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Effect of processing variables on the physico-chemical characteristics and aroma of *borş*, a traditional beverage derived from wheat bran

Antonella Pasqualone[a*], Carmine Summo[a], Barbara Laddomada[b], Elena Mudura[c], Teodora Emilia Coldea[c]

[a]Department of Soil, Plant and Food Sciences, University of Bari 'Aldo Moro', Via Amendola, 165/A, 70126 Bari, Italy
[b]Institute of Sciences of Food Production (I.S.P.A.), C.N.R., via Monteroni, 73100 Lecce, Italy
[c]Department of Food Engineering, University of Agricultural Sciences and Veterinary Medicine, Calea Manastur, 3-5, 400372 Cluj-Napoca, Romania

*Corresponding author. E-mail antonella.pasqualone@uniba.it, phone +39 080 5442225.

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Abstract

Borş is a traditional Romanian beverage obtained by naturally fermenting an aqueous suspension of wheat bran and corn flour, used as flavoring enhancer in local gastronomy since ancient times, and more recently consumed as refreshing drink. To investigate the changes in sensory, physico-chemical, phenolic and aroma composition resulted after two successive fermentations, *borş* samples were subjected to standard, sensory, HPLC and GC/MS analysis. Total phenolic compounds and ferulic acid, the most abundant phenolic compound, were positively influenced by starter addition, increase of fermentation temperature, and thermal treatment, whereas the effect on less abundant phenolic acids was not univocal. The variables had the same effect on antioxidant activity and brown index. Volatiles (alcohols, carboxylic acids, esters), and sensory odor notes of pungent-sour and goat milk-cheese increased at higher fermentation temperature, whereas bran and yogurt odor decreased. The addition of starter at 4 °C allowed balancing odor intensity and antioxidant activity.

Key words: Traditional product; fermented beverage; *borş*; volatile compounds; antioxidant activity

Running title: Effect of processing variables on *borş*

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1. Introduction

Numerous traditional beverages of ancient origin, appreciated in the area of production but almost unknown outside it, are obtained by fermenting aqueous suspensions of cereal meals: *chicha*, derived from corn and consumed in South America (Blandino, Al-Aseeri, Pandiella, Cantero, & Webb, 2003); *bushera*, obtained from sorghum or finger millet flour and consumed in Uganda (Muyanja, Narvhus, Treimo, & Langsrud, 2003); *oshikundu*, from sorghum and pearl millet flour, and *maxau*, from corn flour, both produced in Namibia (Misihairabgwi & Cheikhyoussef, 2017); *boza*, obtained from millet, corn, wheat or rice and consumed in Turkey, Greece, Bulgaria, Albania, and Bosnia Herzegovina (Arici & Daglioglu, 2002); *kvass*, typically produced from rye flour or stale rye bread in Russia and in several Eastern Europe countries (Baschali, Tsakalidou, Kyriacou, Karavasiloglou, & Matalas, 2017); *tarhana*, obtained in Turkey by fermenting wheat flour with yogurt and dried after production, then rehydrated at the moment of use to prepare soups (Kilci & Gocmen, 2014). In addition, the recent interest towards non-diary milk substitutes and functional beverages has prompt the production of innovative fermented beverages from emmer (Coda, Rizzello, Trani, & Gobbetti, 2011).

Another fermented cereal-based beverage is the Romanian *borş de tărâţe*, also called simply *borş*: a sour liquid obtained by natural fermentation of an aqueous suspension of wheat bran – "*tărâţe*" means "bran" in Romanian – and corn flour (Nicolau & Gostin, 2015). *Borş* is traditionally used in Romania (especially in Moldova and, more rarely, in Transylvania) either to prepare a wide range of sour soups, named *ciorbă*, or to be consumed plain as a drink (Grosu-Tudor, Stancu, Pelinescu, & Zamfir, 2014). *Borş* is also referred to as "white *borş*" to distinguish it from the red one (commonly spelled *borsh* or *borsch*), obtained from the juice of beetroot in several countries of Central and Eastern Europe (Nicolau & Gostin, 2015).

Wheat bran, the main ingredient of *borş*, is commonly destined to animal feed, but alternative uses have been recently proposed due to its content of lipophilic and hydrophilic antioxidants such as tocopherols, tocotrienols, and phenolic compounds. In fact, bran oleoresin and bran aqueous extracts – the latter very similar to *borş* but not fermented – have been used to produce functional pasta (Pasqualone et al., 2016).

