



Received for publication, October, 10, 2017
Accepted, December, 15, 2018

Original paper

Microbial and nutritional characteristics of fermented wheat bran in traditional Romanian *borș* production

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Abstract

The heat bran-based fermented beverage *borș*, traditionally prepared in Romania, is used to give a sour taste to traditional soups or to consume as a refreshing drink. Its use has many health benefits, especially due to the high vitamin and mineral contents. Romanian *borș* was investigated for the first time regarding its microbiological and physicochemical characteristics. Commercial products, home-made products, and laboratory-made *borș* were compared. Both culture-dependent and culture-independent methods were used to monitor the microbial species diversity and/or dynamics of these products. The nutritional properties of the home-made and commercial products, as well as the nutritional dynamics of the laboratory-made *borș* were assessed, in particular regarding the protein, amino acid, exopolysaccharide, and phenolic contents. The lactic acid bacterial species diversity of *borș* was restricted, being dominated by lactobacilli, among which *Lactobacillus amylolyticus* was the most frequent. *Lactobacillus amylolyticus* also dominated the microbiota of laboratory-made *borș* from the start of the fermentation. The pH values of all *borș* samples were below 4.0. The protein, amino acid, and phenolic contents of the laboratory-made *borș* increased during fermentation, whereas the exopolysaccharide content remained approximately constant. The varying nutritional profiles and the presence of the acidifying and α -amylase-active *Lb. amylolyticus* may be of importance for the health impact of *borș*.

Keywords

Wheat bran; *borș*; fermented cereals; *Lactobacillus amylolyticus*

To cite this article: GROSU-TUDOR SS, STEFAN IR, STANCU MM, CORNEA CP, de VUYST L, ZAMFIR M. Microbial and nutritional characteristics of fermented wheat bran in traditional Romanian *borș* production. *Rom Biotechnol Lett.* 2019; 24(3): 440-447. DOI: 10.25083/rbl/24.3/440.447



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Introduction

Cereal grains are important sources of dietary proteins, carbohydrates, vitamins, minerals, and fiber for humans all over the world, although the nutritional quality and the sensorial properties of the products made thereof are sometimes poor compared with foods of animal origin (BLANDINO & al. [1], ARENDT & ZANNINI [2]). Several methods are in use to improve the nutritional value of cereal-based foods, encompassing fermentation carried out by both yeasts and bacteria, often leading to a decrease in the level of carbohydrates and non-digestible polysaccharides and a change in their composition regarding fats, minerals, and vitamins (BLANDINO & al. [1]; TODOROV & HOLZAPFEL [3]). Furthermore, fermentation improves the shelf-life and texture, increases the antioxidant activity, and contributes to the taste and aroma of the final products. At present, many cereal-based fermented foods and beverages are produced worldwide, especially in Africa and Asia, at household level or in small industrial companies, often based on a traditional fermentation process (BLANDINO & al. [1]; ARENDT & ZANNINI [2]). These traditionally fermented food products are prepared from the most common cereals (rice, wheat, corn, or sorghum) and are mostly used as colorants, spices, beverages, or light-meal foods. In some of these products, cereals are combined with legumes or milk to improve the quality of the final food product (BLANDINO & al. [1]; TODOROV & HOLZAPFEL [3]). The microbial ecology of many of these cereal-based fermented foods and beverages is quite complex and even not known yet, but it is likely that they involve mixed cultures of yeasts, bacteria, and filamentous molds. Yeasts mainly perform carbohydrate breakdown, whereas bacteria often show proteolytic activity too (TODOROV & HOLZAPFEL [3]).

The bran fraction of the wheat milling process is regarded as a by-product with little commercial value and is mostly used in baked goods to increase the level of insoluble dietary fiber or as a supplement for animal feed (PRÜKLER & al. [4]). However, the potential health benefits of this low-cost and highly accessible product are very important (O'SULLIVAN [5]). From a nutritional point of view, cereal bran has been described as an interesting raw material due to not only its high contents of dietary fiber but also the presence of other biologically active compounds such as vitamins B and E, alkylresorcinols, lignans, phenolic acids, phytosterols, tocopherols, tocotrienols, and folates (STEVENSON & al. [6]).

