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ORIGINAL RESEARCH PAPER

CHARACTERIZATION OF MILLET AND BUCKWHEAT SOURDOUGHS FERMENTING WITH AUTOCHTHONOUS PEDIOCOCCUS PENTOSACEUS STRAINS

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Pediococcus pentosacues strains, isolates from naturally fermenting millet and buckwheat sourdoughs, were used as starters for millet and buckwheat sourdoughs fermentation in order to evaluate their metabolic activity in native sourdough environment. For comparison, fermentation of studied non-wheat sourdoughs with a wheat isolate was done. Generally, sourdoughs fermented with autochthonous isolates are characterized by higher value of TTA and lactic acid compared to sourdoughs fermented with wheat isolates. The observed differences in TTA value between the sourdoughs prepared with autochthonous pediococci and the ones with wheat isolate were more pronounced in the case of millet sourdoughs. Glucose concentration and lactic acid production varied depending on the used pediococci strains and fermentation time.

Keywords: fermentative end-product; residual sugar; lactic acid bacteria; bacterial starter monoculture; gluten free sourdough

Introduction

The use of non-wheat flours in baking industry has recently been one of the most popular trends on the food market. High nutritional value and health benefit, especially the lack of gluten, made them an attractive alternative to wheat. The group of gluten-free (GF) flours consists of flours produced from alternative cereals such as millet, rice, maize as well as pseudocereals such as buckwheat and quinoa. One of the major problems, limiting the use of non-wheat flours, is their low baking quality. It was reported that the fermentation of GF flours may contribute to the improvement of the digestibility, baking performance and organoleptic quality of the baked goods (Vogelmann *et al*., 2009; Moroni *et al*., 2012). The non-wheat sourdough technology required the use of functional starter

cultures for ensuring a constant quality of sourdough (Moroni *et al*., 2010). The selection of the starter strains is determined by their adaptability to the given environment and the ability for survival and dominance over the native microbiota (De Vuyst *et al*., 2009). The commonly used commercial starters are often limited to wheat sourdoughs. Their use for fermentation of non-wheat sourdough was not always successful (Moroni *et al*., 2010). Hence, the application of autochthonous LAB as culture starters for non-wheat sourdoughs was proposed (Vogelmann *et al.*, 2009; Sekwati-Monang *et al.*, 2012). The autochthonous strains are more suitable for use as functional starters, due to their better adaptability to this special environment (Minervini *et al*., 2010).

Studies on the characterization of the microbiota of spontaneously fermenting buckwheat, teff and sorghum sourdoughs allowed for the identification of numerous lactic acid bacteria, belonging mainly to the genera *Lactobacillus, Pediococcus, Leuconostoc.* The selected isolates, mostly from genus *Lactobacillus*, were further employed as starters, and their growth, metabolism and competitiveness in specific sourdough environments were investigated (Moroni *et al*., 2011; Sekwati-Monang *et al*., 2012; Ogunsakin *et al*., 2015). Some studies dealt with the characterization of different non-*Lactobacillus* species belonging to *Leuconostoc*, *Weisella*, *Pedioccocus* and the provided results showed their potential in sourdough fermentation (Settani *et al.*, 2013; Gerez *et al.*, 2006). Moreover, the research done by Gerez *et al.* (2006) revealed that the studied *Pediococcus pentosaceus* strains exhibited proteolytic activity on gluten, during growth in gluten based medium.

Among the non-*Lactobacillus* species, *Pediococcus pentosaceus* has attracted increasing interest due to its probiotic activity. The pediococci strains are able to produce antimicrobial inhibitory compounds (bacteriocins) and, therefore, are widely employed for food preservation. They are commonly used as starter culture in meat, vegetable and dairy fermentation contributing to the specific organoleptic characteristics, and improving hygienic quality of products. Pediococci stranis are not frequently encountered in wheat sourdough ecosystems (De Vuyst *et al*., 2009). They are more often found in non-wheat based traditional fermented products such as Sudanese khamir (Gassem, 1999).

