5.2 ± 0.1^{abcd}	5.3 ± 0.0^{cd}	5.4 ± 0.1^{cb}	5.4 ± 0.1^{abcd}
2.0	4.7 ± 0.1^{bc}	5.1 ± 0.2^{bcde}	5.2 ± 0.1^{de}	5.3 ± 0.1^{cd}	5.4 ± 0.0^{bcd}
2.5	4.6 ± 0.1^{bcd}	4.9 ± 0.2^{cdef}	5.1 ± 0.1^{e}	5.2 ± 0.0^{de}	5.4 ± 0.2^{cd}
3.0	4.6 ± 0.1^{cd}	4.8 ± 0.2^{def}	5.0 ± 0.1^{ef}	5.1 ± 0.0^{ef}	5.3 ± 0.1^{de}
3.5	4.5 ± 0.2^{cd}	4.7 ± 0.2^{def}	4.8 ± 0.1^{fg}	5.0 ± 0.0^{fg}	5.2 ± 0.0^{ef}
4.0	4.4 ± 0.1^{cd}	4.7 ± 0.2^{ef}	4.7 ± 0.0^{gh}	4.9 ± 0.0^{gh}	5.0 ± 0.0^{fg}
4.5	4.4 ± 0.2^{cd}	4.6 ± 0.2^{ef}	4.6 ± 0.1^{gh}	4.8 ± 0.0^{hi}	4.9 ± 0.0^{gh}
5.0	4.4 ± 0.1^{d}	4.5 ± 0.3^{f}	4.6 ± 0.0^{h}	4.7 ± 0.0^{i}	4.8 ± 0.0^{h}

Values are expressed as mean ± standard deviation (n = 3). Values in the same column with the same letters in superscript are not significantly different at the p-value of 0.05.

Tab. 5. Description of samples of fermented millet-based beverages obtained with various structure-forming additives.

Additives / Mixtures	Dose range [g·l^{-1}]	Description of the beverage samples
Modified starch	1–4	Distinct delamination and heterogenity, too thin consistency, favourable taste
Modified starch	5–25	No delamination, consistency similar to the consistency of yoghurt from cows' milk, favourable taste
Native starches	1–5	No effect of thickening or strong delamination and clots formation
Native starches	5–25	No delamination, consistency resembled the consistency of yoghurt from cows' milk, favourable taste
Pectin	1–3	Clear delamination, thin consistency, heterogeneous structure, tasty
Pectin	4–5	Delamination, consistency slightly flocculent, tasty
Carrageenan	1–5	Too thick or too thin, lumpy consistency, off-taste
Agar	1–2	No thickening effect, sometimes light delamination and appearance of curds or clots
Agar	3–4	A semi-liquid, lumpy consistency, without delamination, sour taste
Agar	4–5	Too strong gel, hard consistency, delicate taste, slightly gentian
Guar gum	1–5	Not homogeneous consistency, very often delamination
Guar gum	5–10	Clear delamination or delamination and clots, too weak taste
Xanthan gum	1–5	No thickening effect or not homogenous consistency, very often delamination
Xanthan gum	5–10	Clear delamination or delamination and clots, too weak taste
Fibre from sugar beet or chicory inulin	1–10	Strong delamination and lack of any thickening effect
Agar and guar gum	1–10	Too dense consistency, pudding-like, no delamination, correct acidity, good taste
Agar and carrageenan	1–10	Nice, velvety consistency, correct acidity, too sweet
Native starch and carrageenan	5–35	Gel with a high degree of delamination, good acidity, good taste
Pectin, native starch and xanthan	5–35	Compact consistency, tasty, slightly sour
Pectin and native corn starch	5–35	Thick consistency, very favourable after mixing, no delamination, good acidity, pure taste, without foreign taste

ranging from 0.025 g·l^{-1} to 0.60 g·l^{-1} and measuring the pH values of fermenting beverages at intervals of 0.5 h, it was shown that the minimum starter level for a millet-based beverage was 0.05 g·l^{-1} (Tab. 4).