The incorporation of wheat bran into foods is very limited, mostly due to its adverse technological and sensory properties such as bitterness and grittiness of the resulting food products, but several strategies are nowadays applied to improve the sensory quality of these foods, and one of the most promising is fermentation (PRÜKLER & al. [4]). For instance, the major substrate used for the production of Romanian *borș* is wheat bran, to which warm water and a portion of a previous fermented batch (*huste*) is added. Fermentation can last for 2-3 days and is carried out in

wooden or glass vessels, whereby the liquid can be replaced with warm water every day to get more of the fermented product. *Borș* is an acidic liquid not only used in Romanian cuisine to impart a sour taste to a variety of traditional soups known as *borș* or *ciorba* but it is also consumed as a refreshing drink. Another Romanian cereal bran-based fermented beverage is *braga*, a sweet-sour drink obtained through fermentation of millet (BLANDINO & al. [1]).

Information about Romanian *borș* is scarce, mainly restricted to travel guides and cooking books, as this fermented beverage did not receive any scientific attention up to. However, people preparing and consuming *borș* believe for a long time that, besides its pleasant taste, it may have many health benefits, especially due to its high vitamin and mineral contents. *Borș* is a good revitalizing product, which is used for its perceived beneficial effects in respiratory diseases, digestive problems (indigestion, vomiting), liver and bile diseases, and even cancer treatment. Similarly, it is used to lower the blood pressure and cholesterol level and contribute to the treatment of anemia. A scientific study of *borș*, including the microbiological aspects of its fermentation process and the impact on human health, may bring information not only about the microorganisms involved but also to select new functional microorganisms for targeted applications. Some strains isolated from *borș* have been shown to inhibit the growth of other bacteria [strains of lactic acid bacteria (LAB) and some *Bacillus* species], due to the production of organic acids and bacteriocins (GROSU-TUDOR & al. [7]).

This study aimed to unravel the species diversity and community dynamics of LAB as well as typical physicochemical changes occurring during Romanian *borș* fermentation.

Materials and Methods

1. Microbiological analysis of *borș* samples

Isolation of presumptive LAB. Fifteen end-samples of *borș* were available, namely from two commercial, non-pasteurized products (Danila and Dalco) and thirteen home-made products collected in the Bucharest area. Presumptive LAB were isolated using customized de Man-Rogosa-Sharpe (MRS) agar media, containing either 20 g/l of glucose (further referred to as MRSg), 20 g/l of fructose (MRSf), or 20 g/l of sucrose (MRSs). Appropriate dilutions of the samples were spread onto these agar media, followed by incubation at 37°C for 48 h. Colonies were randomly picked up. The same media (liquid and solid) were used for subsequent growth and purification.

Preliminary identification of the isolates. All isolates (80) were tested for their Gram reaction, catalase activity, and morphology. The Gram-positive and catalase-negative isolates (69) were stored at -85°C in the corresponding liquid isolation medium, supplemented with 25% (v/v) of glycerol. These frozen stocks were used for further identification of the isolates to genus and species level.

Identification of the LAB isolates. Classification and identification of the LAB isolates was performed

through (GTG)₅-PCR fingerprinting, followed by gene sequencing.

(GTG)₅-PCR fingerprinting. Genomic DNA was extracted from overnight cultures, using a Pure Link Genomic DNA kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's guidelines. PCR amplifications of genomic DNA performed with a Mastercycler pro S (Eppendorf, Hamburg, Germany), using the (GTG)₅ primer (5'-GTGGTGGTGGTGGT-3'), and PCR fingerprinting and cluster analysis were performed as described before (RAVYTS & al. [8]).

16S rRNA gene sequencing. From each cluster of identical genomic fingerprints, one to two isolates were selected for further identification by 16S rRNA gene sequencing. PCR amplification of 16S rRNA genes and subsequent purification were performed as described previously (GROSU-TUDOR & al. [7]). Sequencing of the amplification products was performed at Macrogen Europe (Amsterdam, The Netherlands). The DNA sequencing runs were assembled using the BioEdit software. The sequences were compared to those available in databases of the National Center for Biological Information using the BLAST search program.