The objective of this study was to evaluate the potential of selected *Pediococcus pentosaceus* strains for non-wheat sourdough fermentation. For this purpose, the metabolic activity of millet and buckwheat isolates, in terms of acidification and carbohydrate utilization, in their native sourdough environment, was investigated. Additionally, the metabolic activity of the selected wheat isolates in the studied millet and buckwheat sourdoughs was investigated.

Materials and methods

Microorganisms

Three lactic acid bacteria strains (LAB) of *Pediococcus pentosaceus* genus: *Pediococcus pentosaceus* W, *Pediococcus pentosaceus* M, *Pediococcus*

pentosaceus B, previously isolated form naturally (laboratory-scale) fermenting wheat (W), millet (M) and buckwheat (B) sourdoughs, were used as starters in this study. These strains were previously identified by 16S rDNA sequencing (Zawadzka-Skomiał *et al.*, 2009). The LAB strains were grown anaerobically in MRS medium at 30 °C.

Sourdough preparation and fermentation

Millet and buckwheat flours obtained from ecological cultivation (Bio Babalscy, Poland) were used for sourdough preparation. The sourdoughs were prepared by mixing sterile tap water and flour (1:1). The dough yield was estimated as (dough mass/flour mass) x 100, and was 200. The doughs were inoculated with 0.5% of starter culture (10^{10} cfu/g dough). The control samples, prepared without starter cultures, were fermented under the same conditions. The sourdoughs fermenting with starter cultures and the control samples were incubated for 24h at 25 °C, propagated at 25 °C by back-slopping (after 24 h) with 10% of the ripe sourdough. After 24h of initial fermentation and at the 1st refreshment step, samples were withdrawn from the ripe sourdough for metabolite analysis.

Sourdough characteristics

All chemicals used in the study were from Sigma-Aldrich. The chemicals were all of analytical grade.

pH

The pH values were determined on a 1g dough sample blended with 9 mL of distilled water. The pH was measured using a pH Meter (Mettler Delta 320).

Total titratable acidity (TTA)

The total titratable acids (TTA) of the sourdoughs were determined using the standard procedure according to PN 92/A-74100.

Carbohydrate (residual sugar) and lactic acid determinations

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.

The residual sugar levels (maltose, glucose) and lactic acid were determined and 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 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

STATISTICA (Statsoft) software was applied for the statistical analysis of the obtained results. Statistically significant differences between the results (p = 0.05) were evaluated using Tukey's test.

Data was obtained from two independent sourdough fermentations.

Results and discussion

Acidification characteristics (pH, TTA) measured in millet and buckwheat sourdoughs

Acidification (pH, TTA) was monitored before (0h) and after 24h and 48h of fermentation. The effect of the used different pediococci strains on pH and TTA values was compared to the control samples prepared under the same conditions. The pH and TTA values are depicted in Table 1 and Table 2, respectively.

Table 1. pH of the millet and buckwheat sourdough fermenting with and without addition of different *Pediococcus pentosaceus* strains at the beginning and after 24h, 48h sourdough fermentation.

Sourdoughs	pH		
	0 (h)	24 (h)	48 (h)
M	5.03± 0.05	5.35 ± 0.07 $^{a A}$	4.18 ± 0.03 $^{a A}$
MW	5.2 ± 0.01	3.97 ± 0.02 $^{b A}$	4.17 ± 0.01 $^{a B}$
MM	5.2 ± 0.02	3.66 ± 0.01 $^{c A}$	3.87 ± 0.01 $^{b A}$
B	6.6 ± 0.14	4.65 ± 0.07 $^{a B}$	4.06 ± 0.01 $^{a A}$
BW	6.59 ± 0.05	3.93 ± 0.01 $^{b A}$	3.96 ± 0.01 $^{a B}$
BB	6.58 ± 0.06	3.86 ± 0.01 $^{b B}$	3.89 ± 0.01 $^{a A}$

Values are expressed as the mean of two experiments which were twice analysed (± SD). Lowercase (a, b) and uppercase (A, B) letters indicate different statistical significances between different *Pedioccocus* strains in the given type of flour and between the same *Pediococcus* strain in different flours, respectively. According to Tukey's test at p value of <0.05

pH

Generally, millet sourdoughs fermenting with starters and the control sample were characterised by lower initial pH values (5.03 – 5.2), than those of buckwheat sourdoughs (6.58 – 6.6).