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Borş preparation follows a slightly different recipe from one area of Romania to another, and the starter is always obtained through spontaneous fermentation. Several lactic acid bacteria have been isolated from *borş*, able to produce bacteriocins (Grosu-Tudor et al., 2014). After thoroughly mixing bran, corn flour and water, the obtained suspension, put in a not tightly closed container, is kept in a fresh place (approximately 15 °C, but with large seasonal variations) for 2-3 days (Anonymous, 2017). Then, the suspension is filtered, bottled and stored at 4 °C. Part of the solid sediment (called *huşte*) is retained for being used as natural starter to get the successive batch of *borş* (Anonymous, 2011a), which formulation can also include sour cherry leaves, lovage and dill, all with flavoring purposes.

Besides being produced at home for personal consume, many artisanal producers sell unpasteurized *borş* in the produce markets. However, nowadays *borş* is also produced by industrial companies, which usually pasteurize it (Anonymous, 2017). *Borş* acts as a probiotic, when consumed raw, or prebiotic, when consumed pasteurized or cooked in soups (Nicolau & Gostin, 2015).

No studies have been aimed at defining the physico-chemical characteristics of *borş*. Moreover, no study has verified the effect of processing variations on the quality of end product. The aim of this research, therefore, has been to assess the effect of fermentation temperature, pasteurization, and use of starter, on the physico-chemical characteristics and aroma of *borş*.

2. Materials and methods

2.1. Sample production

The experimental design and the sampling points are schematized in Fig. 1. *Borş* was prepared in two successive fermentation stages, according to the artisanal practice that involves repeated fermentations over time, always adding a little amount of sediment from the precedent fermentation. In detail, 300 g of wheat bran (*Triticum aestivum* L.) (carbohydrates 68.8 g/100 g d.m., proteins 2.3 g/100 g d.m., lipids 3.9 g/100 g d.m., fiber 25.1 g/100 g d.m.) and 60 g of corn flour (*Zea mays* subsp. *mays*) (carbohydrates 85.6 g/100 g d.m., proteins 7.7 g/100 g d.m.; lipids

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1.9 g/100 g d.m., fiber 4.9 g/100 g d.m.) were thoroughly mixed with 3 L tap water. The obtained suspension was then divided into three batches - A, B and C - each of it allowing to ferment spontaneously for 3 days, at 4 °C, 14 °C , and 24 °C, respectively, in a not tightly closed glass bottle put in a thermostatic cell (GTest-TM, Fratelli Galli, Milan, Italy), for both the starter preparation (solid fraction) and the recovering of *borş* (liquid fraction). After the first fermentation, the suspension was filtered and a portion of the solid sediment (60 g) was recovered from each batch, to be used as natural starter (traditionally called *huşte* in Romanian) for repitching in the next successive fermentations. The filtered liquid was divided in two aliquots: the first was put in 250 mL bottles and pasteurized at 20 pasteurization units (PU) (samples 1AP, 1BP, 1CP) in a thermostatic bath (Thermovisc 100-F8, Fungilab, Barcelona, Spain), while the second was left unpasteurized (samples 1ANP, 1BNP, 1CNP).

Therefore, the recipe and fermentation were repeated as in the first stage, but with the addition of starter (60 g). Sampling was carried out as described above with pasteurized samples coded 2AP, 2BP and 2CP, and unpasteurized samples coded 2ANP, 2BNP, 2CNP. Experiments were repeated in duplicate.

Two commercial samples, coded COM1 and COM2, were purchased at local retailers and were included in the sample set as reference. All the samples were kept at -20 °C until being analyzed.

2.2. Determination of total phenolic compounds (Folin-Ciocalteu)

Borş samples were centrifuged for 10 min at 5000 × *g*. Then, the total phenolic compounds were determined in the supernatant after Folin-Ciocalteu reaction. The reaction mixture contained 100 μL supernatant, 500 μL Folin-Ciocalteu reagent (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) and 2 mL of 15% (w/v) sodium carbonate. The final volume was made up to 10 mL with distilled water. After 1 h in the dark, the absorbance of the solution was measured at 765 nm by a Cary 60 UV-Vis spectrophotometer (Agilent Technologies Inc., Santa Clara, CA, USA). A calibration curve was built by methanol solutions of ferulic acid (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) at concentrations between 0.1 and 2 g L^{-1} ($y = 0.0007x + 0.0089$; $r^2 = 0.9985$). The results were expressed as ferulic acid. The analysis was carried out in triplicate.

