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GLUCOSE CONSUMPTION AND LACTIC ACID FORMATION IN MILLET SOURDOUGH FERMENTED WITH DIFFERENT STRAINS OF LACTIC ACID BACTERIA

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ABSTRACT

The consumption of glucose and production of lactic acid by selected autochtonous strains of facultatively heterofermentative *Lactobacillus coryniformis*, *Pediococcus pentosaceus* and *Pediococcus acidilactici* species, in millet sourdoughs was studied. Glucose consumption and lactic acid concentration were analyzed after 24 h, 48 h and 96 h of sourdough incubation. They varied significantly depending on the strain. The highest production of lactic acid in all fermentation steps was found in sourdough fermented with *Pediococcus acidilactici* strain, whereas the lowest amount of this metabolite was found in sourdoughs fermented with *Pediococcus pentosaceus* ZFP5. Lactic acid concentration decreased in the successive fermentation steps and after 96 h of sourdoughs fermentation its level was of about 44 % lower than after 24 h of incubation. Glucose was completely consumed in all samples after 96 h of fermentation.

Keywords: lactic acid, millet, sourdough, *Lactobacillus coryniformis*, *Pediococcus pentosaceus*, *Pediococcus acidilactici*

INTRODUCTION

The increasing demand for baked products with a high nutritional value or health benefits is raising the need for the use of alternative (non-wheat) cereals like rice, maize, millet, and sorghum in the baking industry. The alternative cereals have received extensive scientific and technological attention due to better health-promoting composition, especially regarding minor components present in grains (dietary fiber, resistant starch, minerals, vitamins, phenolic compounds) (**Coda *et al.*, 2014**). The greatest advantage of the alternative cereals is a lack of gluten, the causative agent for celiac disease (**Moroni *et al.*, 2009**).
Among the alternative cereals, millet is one of the oldest cultivated plants. In ancient times, it was prevalent in Asia, Africa and central regions of Europe. Millet is unique due to its short growing seasons and its capability of producing good yields of grain under conditions unfavorable to most other cereals. Millet is desirable in man's diet because it is easily digestible and is a rich source of various macroelements including calcium, potassium, magnesium, sodium and valuable unsaturated fatty acids. Moreover, millet contains B vitamins, especially niacin, B6 and folic acid (**Verma *et al.*, 2013**). In terms of protein content and fat, millet grains outweigh popular oat and buckwheat grains. The energy of millet grains is similar to that of rice, buckwheat, but their nutritional values are higher. Effects of millet proteins are comparable to the biological value of proteins of wheat, maize, and beans. Owing to its valuable nutritional values, millet has received a renewed interest among consumers of the European countries. It can be used in several ways, e.g., it may be ground into flour and made into a host of different non-yeast breads. Millet grains and flour are commonly used for making fermented products. Traditional foods prepared with natural fermentation of millet are: gruels, porridges, soups, and fermented beverages. Millet flour can also be used for sourdough preparation. However, the use of the millet-based sourdough in the baking industry is not well standardized. Millet, like the other alternative cereals, is characterized by a low baking quality and final sensory quality (**Gallagher *et al.*, 2004**). These disadvantages limit the use of such flour in the bread making process. One of the ways to overcome these problems is the improvement of fermentation technology, with the main challenge being the design of well-defined starter cultures with functional features for sourdough preparation. It has been reported that lactic acid bacteria fermentation of alternative flours has a relevant influence on sensory as well as baking qualities, providing final products with desirable properties (**Coda *et al.*, 2010**). The huge benefit of the consumption of the alternative flour-based bakery products on health together with increasing demand for healthier products affords researchers the opportunity to find new microbial biodiversity for food processing. Therefore, an increasing number of studies has been recently observed that address the lactic acid bacteria (LAB) microbiota of alternative cereals. On the basis of these studies, it has been found that the selected autochthonous lactic acid bacteria are one of the best candidates to ferment the sourdough. Sourdough fermentation with such species leads to an increased value of *in vitro* protein digestibility and to improvement of the nutritional and sensory potential of non-wheat grains (**Coda *et al.*, 2011; Sterr *et al.*, 2009**). It was also observed that the type of cereals plays an important role in starters selection (**Moroni *et al.*, 2010**). During the selection of the proper starter cultures, consideration should also been given to the sensory characteristics of final products. Therefore, studies on the biochemical pathways of lactic acid bacteria leading to the formation of flavor compounds and their precursors are necessary.
The objective of our study was to evaluate the behavior of the selected facultatively heterofermentative lactic acid bacteria strains i.e.: two different strains of *Lactobacillus coryniformis*, three strains of *Pediococcus pentosaceus* and one strain of *Pediococcus acidilactici* in millet sourdoughs. In particularly, the production of lactic acid by the selected strains was investigated.