2. Microbiological analysis of *borş* made at laboratory scale

Experimental preparation of *borş*. Wheat bran fermentations were carried out in duplicate, according to a traditional recipe and using two identical jars of 5 l. They contained 350 g of *huşte* recovered from the home-made *borş* 11 (Table 1), to which 300 g of wheat bran (from a local mill) were added, together with 3 g of salt, 70 g of white beans, and 70 g of corn kernels. Approximately 200 ml of cold water were added to homogenize the mixture and then 2 l of boiled water were slowly added to each jar, with continuous stirring. The jars were covered with a lid and placed at 37°C. Samples were withdrawn during the fermentation process, namely at the start (0 h) and after 3, 6, 9, and 12 h of incubation. Samples were immediately subjected to a pH measurement and a microbiological analysis.

Microbiological analysis.

Colony enumeration and LAB isolation and identification. For culture-dependent analysis, aliquots of the appropriate decimal dilutions of the samples were plated on MRSg agar medium supplemented with 0.1 g/l of cycloheximide to inhibit fungal growth, violet-red-bile-glucose (VRBG) agar medium (Merck KGaA, Darmstadt, Germany) supplemented with 0.1 g/l of cycloheximide, and yeast extract-peptone-glucose (YPG) agar medium (Merck) supplemented with 0.1 g/l of chloramphenicol to inhibit bacterial growth, for the enumeration of LAB, enterobacteria, and yeasts, respectively. Counts were expressed as colony forming units (CFU) per ml of *borş* sample. MRS and VRBG agar plates were incubated at 37°C and YPG agar plates at 28°C for 48 h. Colonies grown on MRSg agar medium were randomly picked up (about 5-10% of the colonies), purified by two successive cultivation steps in MRSg medium and on MRSg agar medium, respectively, and stored at -85°C in MRSg

medium supplemented with 25% (v/v) of glycerol. Gram-positive, catalase-negative bacterial isolates were identified through (GTG)₅-PCR fingerprinting and 16S rRNA gene sequencing as described above.

Culture-independent analysis. To monitor the bacterial species involved in the laboratory *borş* fermentations as a function of time, 50 ml of each sample were centrifuged at 97 x g for 15 min at 4°C to remove debris. Supernatants were kept at 4°C for 30 min to allow sedimentation of crude particles, followed by centrifugation of 40 ml of the upper part of the samples at 8000 x g for 15 min at 4°C. The pellets and supernatants were used for DNA extraction and physicochemical analysis, respectively. DNA isolation, denaturing gradient gel electrophoresis of 16S rRNA-targeted PCR amplicons making use of LAC and universal V3 primers (rRNA-PCR-DGGE analysis), and gel band processing were done as described previously (WOUTERS & al. [9]). Sequencing was performed at Macrogen Europe.

3. Physicochemical analysis of *borş* samples

The following analyses were performed not only for the laboratory *borş* samples collected after different fermentation times but also for five randomly selected end-samples of *borş*, namely one commercial product and four home-made products.

pH measurements. Samples were analysed for acidification by measuring the pH with an InoLab 720 pH meter (WTW, Weilheim, Germany).

Amino acid and protein profiles. Amino acid profiles were analyzed by thin layer chromatography (TLC), using silica gel plates (Merck, Darmstadt, Germany) and a semi-automatic TLC system (Camag, Muttenz, Germany). Mobile phase and staining solution (0.5%, m/v, ninhydrin) were prepared as described before (PACHUSKI & al. [10]). Fifteen amino acids, with a retention factor (Rf) between 0.108 and 0.646 were used as standards. The protein content of each sample was determined by the method of Bradford. Protein profiles were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The samples were run on 12% (m/v) polyacrylamide gels. Electrophoresis was conducted in a Compact Dual Plate apparatus V20-CDC (Scie-Plas, Warwickshire, UK) at a constant voltage of 90 V in the stacking gel and 180 V in the running gel. A broad-range protein molecular mass marker (Promega, Madison, WI, USA) was used as reference. Gels were stained with Coomassie Brilliant Blue.