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119

120 *2.3. HPLC analysis of single phenolic compounds*

121 Phenolic compounds were extracted from *borş* by acetone/water (80:20, v/v) as reported in
122 Pasqualone et al. (2018), and then the extract was dried by rotary evaporator and dissolved in 2
123 mL of 80:20 methanol/water (v/v). An aliquot of 50 µL of phenolic extract was filtered on 0.45
124 µm polytetrafluoroethylene (PTFE) filters (Teknokroma, Barcelona, Spain) and quali-
125 quantitatively analyzed using an Agilent 1100 Series HPLC-DAD system (Agilent Technologies,
126 Santa Clara, CA, USA), equipped with a reversed phase C18(2) Luna column (Phenomenex,
127 Torrance, CA, USA) (5 µm, 250 × 4.6 mm), in the conditions reported in Laddomada et al.
128 (2017). Peaks were identified by comparing their retention times and UV-Vis spectra to those of
129 authentic phenolic standards. All phenolic compounds were quantified via a ratio to the internal
130 standard (3,5-dichloro-4-hydroxybenzoic acid) added to every sample and using calibration
131 curves of phenolic standards. The analysis was carried out in triplicate.

132

133 *2.4. Determination of* in vitro *antioxidant activity*

134 *Borş* samples were centrifuged for 10 min at 5000 × *g*. The *in vitro* antioxidant activity was
135 assessed on supernatant by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging
136 capacity assay in the conditions reported in (Pasqualone et al., 2015), and was expressed as 6-
137 hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox). The analysis was carried out in
138 triplicate.

139

140 *2.5. Color and pH determination*

141 *Borş* was centrifuged for 10 min at 5000 × *g*, then the supernatant was put into a Petri dish placed
142 on a white paper sheet and the color of a 1-cm thick layer of sample was determined by
143 Chromameter CR-300 (Minolta, Osaka, Japan), under the illuminant D65. Brown index was
144 calculated as 100 – *L**. The determination of pH was made by a Basic20 pHmeter (Crison
145 Instruments, Alella, Spain). The determinations were carried out in triplicate.

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147 *2.6. Determination of volatile compounds*

148 Volatile compounds of *borş* samples were determined by solid phase micro-extraction (SPME)
149 coupled to gas-chromatography/mass spectrometry (GC/MS). Five mL of *borş* were put in a 20-
150 mL vial that was sealed by butyl rubber septa and aluminum crimp caps. The extraction of
151 volatile compounds was carried out by exposing a 75 μm carboxen/polydimethylsiloxane
152 (CAR/PDMS) SPME fiber (Supelco, Bellefonte, PA, USA) in the headspace of the sample at 50
153 °C for 40 min. The fiber was then desorbed for 2 min in the injection port of the gas-
154 chromatograph, operating in split-less mode. An Agilent 6850 gas-chromatograph equipped with
155 an Agilent 5975 mass-spectrometer (Agilent Technologies Inc., Santa Clara, CA, USA) was
156 used. The volatile compounds were separated on a HP-Innowax (Agilent Technologies Inc.,
157 Santa Clara, CA, USA) polar capillary column (60 m length × 0.25 mm i.d. × 0.25 μm film
158 thickness) in the conditions reported in Cosmai, Caponio, Pasqualone, Paradiso, & Summo
159 (2017). Peak identification was performed by computer matching with the reference mass spectra
160 of National Institute of Standards and Technology (NIST) and Wiley libraries. Semi-quantitative
161 data (peak areas expressed as total ion counts - TIC) were considered. The analysis was carried
162 out in triplicate.
163
164 *2.7. Sensory evaluation of main odor notes*
165 The intensity of the main odor notes of *borş* samples was determined by 8 trained panelists (four
166 males, four females, aged between 24 and 49 years), selected for their reliability, consistency and
167 discriminating ability as in Pasqualone, Piergiovanni, Caponio, Paraidso, Summo, & Simeone
168 (2011). All the samples, identified by alphanumerical codes, were randomly served to panelists
169 into plastic cups covered by an aluminum foil. The odor notes “bran”, “yogurt”, “goat milk-
170 cheese”, “pungent-sour”, “ripe/fermented fruit” (banana-like, cider-like), and “herbal/spicy” were
171 rated on an anchored line scale that provided a 0-9 score range, in the conditions reported in
172 Pasqualone et al. (2011).
173
174 *2.8. Statistical analysis*

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Data were submitted to three-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant Differences (HSD) test for *post hoc* multiple comparisons, as well as to Principal Component Analysis (PCA), by using the XLStat software (Addinsoft SARL, New York, NY, USA).