Further experiments concerned the development of the recipe composition of the mixture intended for the fermentation process of millet beverage. Twenty independent experiments were performed, in which, one day after the end of the fermentation process, acidity (pH) was measured and sensory evaluation of samples of fermented millet-based beverages obtained with various structure-forming additives and their doses was carried out. The final pH of the samples (one day after the end of fermentation) was lower than that measured immediately after the completion of the fermentation process, being in the range of 4.2–4.4. Most of the tested substances used alone did not cause the intended thickening of the beverage with a homogeneous structure without clots and precipitates. Different effects on appearance, consistency, taste and smell were noted for individual additives or their mixtures (Tab. 5). From the variants tested, the mixture containing pectin and native maize starch was selected for further experiments at a dose of 2% g·l^{-1} and 20 g·l^{-1}, respectively. The use of such the stabilizing mixture allowed to obtain the dense consistency of the product, which was a potable fermented millet beverage, without the effect of delamination and clots. Repetitions were made on the basis of the chosen recipe to check repeatability and reproducibility of the recipe composition. The obtained samples were subjected to a storage test and were analysed for sensory characteristics, one day after the end of fermentation, and in weekly intervals for 28 days of storage under refrigeration conditions (at 6 °C). The samples obtained were characterized by a thick consistency, velvety structure, light clotting density, slightly acidic good taste and a smell resembling natural cows' yoghurt. These

experiments demonstrated that the selected additives gained reproducible quality of the drinking fermented millet-based beverage.

Microbiological analysis

Fermentation of plant-derived beverages depends on the type of sugars found in the raw material from which the drink was obtained. Millet-based beverage is produced from millet, which, regarding its carbohydrates, is comparable to other popular cereals, such as rice, wheat or barley. It mainly contains starch [10–12]. In the case of a millet beverage fermented using the Yo-Mix 495 LYO starter, positive survival results of lactic streptococci were obtained (Tab. 6). As for the population of lactobacilli, it was significantly weak since the first few days of refrigerated storage of the product. The total cells number of microbial flora was greater than 10^6 CFU·g^{-1} for 28 days of refrigerated storage. The analysis proved that the developed recipe of fermented millet-based beverage allowed obtaining the product with a 28-day shelf life. The high population level of microorganisms used in its production for the whole shelf life is one of the indicators of the quality of such products and of their health-promoting properties.

Good viability of the microbial starter culture is the first and the most important criterion to ensure good quality and health-promoting properties of the product. The careful selection of starter cultures as well as of storage parameters is a guarantee of good organoleptic properties of the final product, which is determined by the metabolites of lactic acid bacteria produced during the fermentation process and during storage time [13, 14]. Several studies were devoted to the use of lactic acid bacteria for the fermentation of a millet product, including bread dough, porridge and beverages [15–19]. Less data are available on the survival of these bacteria in fermented millet beverages stored under refrigeration conditions, which makes it difficult to discuss our results. Kocková et al. [16] evaluated the stability of millet porridges fermented by the probiotic strain *Lactobacillus rhamnosus* GG at 37 °C during 10 h. They observed no lag phase during the fermentation of a 100 g·l^{-1} mixture of millet with water, which indicated that the bacteria did not need time to adapt to the new environment. After the fermentation process, the bacterial contents was at a level of 10^8 CFU·g^{-1}. During this time, pH of the samples changed from the initial 5.91 to the final 5.06, which meant a minor change considering the on-going fermentation process. In the case of our experiments, changes in acidity caused by the activity of microflora were much more intense. Walkowiak-Tomczak and Zielińska [20] studied the population of the *Lactobacillus plantarum* T106 strain during fermentation of beetroot juice and observed a significant increase in the population of bacteria during fermentation. However, it should be noted that beetroot juice has a different carbohydrate profile than millet. Ziarno and Zaręba [21] studied the viability of yoghurt bacteria in selected plant beverages based on soya, rice or coconut, which are, in terms of carbohydrate composition, more similar to millet than beetroot juice. Some researchers observed an increase in the bacterial population for a few first days of refrigerated storage, and later reduction in the population [21, 22]. Kocková et al. [16] observed a slight reduction of *Lb. rhamnosus* GG population during storage of fermented millet substrate (from the initial 7.83 log CFU·g^{-1} to the final 7.60 log CFU·g^{-1}) without any significant change in pH (within the range of 5.23–5.30). On this basis, we can suppose that the observed reduction of population of lactobacilli in our experiments could have been accelerated by the low pH of the fermented millet beverage samples.