MATERIAL AND METHODS

Microorganisms

Six lactic acid bacteria strains (LAB), previously isolated form homemade millet sourdoughs, were used in this study: *Lactobacillus coryniformis* ZFP1 and ZFP4; *Pediococcus pentosaceus* ZFP2, ZFP3 *and* ZFP5*; Pediococcus acidilactici* ZFP6. These strains were identified by 16S rDNA sequencing. LAB were grown anaerobically in MRS medium at 30 °C.

Sourdough preparation and fermentation

Millet flour (Bio Babalscy, Poland) was used for fermentation of sourdoughs. Sourdoughs were prepared by mixing sterile tap water and whole meal flour of millet in a 1:1 (w/w) ratio. The dough yield was 200. The fermentations were carried out at 25 °C for 24h. Sourdoughs were inoculated with 0.5% of starter culture (10^{10} cfu/g dough). The fermentations were continued with 3 back-sloppings (once every 24 h) at 25 °C using 10% of the ripe sourdough as the inoculum. After 24h of initial fermentation and at the 1st and 3rd refreshment step, samples were taken from the ripe sourdough and analysed. An uninoculated (control) sourdough was prepared under the same conditions. Data were obtained from two independent sourdough fermentations.

Lactic acid and glucose analysis in sourdough

All chemicals used in the study were from Sigma-Aldrich. The chemicals were all of analytical grade.
Prior to analyses, dough extracts were treated as described previously (**Robert *et al*., 2006**) with some modifications. Sourdough was homogenized with redistilled water (1:7). Then, the solution was stirred with 2.5 mL of Carrez I solution and 2.5 mL of Carrez II solution and was centrifuged for 3 min at 150 rpm. The supernatant was filtered through a 0.45 µm filter (Membrane Solutions, USA) prior to analysis.
Glucose and lactic acid were quantified by HPLC apparatus (Gilson, Inc. Meddleton, USA) with an Aminex 87HP column (300 mmx 7.8 mm, Bio-Rad, Mississauge, Canada) at a temperature of 20 °C and a flow rate of 0.6 mL min^{-1} with H_2SO_4 (pH = 3.4) as the eluent. The quantification was based on a refractive index detector and performed with external standards in duplicate. The results were expressed as mean values. Errors are represented as standard deviations.

Statistical analysis

Data were compared by Tukey's test. Statistical significance ($p<0.05$) was determined with STATISTICA (Statsoft) software.

RESULTS AND DISCUSSION

The selected autochthonous LAB strains of *Lactobacillus coryniformis*, *Pediococcus pentosaceus* and *Pediococcus acidilactici* applied in this study are facultatively heterofermentative microorganisms that degrade mainly hexoses via the Embden-Meyerhof-Parnas (EMP) pathway and the lactic acid is the major end product (Gänzle et al. 2007). *Pediococcus pentosaceus* and *Pediococcus acidilactici* are important LABs involved as starter cultures in meat, vegetable and dairy fermentation and causing characteristic flavor changes, improving hygienic quality and extending the shelf life of several products (Irmler et al. 2013). *Lactobacillus coryniformis* has been recently reported to display a variety of potential probiotic properties, to be characterized by a strong antifungal activity and potential to be used as a biopreservative in feed systems (Magnusson, Schnurer, 2001; Sekwati-Monang et al. 2012).
Glucose consumption and lactic acid formation in millet sourdoughs are depicted in Table 1.

Table 1 Glucose utilization and lactic acid formation after 24 h, 48h (1st refreshment) and 96 h (3rd refreshment) of millet sourdough fermentation. Sourdoughs were inoculated with approximately 10^{10} cfu/g of each of the six strains, incubated at 25 °C and back-slopped every 24 h with 10% inoculum. Results are shown as means ± standard deviation of duplicate independent experiments analyzed in duplicate.

Strains	Incubation time [h]	Glucose utilization [mmol kg^{-1}]	Lactic acid formation [mmol kg^{-1}]
L. coryniformis ZFP1	24h	8,5 ± 0,44 a B	130,4 ± 5,41 a F
	48h	46,4 ± 1,09 b C	116,0 ± 8,13 b D
	96h	n.d.	65,8 ± 1,33 a AB
L. coryniformis ZFP4	24h	18,4 ± 0,61 b D	99,9 ± 3,49 b D
	48h	n.d. A	87,3 ± 2,18 a C
	96h	n.d.	65,5 ± 1,08 a AB
P. pentosaceus ZFP2	24h	n.d. A	89,0 ± 2,50 a C
	48h	63,2 ± 0,20 D	106,0 ± 2,72 a AB
	96h	n.d.	54,2 ± 3,37 a BD
P. pentosaceus ZFP3	24h	47,7 ± 2,90 a G	115,4 ± 5,01 b E
	48h	n.d. A	105,4 ± 1,08 a AB
	96h	n.d.	70,3 ± 3,81 b AC
P. pentosaceus ZFP5	24h	26,3 ± 0,75 b E	76,0 ± 3,12 c B
	48h	n.d. A	67,2 ± 1,54 b E
	96h	n.d.	41,9 ±1,46 a D
P. acidilactici ZFP6	24h	14,7 ± 1,29 C	180,9 ± 5,71 G
	48h	16,1 ± 0,21 B	113,5 ± 4,15 BD
	96h	n.d.	77,8 ± 3,81 AC
control (uninoculated dough)	24h	35,9 ± 0,80 F	52,8 ± 1,94 A
	48h	n.d. A	96,7 ± 2,98 AC
	96h	n.d.	79,7 ± 7,73 C

n.d.-not detect
*Means between strains of the same LAB species followed by the different small letters and means between the different LAB species followed by the different capital letters are significantly different at $p < 0,05$ (Tukey test)