After 24h of fermentation, pH values of millet sourdoughs fermenting with millet isolate (MM) and wheat isolate (MW) decreased significantly (from 5.2 down to 3.66 and 5.2 down to 3.97, respectively), whereas those of the control samples remained unchanged. After the next 24h of fermentation, the pH of MW increased (up to 4.17) while the pH of MM remained nearly constant (3.87 versus 3.66).

Considering the changes in the pH values of buckwheat sourdoughs after 24 h of fermentation, a significant decrease of pH values of both the control sample (B) (from 6.6 down to 4.65) and fermenting with wheat (BW) and buckwheat (BB) isolates (from 6.59 down to 3.93; and from 6.58 down to 3.86, respectively) was observed. After 48h of fermentation, the pH of the control sample slightly dropped to 4.06, while the pH values of samples fermenting with starters remained nearly constant. The pH values of the samples prepared with wheat and buckwheat isolates did not significantly differ from each other.

Table 2. TTA of millet and buckwheat sourdough fermenting with and without addition of different *Pediococcus pentosaceus* strains at the beginning and after 24h, 48h sourdough fermentation.

Sourdoughs	TTA		
	0 (h)	24 (h)	48 (h)
M	2.062± 0.03	4.15 ± 0.05 a B	13.30 ± 0.08 a A
MW	2.061 ± 0.04	7.42 ± 0.06 b B	12.72 ± 0.14 b B
MM	2.065 ± 0.04	11.90 ± 0.06 c B	15.23 ± 0.16 c B
B	6.55 ± 0.09	13.30 ± 0.07 a C	25.95 ± 0.20 a B
BW	6.50 ± 0.08	27.96 ± 0.11 b C	24.98 ± 0.16 a C
BB	6.54 ± 0.08	29.02 ± 0.14 c B	27.04 ± 0.14 a B

Values are expressed as the mean of two experiments which were twice analysed (± SD). Lowercase (a, b) and uppercase (A, B) letters indicate different statistical significances between different *Pedioccocus* strains in the given type of flour and between the same *Pediococcus* strain in different flours, respectively. According to Tukey's test at p value of <0.05.

TTA

Generally, the highest TTA values were found in buckwheat sourdoughs both at the beginning of the fermentation (~ 6.53) and after 24h and 48h of fermentation (Table 2).

The millet and buckwheat sourdoughs prepared with autochthonous *P.pentosaceus* strains had higher TTA values compared to the samples prepared with wheat isolates. The observed differences in TTA value between the sourdoughs prepared with autochthonous pediococci and with wheat isolate were more pronounced in the case of millet sourdoughs.

Considering the differences in the TTA values between the control samples and the ones fermenting with the starter cultures, the control samples were characterised by significantly lower TTA values.

Carbohydrate utilization and lactic acid production

Immediately after mixing, maltose was the dominant carbon source in the studied non-wheat sourdoughs (Figure 1). After 24h of fermentation, maltose was not found in millet sourdoughs and distinctly depleted in buckwheat sourdough. The increase of maltose and/or glucose could be related to the hydrolytic activity of

cereal amylases on the starch fraction during sourdough preparation (Robert *et al.*, 2006).

The inoculated millet sourdoughs were characterised by a higher content of glucose (about 5.5 g kg^{-1} sourdough) and a lower content of lactic acid (about 9.55 g kg^{-1} sourdough) than the inoculated buckwheat sourdoughs (about 2.0 g kg^{-1} sourdough for glucose and about 11.5 g kg^{-1} sourdough for lactic acid) (Figure1, Figure2).