3. Results and discussion

3.1. Effect on phenolic compounds

In this study, *borş* samples were produced at three different fermentation temperatures (4, 14, and 24 °C), in order to represent the variability of an artisanal process generically carried out "in a fresh place" that is largely conditioned by season.

The total phenolic compounds (TPC) of *borş* samples were estimated by Folin-Ciocalteu assay, a fast and widely used method. However, this method is not very accurate because it measures also other reducing substances that may be present in the sample. For this reason, the samples were submitted also to determination of the single phenolic compounds by HPLC.

The levels of TPC of experimental samples ranged from 190.1 mg/L to 498.9 mg/L, expressed as ferulic acid (Table 1). Six phenolic acids, typically present in cereals, were identified: 4-hydroxybenzoic, vanillic, syringic, *p*-coumaric, sinapic, and ferulic acid as the most abundant. Ferulic acid is known to be the most represented phenolic compound in wheat bran (Klepacka & Fornal, 2006), which is the main ingredient of *borş*. Among the commercial samples, COM1 showed higher TPC levels than COM2, but the amounts of single phenolic acids determined by HPLC were similar in these two samples.

Although with a different profile, the levels of single phenolic acids observed in *borş* were similar to those detected in a previous study in almond blanching water, a by-product of almond industry. Almond blanching water is useful for enriching the dough of bakery products in antioxidant compounds (Pasqualone et al., 2018). Similarly, *borş* could be effectively used in bread-making, due to its lactic bacteria content able to confer good sensory and healthy features to the end product (Katina, Heiniö, Autio, & Poutanen, 2006).

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To estimate the influence of each processing variable – fermentation temperature, use of starter, and pasteurization – and of their interactions, three-way ANOVA and Tukey's HSD test for multiple comparisons were carried out. A significant effect of all the variables was observed, and also the interactions between variables were significant in the majority of cases.

Among the variables considered, the use of starter had the most significant effect on phenolics (P<0.001 for all the compounds). In fact, both TPC and single phenolic acids generally increased from first to second fermentation, with the exception of *p*-coumaric acid. A possible explanation for *p*-coumaric acid decreasing could be the fact that it is degraded to 4-hydroxybenzoic acid (Morón, Pozo-Morales, Benito Mora, Garvi, & Lebrato, 2018) in the second fermentation. The phenolic compounds of cereals are known to be mostly bound to the cell wall structures, which render them insoluble in water, difficult to extract and not bio-available (Klepacka & Fornal 2006). However, bioprocessing techniques involving fermentation or enzymatic treatments of wheat bran are able to release free soluble phenolics and to improve their bioaccessibility (Mateo Anson et al., 2009). Therefore, similar mechanisms probably occurred during *borş* fermentation, and were more intense in the second fermentation, when an already active starter was used. Moreover, it is popular knowledge that over time the starter becomes even more active, accelerating the productive process to maximum 1-2 days and making *borş* become more sour and concentrated (Anonymous, 2011b).

Also pasteurization had a significant effect on both TPC and single phenolic acids, but with a not univocal trend. TPC, *p*-coumaric acid, sinapic acid and ferulic acid tended to increase with thermal treatment (P<0.001), particularly in the second fermentation (the interaction "use of starter"∗"pasteurization" was significant, apart for *p*-coumaric acid), where acetic acid registered higher values (Table 2). Studies found acetic acid used in different solvent mixtures significantly contributes to extraction yield of polyphenols (Złotek, Mikulska, Nagajek, & Świeca, 2016). On the contrary, the other phenolic acids tended to decrease in the second fermentation pasteurized *borş*. Also other authors found a diverse behavior of different phenolic acids during thermal treatments similar to pasteurization. During the hydrothermal processing of oat, for example, in particular in the steaming step, has been reported an increase of ferulic acid but a negligible

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variation of *p*-coumaric and vanillic acids (Bryngelsson, Dimberg, Kamal-Eldin, 2002). Moreover, phenolics can also undergo oxidative phenomena, therefore the effects of heat are diverse in presence of oxygen: drum drying of oat has been reported to cause a decrease of phenolics (Bryngelsson et al., 2002). On the other hand, heat treatments could also mobilize and release the bound fraction of phenolics, which prevails in cereals (Wang, He, & Chen, 2014). Therefore, different equilibrium between oxidative degradation and mobilization of bound forms could explain the diverse trends observed for different phenolic compounds during pasteurization of *borş*.