Exopolysaccharide (EPS) content. EPS were isolated by acetone precipitation and quantified gravimetrically, as described previously (DE VUYST & al. [11]). They were purified by ultrafiltration and hydrolysed with 8 N HCl at 100°C for 6 h (GROSU-TUDOR & al. [12]). The carbohydrate composition of the hydrolysed samples was determined using a high-performance liquid chromatography (HPLC) system (Jasco, Tokyo, Japan), equipped with a Carbo Sep Coregel 87P column (Teknokroma, Barcelona, Spain), kept at 85°C, and coupled to a RI-2031 refractive index detector (Jasco). Elution was performed with ultrapure water, at a flow rate of 1 ml/min. Arabinose, fructose, galactose, glucose, maltose, mannose, rhamnose,

ribose, sorbose, sucrose, and xylose, at a concentration of 0.1 mg/ml, were used as standards.

Free phenolics. The free phenolic content was measured using the Folin-Ciocalteu method (SLINKARD & SINGLETON [13]) and the results were expressed as mg of gallic acid equivalents per ml of sample.

Results

Microbiological analysis and acidification extent of borş samples

End-samples of borş contained one to five *Lactobacillus* species (Table 1). About half of the end-samples displayed a very low species diversity (one or two *Lactobacillus* species were found), whereas in other end-samples three or more *Lactobacillus* species were found. *Lactobacillus amylolyticus* was found in eleven end-samples of borş (Table 1), being present on all isolation agar media used, but mostly on MRS-g agar medium (results not shown). Other lactobacilli frequently found in four to five borş samples were *Lactobacillus fermentum*, *Lactobacillus plantarum*, *Lactobacillus casei* and *Lactobacillus buchneri*, without a direct correlation with the isolation agar medium. *Lactobacillus panis*, *Lactobacillus brevis*, *Lactobacillus oris*, and *Enterococcus durans* were only found in one or maximum two borş end-samples, and only in combination with another *Lactobacillus* strain.

In some samples, *Lb. amylolyticus* (four samples) or *Lb. fermentum* (one sample) was the sole *Lactobacillus* species present.

The pH values of the end-samples of borş were in the range of 3.4 to 3.9.

Microbiological analysis and acidification of borş made at laboratory scale

During the laboratory borş fermentations, the pH dropped fast during the first 3 h, from about 5.0 to about 4.0, while from 6 h of fermentation, it reached a value of about 3.5 (Table 2). Culture-dependent and culture-independent analysis did not show the presence of enterobacteria or yeasts (results not shown). LAB were present in high counts (about 8.0 log CFU/ml) from the start of the fermentation (Table 2). The LAB counts increased fast during the first 6 h of fermentation, reaching about 12 log CFU/ml, with a concomitant pH drop to 3.5. After 6 h of fermentation, the bacterial cells entered the stationary growth phase, during which their counts showed a slightly decreasing trend and the pH remained approximately constant until 12 h, when the fermentation was stopped and the liquid was removed and stored at 4°C.

All isolates recovered from the MRSg agar media were identified as *Lb. amylolyticus*. This LAB species was also the only taxon found in the rRNA-PCR-DGGE community profiles when using both the LAC and universal V3 primers (Fig. 1).

Table 1. Lactic acid bacterial (LAB) species diversity of end-samples of borş; samples 1 and 2 are commercial products, samples 3-15 are home-made products

LAB species	Samples														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
<i>Lb. amylolyticus</i>	+	+	+	-	-	+	-	+	+	+	+	+	+	+	-
<i>Lb. casei</i>	+	+	-	-	-	+	-	-	-	+	-	-	-	-	-
<i>Lb. buchneri</i>	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-
<i>Lb. fermentum</i>	-	+	-	+	+	-	-	+	-	-	-	-	-	-	+
<i>Lb. plantarum</i>	-	-	+	-	-	-	+	+	-	-	-	-	-	-	+
<i>Lb. oris</i>	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-
<i>Lb. panis</i>	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Lb. brevis</i>	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-
<i>Ent. durans</i>	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-

Lb., *Lactobacillus*; *Ent.*, *Enterococcus*

Table 2. Growth parameters and physicochemical properties of borş samples collected at different time points during laboratory fermentations (values ± SD)