A very low survival of lactobacilli in the observed samples and a fast decrease in their counts are interesting issues. In the case of other plant beverages, such as those based on soya, rice and coconut drink, this phenomenon was not observed [21, 23]. Also, the counts of lactic streptococci were not stable and significantly decreased during the time of storage in refrigeration conditions. Good survival of starter bacterial cells is reflected in longer shelf life of the product, therefore it is so important to determine an effect of yoghurt storage time on the survival of the microorganisms

Tab. 6. Changes in the population of starter bacterial microflora and pH value in fermented millet-based beverage samples during storage at 6 °C.

Storage time [d]	Streptococci	Lactobacilli and bifidobacteria	pH
	[log CFU·g^{-1}]		
1	8.3 ± 0.2^{a}	7.7 ± 0.2^{a}	4.2 ± 0.1^{a}
7	7.9 ± 0.3^{a}	5.6 ± 0.1^{b}	4.1 ± 0.1^{ab}
14	7.4 ± 0.2^{a}	3.4 ± 0.3^{c}	4.0 ± 0.1^{ab}
21	6.4 ± 0.2^{ab}	3.2 ± 0.1^{c}	4.0 ± 0.2^{ab}
28	6.1 ± 0.3^{b}	3.1 ± 0.3^{c}	3.9 ± 0.2^{b}

Values are expressed as mean ± standard deviation (n = 3). Values in the same column with the same letters in superscript are not significantly different at the p-value of 0.05.

contained in it. Initially, it was supposed that the reason for such poor survival of the starter bacteria was the low pH of the samples. Yoghurt bacteria are able to produce small amounts of lactic acid at low temperatures [16]. It can therefore be assumed that the trend observed in the conducted studies would be reversed as a consequence of the progressive acidity of yoghurts, since the development of lactic streptococci is suppressed at pH 4.2, while lactobacilli tolerate much lower pH values reaching 3.5–3.8 [24]. However, no strong decrease in pH was observed (Tab. 6). Initially, the pH value of fermented millet-based beverage samples was significantly lower at the end of the storage period than at its beginning, but these changes were not so radical as to explain the strong decrease in counts of lactobacilli. Millet is rich in polyphenolics, proteins and peptides with antimicrobial properties also against the Gram-positive bacteria [25, 26]. Perhaps the worse survival of lactobacilli than streptococci was the result of the total negative impact of antimicrobial substances derived from millet, low pH as well as refrigeration storage conditions of the samples. However, this requires further research. The observed changes in the pH value of the fermented millet-based beverages during the storage time could be the evidence that starter bacteria found more sugars in biochemical pathways useful for conversion into the energy required for the growth of the bacterial cells [27–29]. However, the extinction of starter bacteria present in the samples, in particular lactobacilli, contradicts this theory. A similar trend for pH value reduction in fermented beverages was noted by many researchers, and it is known that acidity is a factor important for bacterial cell viability [20, 29–31].

Organoleptic analysis

Products of plant origin can be fermented with the same effectiveness as dairy products, but the fermentation process allows to obtain a completely new range of products that are an excellent alternative to fermented food products of animal origin. Sensory features of food products, such as colour, smell, taste and consistency, are not only a determinant of quality but also play a key role in the choice of consumers. However, it should be noted that the taste, smell, colour and, in particular, the consistency of the products are usually subjected to deterioration during long storage. Sensory analysis carried out by four groups of consumers, including vegetarians and the indivisuals on a lactose-free diet, showed that, regardless of the panel assessing the samples of the fermented millet-based beverage, they were evaluated positively (Tab. 7).

During the refrigerated storage of fermented millet-based beverage samples, a gradual but statistically significant deterioration of consistency, taste and smell were observed (Tab. 8). The consistency became more fluid, described by many people as ‘too thin’ for a potable drink. Foreign tastes, bland, diluted, gentian taste, sour, tart or grassy odour appeared in the taste and smell of the samples.

Tab. 7. Organoleptic analysis scores of one day-old fermented millet-based beverages.