In the studied millet sourdoughs, glucose was the dominant utilized carbohydrate. The consumption of glucose, as a major carbon source, was observed in sorghum sourdough (Sekwati-Monang et al. 2012). After the first 24 h of fermentation, the level of glucose varied depending on the LAB strains used. Moreover, statistically significant differences were observed between strains of the same LAB species (Table 1). In case of *P. pentosaceus* strains ZFP3 and ZFP5, the level of glucose was 47.7 and 26.3 mmol kg^{-1}, respectively, whereas no glucose was detected in sourdough fermented with *P. pentosaceus* ZFP2. Significantly lower amounts of glucose were found in sourdoughs inoculated with *L. coryniformis* ZFP1 and ZFP4 (8.5 and 18.4 mmol kg^{-1}), and *P. acidilactici* (14.7 mmol kg^{-1}). Glucose level in the spontaneously fermented millet dough was relatively high (35.9 mmol kg^{-1}), compared to the inoculated samples.
The amount of synthesized lactic acid, after 24 h of fermentation, ranged between 76.0 and 180.9 mmol kg^{-1}, depending on the strain used. The highest amount was detected in the sourdough fermented with *P. acidilactici* ZFP6 (180.9 mmol kg^{-1}), whereas the lowest was found in sourdough with *P. pentosaceus* ZFP5 (76.0 mmol kg^{-1}).
After 48 h of incubation (1st refreshment), a successive decrease of lactic acid was observed in almost all sourdoughs, except the sample inoculated with *P. pentosaceus* ZFP2 and the non-inoculated one. The differences in the amount of produced lactic acid between the strains of the same LAB species as well as between different LAB species diminished and in few cases became statistically less significant. No significant differences were observed between the sourdoughs fermented with *P. pentosaceus* ZFP2 and *P. pentosaceus* ZFP3. However, significantly lower amount of lactic acid was synthesized in sourdough fermented with *P. pentosaceus* ZFP5. Compared to the other millet sourdoughs, *L. coryniformis* ZFP1 and *P. acidilactici* ZFP6 exhibited the highest level of lactic acid production.
Considering glucose consumption after 48h of incubation, its level varied between the samples and in case of the control and sourdough fermented with *L. coryniformis* ZFP4, *P. pentosaceus* ZFP3, and *P. pentosaceus* ZFP5 was not detected. In other samples, its level was higher compared to the samples obtained after 24h of incubation.
During 96 h of sourdough fermentation glucose was completely consumed in all the studied samples. The level of lactic acid ranged from 41.9 mmol kg^{-1} to 79.7 mmol kg^{-1}, and was of about 44% lower than after the first 24h of fermentation. No statistically significant differences were observed between the sample inoculated with strains of the *L. coryniformis* species as well as between the *P. pentosaceus* ZFP2 and *P. pentosaceus* ZFP5.
Carbohydrate metabolism of LAB and formation of lactic acid during sourdough fermentation is a very complex process. The nature of sourdough microbiota as well as the type of flour used are one of the main factors determining the metabolite kinetics of sourdough fermentation. The metabolic activity of various

LAB species and their interaction with other sourdough microorganisms are flour specific. The unique chemical composition of wheat, rye and non-wheat flours affect the observed varying behavior of the species participating in the fermentation process. Moreover, the observed differences in the carbohydrate metabolism are not only between the strains of various LAB species but also between the strains of the same LAB species. In our study, statistically significant differences in glucose and lactic acid level between the applied selected LAB starters were detected predominantly after 24h of fermentation. During the next fermentation steps, the differences diminished and finally at the end of fermentation were almost comparable. The observed behavior of the applied autochthonous starters results probably from their interaction and competitive activities with other millet sourdough microorganisms.

CONCLUSION

The present study has shown varied behavior of selected autochthonous LAB starters during millet sourdough fermentation. Among the used starters, *P. acidilactici* ZFP6 exhibited the highest level of lactic acid production in all fermentation steps, compared to the other inoculated sourdoughs. Whereas, the lowest amount of this metabolite was found in sourdoughs fermented with *Pediococcus pentosaceus* ZFP5.
The obtained results will be a useful tool to better understanding of the carbohydrate metabolism of LAB in the millet sourdough and to the further studies on design of proper LAB starter cultures for millet sourdough preparation.

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