Time (h)	pH	Viable counts (log CFU/ml)	Protein content (mg/ml)	Exopolysaccharides (g EPS/l)	Free phenolics (µg/ml)
0	4.9 ± 0.1	8.3 ± 0.2	0.8 ± 0.1	1.8 ± 0.1	147 ± 2
3	4.0 ± 0.0	10.7 ± 0.1	1.3 ± 0.1	1.8 ± 0.1	190 ± 9
6	3.5 ± 0.0	12.4 ± 0.3	1.8 ± 0.1	1.7 ± 0.1	261 ± 1
9	3.4 ± 0.0	12.3 ± 0.3	1.9 ± 0.1	1.7 ± 0.1	320 ± 2
12	3.4 ± 0.0	11.9 ± 0.1	1.9 ± 0.0	1.9 ± 0.1	332 ± 1

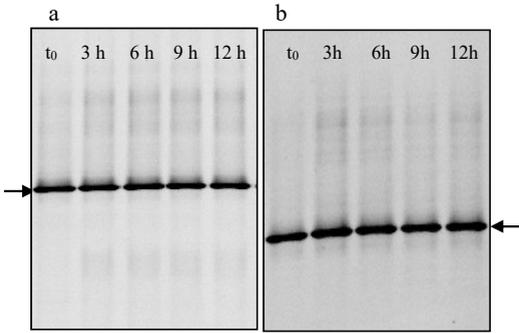


Figure 1. rRNA-PCR-DGGE community profiles (35–60% denaturing gradient from top to bottom of the gels) of total DNA extracted from the samples collected from laboratory-scale *borş* fermentations, using LAC primers (a) and universal V3 primers (b). The arrows show the bands that were cut out from the gel and sequenced. The closest relative (99% identity) of the sequenced fragments was *Lactobacillus amylolyticus* strain L6 (16S ribosomal RNA gene, partial sequence, accession number KT996124.1).

Physicochemical analysis of borş

Several amino acid bands were detected by TLC in all the end-samples of *borş* and in the samples collected during laboratory *borş* fermentation. However, there were no differences between the end-samples, except for the intensity of the bands (results not shown). In the case of samples collected after different laboratory *borş* fermentation times, an increase of the band intensity could be observed from the first toward the last sample, indicating amino acid accumulation upon fermentation (Fig. 2).

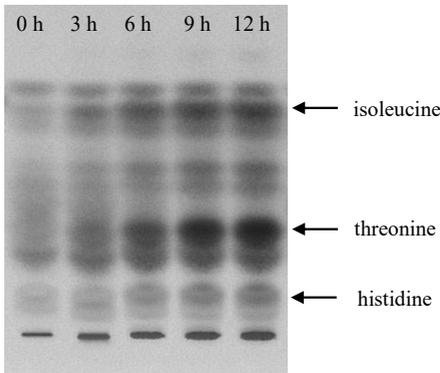


Figure 2. TLC chromatogram of the amino acids from samples collected at different time points during laboratory-scale *borş* fermentations. Bands were stained with 0.5% (v/v) ninhydrin.

During laboratory *borş* fermentation, the protein content increased from about 0.8 mg/ml at the start to about 1.9 mg/ml after 9 h of fermentation, remaining approximately constant afterwards (Table 2). SDS-PAGE revealed differences in the protein profiles of the samples collected after different fermentation times (Fig. 3). In general, at the beginning of the fermentation (0–3 h), there were more protein bands compared with later samples.

However, the low-molecular-mass proteins (10–15 kDa) remained approximately constant in diversity and concentration in all samples. The protein concentrations of the home-made *borş* samples were, in general, lower than 0.5 mg/ml and variable (Table 3), depending most probably on the recipe used for manufacturing.

The EPS content of the samples collected during laboratory fermentation was in the range of 1.7–1.9 g/l (Table 2). However, there were differences in the EPS contents among the end-samples of *borş* from different origins (Table 3). The EPS material isolated from three randomly selected *borş* samples was subjected to acid hydrolysis and revealed the presence of glucose, xylose, and arabinose after HPLC analysis (results not shown).

The concentration of the phenolic compounds increased during the laboratory *borş* fermentations, from approximately 150 µg/ml to approximately 330 µg/ml (Table 2). The home-made *borş* samples displayed variable phenolic contents, ranging from approximately 340 µg/ml to approximately 790 µg/ml (Table 3).