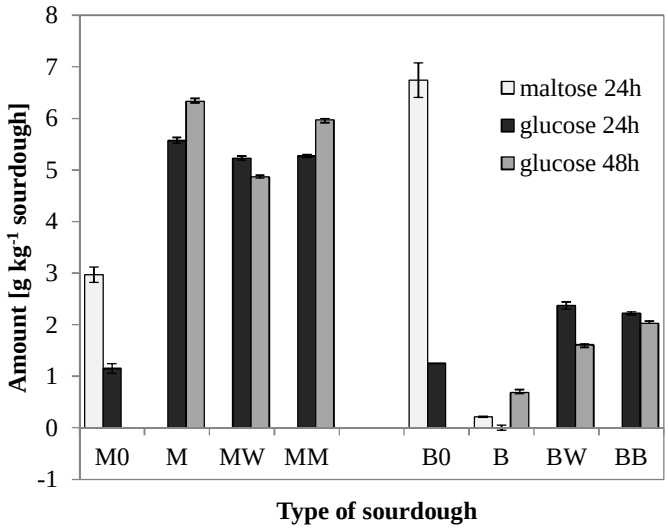

Figure 1. Concentrations of glucose and maltose in millet and buckwheat sourdoughs, at the beginning and after 24h and 48h of fermentation. M, B control millet and buckwheat sourdough, respectively. MW, BW millet and buckwheat sourdoughs inoculated with wheat isolates; MM millet sourdough inoculated with millet isolates; BB buckwheat sourdough inoculated with buckwheat isolates. In the case of M0, B0 millet and buckwheat sourdoughs, the concentration of maltose and glucose at the beginning of fermentation is shown.

After the first 24h of fermentation, glucose concentrations in the millet and buckwheat sourdoughs prepared with autochthonous *P. pentosaceus* strains and those prepared with wheat isolate were at a similar level. Significant differences in glucose concentration were observed after the next 24h of fermentation. In particular, after 48h of fermentation, glucose concentration in millet sourdough prepared with millet isolate (MM) remained nearly constant 95.27 versus 5.97 g kg^{-1} sourdough), while its content in the sample prepared with wheat isolate (MW) decreased (from 5.23 down to 4.87 g kg^{-1} sourdough). Similar behavior was found for buckwheat sourdoughs. In the case of sourdough inoculated with autochthonous *P. pentosaceus* strain (BB), glucose content remained nearly constant (2.22 versus 2.02 g kg^{-1} sourdough), while in sourdough prepared with wheat isolate (BW) glucose content decreased from 2.37 down to 1.61 g kg^{-1} sourdough.