As regards the influence of fermentation temperature, it was significant except for sinapic acid. Raising the fermentation temperature induced an increase of TPC level. The effect of temperature on the single phenolic acids, instead, was again positive for some compounds (syringic, ferulic, and 4-hydroxybenzoic acids) and negative for others (vanillic and *p*-coumaric acids), and generally was more evident in the second fermentation.

3.2. Effect on antioxidant activity, color features and pH

Table 2 shows the values of the *in vitro* antioxidant activity, color and pH of *borş* samples, and the results of three-way ANOVA with Tukey's HSD test for multiple comparisons.

The antioxidant activity of *borş* ranged from 3.05 to 8.52 mmol/L Trolox and was similar to the values reported for herbal medicinal products such as sage tea (aqueous infusions of *Salvia officinalis* L.), which displays an antioxidant activity accounting for 4-18 mmol/L Trolox (Walch, Ngaba Tinzoh, Zimmermann, Stühlinger, & Lachenmeier, 2011). Some commercial pharmaceutical tinctures, such as those of mint, salvia and melissa, have higher antioxidant activity, ranging from 23.5 to 35.6 mmol/L Trolox (Kowalczyk, Biskup, & Fecka, 2012). Among the commercial *borş* samples, COM1 showed antioxidant activity markedly higher than COM2.

Borş is a brown-yellowish liquid, therefore brown index ranged from 24.6, in the sample where yellowness prevailed, to 46.0, in the brownest sample. COM1 was darker than COM2, inducing to hypothesize that a more intense thermal treatment – either in raw materials or during *borş*

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processing – was carried out in COM1, and so explaining also its higher antioxidant activity, probably due to the formation of antioxidant Maillard reaction products.

The pH ranged from 3.3 to 4.2, evidencing the role played by lactic bacteria in fermentation. Values of pH varying from 2.5 to 3.5 have been reported in *borş* by other authors (Nicolau & Gostin, 2015). The commercial samples had similar pH to the experimental ones, especially to those fermented at higher temperature.

Fermentation temperature, use of starter, and pasteurization showed a significant effect on the considered parameters. In detail, both antioxidant activity and brown index significantly increased when the starter was added, whereas pH decreased (P<0.001 for all the parameters). The fermentative processes are able to improve the antioxidant activity mainly due to an increase in the amount of free phenolic compounds consequent to the structural breakdown of plant cell walls (Hur, Lee, Kim, Choi, & Kim, 2014). The second fermentation involved starter addition and was therefore more effective, as indicated also by lower pH values. The variations induced by the use of starter were more evident at 14 °C and 24 °C than at 4 °C.

Also the fermentation temperature had a significant effect, particularly evident by comparing the results obtained at 4 °C with those at 14 °C. Both antioxidant activity and brown index increased with fermentation temperature, whereas pH decreased (P<0.001 for all the parameters).

Pasteurization had a significant effect on antioxidant activity and brown index, which increased both, but not on pH. Thermal processing in general results in browning and increase of free radical scavenging-linked antioxidant activity (Randhir, Kwon, & Shetty, 2008).

A strong correlation was found between antioxidant activity and TPC or ferulic acid (r = 0.9491 and r = 0.8301, respectively, P < 0.001). A slightly weaker correlation was observed between TPC or ferulic acid and brown index (r = 0.8966 and r = 0.7843, respectively, P < 0.001), because brownness is partly imputable to the enzymatic oxidation of phenolic compounds mediated by polyphenol oxidase and partly due to non-enzymatic browning involving Maillard reaction and caramelization.

3.3. Effect on volatile compounds and odor notes

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We investigated also the volatile compounds, responsible of the strong characteristic aroma of *borş*. During the fermentation of cereals several volatile compounds are formed, which contribute to the aroma of the end products. The presence of aromas related to diacetyl, acetic and butyric acids make fermented wheat products more appetizing (Blandino et al., 2003). The proteolytic activity of microorganisms is capable of producing the precursors of aromatic compounds, such as amino acids which may be deaminated or decarboxylated with formation of aldehydes, which in turn can be oxidized to carboxylic acids or reduced to alcohols (Mugula, Nnko, Narvhus, & Sørhaug, 2003).

The most represented volatiles of the examined *borş* samples were low-chain alcohols (ethanol and 1-butanol) and carboxylic acids (mainly acetic, followed by butyric), as well as the corresponding esters and several carbonyl compounds (Tables 2 and 3). All the detected compounds are typical of fermented cereal-based foods and beverages (Blandino et al., 2003), although with profiles changing from a product to another. The same compounds have been detected also in a proposed functional beverage obtained by fermenting emmer with lactic bacteria (Coda et al., 2011).