Panel group	*n*	Appearance	Consistency	Taste	Smell
Female, standard diet	18	4.9 ± 0.5^{a}	4.8 ± 0.4^{a}	4.1 ± 1.0^{a}	4.6 ± 0.6^{a}
Female, vegetarian diet	6	5.0 ± 0.0^{a}	5.0 ± 0.0^{a}	4.7 ± 0.5^{a}	4.8 ± 0.4^{a}
Female, lactose free diet	8	4.5 ± 0.8^{a}	4.6 ± 0.7^{a}	4.5 ± 0.5^{a}	5.0 ± 0.0^{a}
Male, standard diet	28	4.6 ± 0.7^{a}	4.7 ± 0.5^{a}	4.4 ± 0.8^{a}	4.7 ± 0.7^{a}

Values are expressed as mean ± standard deviation. Values in the same column with the same letters in superscript are not significantly different at the *p*-value of 0.05.

Tab. 8. Results of organoleptic analysis of fermented millet-based beverages during storage at 6 °C.

Storage time [d]	Appearance	Consistency	Taste	Smell
1	5.0 ± 0.0^{a}	5.0 ± 0.0^{a}	4.7 ± 0.5^{a}	4.9 ± 0.3^{a}
7	4.8 ± 0.4^{a}	4.8 ± 0.4^{ab}	4.3 ± 0.6^{ab}	4.8 ± 0.4^{a}
14	4.7 ± 0.5^{a}	4.8 ± 0.4^{ab}	4.0 ± 0.4^{ab}	4.6 ± 0.4^{ab}
21	4.7 ± 0.5^{a}	4.3 ± 0.5^{b}	4.0 ± 0.6^{b}	4.4 ± 0.5^{ab}
28	4.5 ± 0.5^{a}	4.3 ± 0.5^{b}	3.8 ± 0.5^{b}	3.8 ± 0.4^{b}

Values are expressed as mean ± standard deviation ($n = 12$). Values in the same column with the same letters in superscript are not significantly different at the *p*-value of 0.05.

Nutritional and calorific values

The nutritional value of millet grains in terms of proteins, carbohydrates and energy is comparable to other more popular cereals such as rice, wheat or barley. Millet contains 80–120 $g \cdot l^{-1}$ protein, 20–40 $g \cdot l^{-1}$ fat and 650–750 $g \cdot l^{-1}$ carbohydrates, mainly starch (over 550 $g \cdot l^{-1}$), and fibre (approximately 90 $g \cdot l^{-1}$) [5].

The recipe composition developed in the present experiments made it possible to calculate the nutritional and energy value of the fermented millet-based beverage. The calculated energy value of the fermented millet-based beverage was 293 kJ per 100 g (67 kcal per 100 g). Fat content was 1.1 g per 100 g, including saturated fat 0.1 g per 100 g. Carbohydrates content was 13.4 g per 100 g, including sugars 7.0 g per 100 g. Protein content was 0.7 g per 100 g. The calculated salt content was 0.09 g per 100 g. On the basis of the recipe ingredients, the beverage was free from any of the 14 allergens required to be declared by legislation, including gluten, milk proteins and lactose.

CONCLUSIONS

Using a starter containing typical lactic acid bacteria for yoghurt production, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, it is possible to obtain an attractive potable fermented millet-based beverage. The final product obtained on the basis of the developed recipe contained total microflora at a level of $> 10^6$ CFU$\cdot ml^{-1}$ for 28 days of storage at 6 °C. However, confirmation of the hypothesis explaining the intensive decrease of lactobacilli during the refrigerated storage requires further studies. Potable fermented millet-based beverage was positively evaluated in a sensory assessment carried out by four groups of consumers, including vegetarians and people with lactose-free diet. Gradual but statistically significant deterioration of consistency, taste and smell was observed during the refrigerated storage. The possibility of the use of flavour additives for the developed recipe of potable fermented millet-based beverage and the tendency of changes in selected quality traits during the refrigerated storage of the product should be examined in the future.

Acknowledgements

The authors disclose receipt of the financial support for the research, authorship, and/or publication of this article from the regional operational program 2014–2020 for Dolnośląskie Voivodeship (RPO WD 2014–2020) in Poland within the frame of the European Regional Development Funding (EFRR).

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Received 14 May 2018; 1st revised 31 July 2018; 2nd revised 13 August 2018; accepted 21 August 2018; published online 4 December 2018.