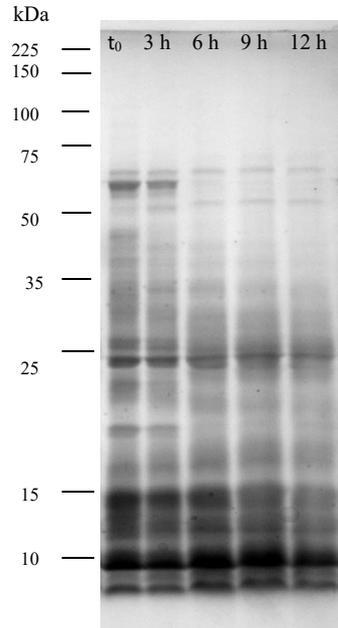


Figure 3. SDS-PAGE of proteins from samples of *borş* collected at different time points during laboratory-scale fermentations.

Table 3. Physicochemical properties of selected end-samples of commercial (sample 1) and home-made (samples 12-15) *borș*

Sample	pH	Protein content (μg/ml)	Free phenolics (μg/ml)	Exopolysaccharides (g EPS/l)
1	3.9	342	607	1.2
12	3.4	413	337	0.5
13	3.4	182	499	0.4
14	3.5	504	789	1.1
15	3.6	198	451	0.8

Discussion

Fermented cereals, including fermented cereal-based beverages, represent a potential source of new functional LAB species, besides interesting nutrients and bioactive compounds, all with beneficial effects on human health (PALLIN & al. [14]). In the present study, the cereal bran-based fermented beverage *borș*, traditionally prepared in Romania, was investigated for the first time regarding its microbiological and physicochemical characteristics. In the review of BLANDINO & al. [1] on indigeneous cereal-based fermented foods and beverages produced worldwide, *borș* was not included, but it has probably been taken for *boza*, which is mentioned as a cereal-fermented beverage from Romania, along with *braga*. Several *boza* types have been described in the literature, with various compositions and physicochemical properties, depending on the cereals used as raw materials and the recipes applied for their production, influencing the amount and quality of carbohydrates available as fermentation substrates, nitrogen sources, and growth factors for microbial activity (TODOROV & HOLZAPFEL [3]).

The pH values of the final *borș* samples (below 4.0) were highest for the commercial products and were similar to those found for other fermented cereals (SALMERÓN & al. [15]). This low pH contributes to the improvement of the product shelf-life, since a pH below 3.8 inhibits the growth of most food spoilage bacteria. Indeed, *borș* can be stored at 4°C for several weeks without being spoiled or losing its organoleptic properties, provided the recipients are well sealed.

The LAB species diversity of *borș* consisted of various lactobacilli. Among them, the most abundant species was *Lb. amylolyticus*. This LAB species was present in almost all *borș* samples investigated, probably due to its high competitiveness by its simultaneous acidifying and α -amylase activities. It is indeed known that LAB are fastidious microorganisms and usually grow in rich environments, in the presence of high amounts of mono- and disaccharides and amino acids. During the fermentation of cereals, in particular wheat bran fermentation, endogenous grain amylases continuously generate fermentable carbohydrates that serve as a constant source

of energy for LAB (BLANDINO & al. [1]). Alternatively, a few LAB species, such as *Lb. amylolyticus*, *Lb. amylophilicus*, and *Lb. amylovorus*, and even strains of the LAB species *Lactococcus lactis* and *Lb. plantarum*, have the capability to degrade starch in the presence of easier fermentable carbohydrates (REDDY & al. [16]). Such amylolytic strains have been isolated from fermented cassava, maize, sorghum, rice, beer malt, etc. (HATTINGH & al. [17]).

LAB species such as *Lb. oris* and *Ent. durans* are most probably environmental contaminants, since for instance *Lb. oris* has been found in the human oral cavity (FARROW & COLLINS [18]). Alternatively, *Lb. panis* has been associated with sourdough, whereas *Lb. brevis* along with *Lb. buchneri* is commonly found not only in dairy products, vegetables, and sourdoughs, but also in sour starch fermentation and silage (PREEDY & al. [19]).

The variability of the *Lactobacillus* species diversity among the *borș* samples may be due to the origin of the raw materials, the milling process, the house microbiota, etc., as has been seen for other cereal fermentation processes such as sourdough (DE VUYST & al. [20]). Alternatively, it is important to notice that part of a previous batch is always used as the inoculum for *borș* preparation, in which a stable microbiota has been established over time based on a natural selection. This microbiota may differ from one household to another.