Ethanol is an important flavor component. Together with 1-butanol, acetic and butyric acids, and ethyl-acetate, it typically derives from the glucidic microbial metabolism (Hansen & Schieberle, 2005). Among esters, ethyl-acetate is one of the most significant compounds affecting flavor in fermented beverages. Produced from acetyl-CoA and ethanol, is the most abundant ester in cider (Herrero, García, & Díaz, 2006). Hexanal and nonanal are considered markers of lipid oxidation (Frankel, 1983) and have been previously reported in cereal-based foods such as biscuits and bread (Pasqualone et al., 2015).

A significant effect of the variables “fermentation temperature” and “use of starter” on the levels of volatile compounds was observed, whereas the variable “pasteurization” had a significant effect only on ethanol, butyric acid, ethyl acetate, and hexanal. The interactions of first and second order among these variables also had a significant effect in several cases.

Fermentation temperature had the most significant influence ($P<0.001$), and generally its increase made the volatile compound levels become higher. Ethanol had a peculiar behavior because when

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temperature raised it first increased and then decreased, probably due to oxidative conversion to acetic acid, particularly at the highest fermentation temperature. The associated compound acetaldehyde behaved similarly to ethanol. Evidently, low fermentation temperatures slowed down cellular metabolism, leading to rather low levels of volatile compounds. In fact, especially when sourdough is utilized, a careful temperature control is required to avoid excessive acidity (perceived as a sour or pungent flavor) (Katina et al., 2006).

Hexanal and nonanal levels were quite low. Slightly higher levels were observed at 4 °C for hexanal, which derives from oxidative phenomena affecting linoleic acid, abundant in the lipid fraction of raw materials. Nonanal, derived from the less oxidizable oleic acid, was always negligible.

The use of starter tended to increase the amounts of volatiles compared to the first fermentation, with the most evident effects for acetic acid at fermentation temperatures higher than 4 °C. Pasteurization was associated to a general decrease of volatile levels which, however, was significant only in few cases.

In the samples were perceived several different sensory notes: odor of bran, yogurt, goat milk-cheese, pungent-sour, and reminiscent of ripe-fermented fruit. A herbal-spicy note was also scored, because its intensity was relatively strong in the two commercial samples collected. However, this odor note was very weak in the experimental samples. The intensity of each odor descriptor is reported in Table 4.

Fermentation temperature, use of starter, pasteurization, and their interactions, had a significant effect on the sensory odor notes in the majority of cases. As already observed for the volatiles, the variable “fermentation temperature” had the most significant influence (P<0.001), and its increase made all the odor notes, except bran and yogurt, become stronger. Pasteurization was generally associated to a decrease of intensity of the odor notes. The use of starter caused an increase of odor notes, more significant for pungent-sour, especially at higher temperature.

Therefore, the odor notes perceived agreed with the instrumental data of volatile compounds. In fact, a marked ripe-fermented fruit odor note, reminiscent of banana and cider, was perceived in the samples fermented at 14 °C and 24 °C, that were rich of alcohols and esters typically

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responsible for such fruity odor. The odor note of ripe-fermented fruit is related to esters and alcohols such as butanol, ethyl acetate, and butyl acetate. Butyl acetate and ethyl acetate are among the main volatile compounds present in banana and responsible for its characteristic sweet aroma (Salmon, Martin, Remaud, & Fourel, 1996). Ethyl acetate, ethanol, and 1-butanol, are among the main volatiles of cider (Herrero et al., 1996). More in detail, 1-butanol odor comes from a blend of banana and fusel odors, also described as a sweet-rancid odor (Salmon et al., 1996).

Moreover, the *borş* samples fermented at 14 °C and 24 °C showed also a pungent-sour note, typical of vinegar, and a goat milk/cheese note, that agreed with the detection of relevant amounts of acetic acid and butyric acid, respectively.

The *borş* samples fermented at 4 °C, instead, showed more intense bran and yogurt notes, having higher levels of diacetyl, acetaldehyde and hexanal, coupled to lower levels of butyric and acetic acids than other samples. Acetaldehyde and diacetyl contribute most to the typical aroma of yogurt (Cheng, 2010), and diacetyl is known for its intense buttery aroma. The bran odor note could be related to hexanal, which characterizes relatively fatty and polyunsaturated raw materials such as bran.