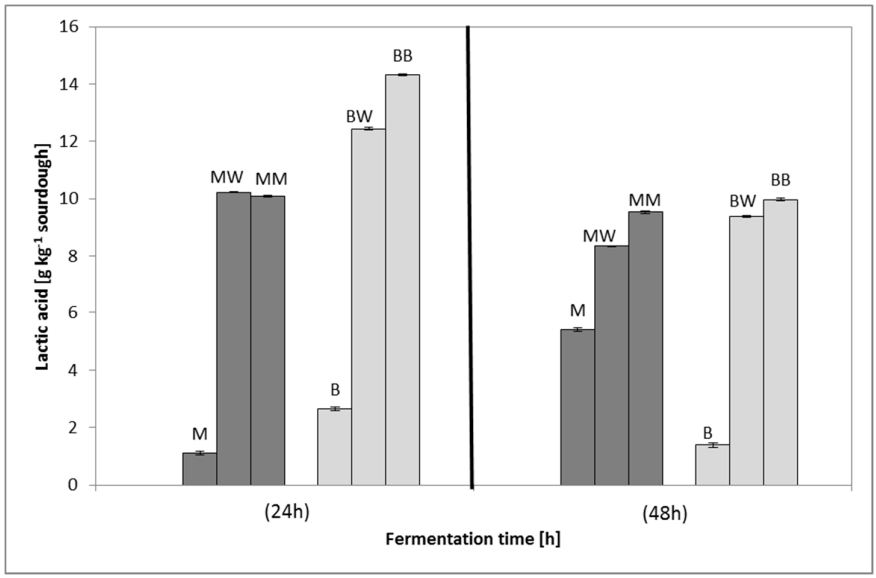

Figure 2. Concentration of lactic acid in millet and buckwheat sourdoughs, after 24h and 48h of fermentation. M, B control millet and buckwheat sourdough, respectively. MW, BW millet and buckwheat sourdoughs inoculated with wheat isolates; MM millet sourdough inoculated with millet isolates; BB buckwheat sourdough inoculated with buckwheat isolates.

Lactic acid

The inoculated millet and buckwheat sourdoughs showed distinctly higher content of lactic acid compared to that of the control samples, over the studied fermentation period.

Considering that the differences in the lactic acid content depend on the nature of the added *Pediococcus pentosaceus* strains, the addition of each autochthonous pediococci strain led to a distinct increase of the lactic acid compared to the sourdoughs prepared with wheat isolate. In particular, the increase in buckwheat sourdough was observed already after 24h of fermentation, whereas in millet sourdough the increase was noticed after 48h of fermentation.

The conducted studies showed differences in acidification properties, carbohydrate consumption and lactic acid production depending on the nature of the used strains and the type of flour. Moreover, the content of the analysed metabolite was variable during the fermentation period. The studied sourdoughs were prepared under the same technological conditions as mentioned above. Hence, the metabolic activity of the studied pediococci strains in the specific sourdough environment should depend mainly on the nature of the substrate (type of used flours) and the microbial interaction of used pedioccoci strains with indigenous microbiota. The type of substrate and the existence of indigenous microbiota are widely recognized

as driving ecological factors in the selection of dominant sourdough species (Hammes *et al*., 2005). Although it is not known exactly to which extent, both mentioned factors determine the selection (De Vuyst *et al.*, 2009).

The addition of the autochthonous *Pediococcus pentosaceus* isolates led to a distinct increase of lactic acid in the millet and buckwheat sourdoughs mainly after 48 h of fermentation, compared to the sourdough prepared with wheat isolate. Thus, the above findings showed that the lactic acid production was better when using autochthonous pediococci strains for the given type of sourdough. An advantage of using the autochthonous LAB strains as starter in wheat and non-wheat sourdoughs fermentation was previously postulated by Vogelmann *et al.* (2009) and Sekwati-Monang *et al*. (2012). Our findings will support this thesis. However, it must be emphasized that the content of analysed metabolites was variable during the fermentation period and differed depending on the used flour. As it was already mentioned, the positive effect of the autochthonous strains on the lactic acid production in millet sourdoughs was found after 48h of fermentation, whereas in buckwheat sourdoughs over the all tested fermentation period. It could be assumed that at the first stage of millet sourdough fermentation, the metabolic activity of LAB strains was affected more by the interactions with the indigenous biota than by the substrate quality.

Conclusions

Generally, the millet and buckwheat sourdoughs fermenting with autochthonous isolates were characterized by a higher amount of lactic acid and higher TTA values, than the sourdoughs prepared with wheat isolates. The carbohydrate consumption and lactic acid production for both wheat and non-wheat *Pediococcus pentosaceus* strains, varied depending on the used flour and changed during the fermentation period.

The obtained results support previous observations, namely that the specific behavior of autochthonous LAB starters is associated most likely with both high affinity of these strains to carbohydrate substrates and amino acids of flour, and their interaction with indigenous microorganisms.

More research on the metabolic activity of the studied non-wheat pediococci strains is needed to provide information on their competitiveness in non-wheat sourdoughs and to give evidence of their potentiality in sourdough fermentation. This will be further useful for the development of tailor made starter cultures for the specific substrates (flours).

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