The absence of enterobacteria in the laboratory-made *borș* guaranteed a good fermentation quality of the end-products. The absence of yeasts may be ascribed to their absence in the inoculum or their inhibition by the strongly acidifying lactobacilli during the fermentation period (TODOROV & HOLZAPFEL [3]). Alternatively, the high LAB counts could be ascribed to their introduction through the inoculum (previous batch of *borș*) and their fast growth during fermentation. Indeed, the LAB species diversity of the laboratory *borș* fermentations of the present study was dominated by *Lb. amylolyticus* from its start, as was demonstrated by both the culture-dependent and culture-independent methods. The data support the above postulated hypothesis that a stable microbiota was established in the inoculum used (in casu *huște* from previous *borș*) and that *Lb. amylolyticus* was very well adapted to the *borș* environment, in particular with respect to its α -amylase activity, being able to compete with other microorganisms that may be present in the wheat bran.

Borș fermentation was characterized by an increase of available amino acids and free phenolics, whereas the EPS content was more or less constant. An increase of amino acids has been reported previously for the fermentation of various cereals, although to a variable extent (BLANDINO & al. [1]). Matrix proteins are probably degraded over time by the proteolytic enzymes from the outer bran layers (KATINA & al. [21]) or by those produced by the microorganisms involved in the fermentation (POUTANEN & al. [22]). Controversial reports have been published concerning the effect of fermentation on the protein levels of maize, millet, sorghum, and other cereals (BLANDINO & al. [1]). Yet, many studies have proven that fermentation

of cereals by LAB improves the protein quality as well as the level of certain free amino acids by enhanced endogenous proteolysis and/or microbial action (WANG & al. [23]). The monosaccharides detected after hydrolysis of the EPS present in the *borş* samples indicated that these EPS are, most probably, originating from the soluble cereal fibers that consist of β -glucans, arabinoxylans, oligosaccharides, and starch (TEJADA-ORTIGOZA & al. [24]).

The increase of the concentrations of the phenolic compounds might not be caused by fermentation (PRÜCKLER & al. [4]), although it is known that LAB and yeasts elaborate phenolic compounds through enzymatic conversions (WANG & al. [25]). However, it can partly indicate proteolysis during or after the fermentation process, with a consequent release of aromatic amino acids (KATINA & al. [21]). This is also suggested by the lower protein concentrations and higher phenolic contents in the end-samples of *borş* compared with the samples obtained during laboratory *borş* fermentations. Although responsible for the bitterness and aftertaste of cereals, phenolic compounds have some health benefits, including antioxidant activity (VAZQUEZ-OLIVO & al. [26]; BORS *et al.* [27]), antimicrobial activity (MARINAS & al. [28]) as well as a glycemic index-lowering effect (POUTANEN & al. [22]).

Conclusions

Wheat bran-based, Romanian *borş* fermentation was mainly carried out by lactobacilli, among which *Lb. amylolyticus* was the most frequent LAB species. The use of amylolytic LAB strains as starter cultures may offer the advantage of combining both amylase production and acidification within one microorganism and may be of importance for the health impact of *borş* (SANTOYO & al. [29]). The fermentation of wheat bran during the production of *borş* led to an increase of the concentrations of available amino acids and phenolic compounds. Moreover, the ability of certain strains of the lactobacilli involved in *borş* fermentation to repress the growth of other microorganisms (GROSU-TUDOR & al. [7]), including fungi and spoilage bacteria, may find biotechnological applications. These strains may be used as starters or co-starters for controlled fermentations to get an improved shelf-life of the final products.

Conflict of interest disclosure

There are no known conflicts of interest in the publication of this article. The manuscript was read and approved by all authors.

Acknowledgements

This work was supported by a grant of the Romanian National Authority for Scientific Research, CNDI-UEFISCDI, project number 105/2012 (PLANTLAB) and a bilateral project in the framework of the interacademic

exchanges between the Romanian Academy and the Research Foundation Flanders (4271/2014). The authors acknowledge the project RO1567-IBB05/2017 from the Institute of Biology Bucharest of Romanian Academy. LDV acknowledges its grants from the Research Council of the Vrije Universiteit Brussel (SRP7 and IOF342 projects).

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