The commercial samples COM1 and COM2 displayed a herbal-spicy odor note probably due to the addition of flavoring herbs, which were not added to the experimental samples. Other odor notes observed in the commercial samples, more evident in COM2 than in COM1, were the fruity and the pungent ones, imputable to a relevant presence of ethyl acetate and acetic acid (the highest levels observed among all samples tested). Moreover, COM1 was dominated by a caramel odor note probably coming from thermally treated/malted raw material. Despite this study was not aimed at giving an insight of the qualitative features of commercial *borş*, it is worth noting that these two samples showed different sensory and physico-chemical features, demonstrating that there is a real need for a more effective control and standardization of the productive process.

3.4. Principal component analysis (PCA)

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The whole analytical dataset was submitted to PCA (Fig. 2) to point out the main factors influencing contemporarily the physico-chemical data and aroma of experimental samples of *borş*, so as to obtain useful information for balancing desired (such as antioxidant activity and content of phenolic compounds) and undesired (such as strong pungent-sour or goat milk-cheese odors) characteristics.

The first and second main components described 62% of system variability. The distribution of samples appeared mostly influenced by the use of starter and by fermentation temperature, whereas pasteurization played a minor role. The use of starter discriminated the *borş* samples along the F2 and was positively correlated with antioxidant activity and content of phenolic compounds (especially ferulic, sinapic and vanillic acids). The F1 discriminated the samples principally as a function of temperature, distinguishing the samples produced at 4 °C (1AP, 1ANP, 2AP, 2ANP) from those obtained at 14 °C and 24 °C, which were mixed altogether. Higher temperatures were positively correlated with the levels of carboxylic acids and esters, as well as with the intensity pungent-sour, goat milk-cheese and ripe-fermented fruit odor notes, whereas were negatively correlated with yogurt odor and bran odor. Therefore, the addition of starter, coupled with fermentation at low temperature (4 °C), allowed to smooth the intensity of pungent-sour, goat milk-cheese and ripe-fermented fruit odor notes, while increasing the level of phenolic compounds and diacetyl, enhancing the antioxidant activity.

The commercial *borş* samples COM1 and COM2 were very different from each other and grouped with 4 °C fermented-starter added and with 14 °C fermented-no starter added experimental samples, respectively, evidencing the heterogeneity of processing conditions.

4. **Conclusions**

This research allowed ascertaining that the main parameters of the productive process of *borş* have a significant effect on the physico-chemical characteristics of the end product, therefore their control would allow to effectively balance the content of phenolic compounds and the aromatic profile. Specifically, the addition of starter, coupled with a fermentation temperature of 4 °C, allows reducing the intensity of some, less desired, odor notes – such as pungent-sour and

398 goat milk-cheese – while increasing the level of phenolic compounds and enhancing the
399 antioxidant activity.
400 The fermentation temperature is affected by both the temperature of raw materials, water and
401 flour-bran mixture, and the external temperature. In practice, these are not strictly controlled but
402 are subject to changes on a daily and seasonal basis. A more stringent control of fermentation
403 temperature of *borş* is highly recommended to obtain the desired aromatic attributes without an
404 excessive pungent note.
405 Traditional cereal-based beverages such as *borş* could fulfill the increasing demand of consumers
406 for non-dairy milk substitutes with high acceptability and functionality, given by their pleasant
407 flavor and antioxidant compounds, respectively. With a more strict control of processing
408 parameters, this product could be appreciated also in the international market, far beyond the area
409 of production.
410

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415

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Table 1. Total phenolic compounds, determined by Folin Ciocalteu reaction, and HPLC-determined single phenolic acids of experimental samples of *borş* derived from two subsequent fermentations (1 and 2, the second in presence of starter) carried out at three different fermentation temperatures (4, 16 and 24 °C, indicated with the letters A, B, and C, respectively). Samples were either pasteurized (P) or not pasteurized (NP). Commercial samples (COM1 and COM2) were inserted for comparison.

	Total phenolic compounds (mg/L ferulic acid)		**4-Hydroxybenzoic acid (mg/L)**		**Vanillic acid (mg/L)**		**Syringic acid (mg/L)**		***p*-Coumaric acid (mg/L)**		**Sinapic acid (mg/L)**		**Ferulic acid (mg/L)**	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Fermented at 4 °C														
ANP	190.1±8.3i	212.4±10.3hi	1.7±0.3de	4.8±0.7b	0.9±0.1f	3.2±0.1a	0.7±0.1de	1.9±0.2b	1.0±0.1b	0.5±0.1c	0.6±0.1c	1.3±0.3bc	13.7±2.1d	23.4±3.6bcd
AP	207.5±6.1hi	227.2±9.6gh	0.9±0.3e	2.6±0.4cd	1.0±0.1f	3.0±0.2ab	0.8±0.1de	0.9±0.1cde	1.5±0.2a	1.1±0.2b	1.0±0.2bc	2.4±0.3a	15.5±2.6d	37.3±6.1ab
Fermented at 14 °C														
BNP	242.3±4.2fg	371.3±9.5c	1.7±0.5de	5.9±0.8ab	1.0±0.1f	2.3±0.6abcd	0.7±0.1de	2.0±0.3b	0.5±0.1c	0.0±0.0d	0.8±0.1bc	1.4±0.4b	14.6±2.3d	26.1±3.7bcd
BP	327.8±7.7d	498.9±10.1a	1.0±0.2e	3.3±0.6c	1.3±0.2ef	1.4±0.3cdef	0.5±0.1e	1.3±0.1c	0.6±0.1c	0.0±0.0d	0.9±0.3bc	2.5±0.4a	16.7±2.6cd	46.2±7.2a
Fermented at 24 °C														
CNP	256.5±7.1ef	379.1±9.8c	2.6±0.5cd	6.3±0.7a	1.4±0.1def	2.4±0.6abc	1.0±0.1cd	2.5±0.1a	0.5±0.1c	0.0±0.0d	0.8±0.2bc	1.4±0.4b	17.1±2.5cd	30.1±6.2bc
CP	275.5±5.5e	472.6±13.5b	0.9±0.3e	5.6±0.6ab	0.6±0.2f	2.2±0.6bcde	0.5±0.1e	2.0±0.2b	0.4±0.1c	0.0±0.0d	1.1±0.1bc	2.7±0.2a	19.1±2.1cd	47.8±9.8a
Commercial samples														
COM1	439.4±16.5		1.8±0.2		1.4±0.1		0.6±0.2		1.1±0.2		1.9±0.3		19.9±1.1	
COM2	290.5±4.5		0.7±0.1		0.2±0.1		0.3±0.1		0.8±0.2		1.0±0.2		17.7±0.5	
Significance of variables and interactions														
Fermentation temperature (T)	*P*<0.001		*P*<0.001		*P*<0.01		*P*<0.001		*P*<0.001		NS		*P*<0.05	
Use of starter (S)	*P*<0.001		*P*<0.001		*P*<0.001		*P*<0.001		*P*<0.001		*P*<0.001		*P*<0.001	
Pasteurization (P)	*P*<0.001		*P*<0.001		*P*<0.05		*P*<0.001		*P*<0.001		*P*<0.001		*P*<0.001	
T*S	*P*<0.001		*P*<0.01		*P*<0.001		*P*<0.001		NS		NS		NS	
T*P	*P*<0.001		NS		NS		NS		*P*<0.001		NS		NS	
S*P	*P*<0.001		*P*<0.05		NS		*P*<0.001		NS		*P*<0.001		*P*<0.001	
T*S*P	*P*<0.001		*P*<0.01		*P*<0.05		*P*<0.01		NS		NS		NS	

Different letters per each compound indicate significant differences at $P < 0.05$.

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Table 2. *In vitro* antioxidant activity, color, pH, volatile alcohols and volatile carboxylic acids (peak area, TICs*10^6) of experimental samples of *borş* derived from two subsequent fermentations (1 and 2, the second in presence of starter) carried out at three different fermentation temperatures (4, 16 and 24 °C, indicated with the letters A, B, and C, respectively). Samples were either pasteurized (P) or not pasteurized (NP). Commercial samples (COM1 and COM2) were inserted for comparison.

	Antioxidant activity (mmol/L Trolox)		**Brown index (100-*L**)**		**pH**		**Ethanol**		**1-Butanol**		**Acetic acid**		**Butyric acid**	
	1	2	1	2	1	2	1	2	1	2	1	2	1	2
Fermented at 4 °C														
ANP	3.05±0.06f	3.56±0.24e	24.6±0.5i	28.5±0.4g	4.1±0.1a	3.7±0.1bcd	3.4±0.3cd	3.0±0.7cde	1.8±0.5d	1.0±0.3d	6.2±0.2d	6.9±1.2d	0.9±0.7de	3.0±0.9de
AP	3.14±0.15f	4.33±0.03d	28.7±0.4g	32.4±0.4e	4.2±0.1a	3.6±0.1cd	0.4±0.1fg	2.5±0.2de	1.5±0.2d	0.3±0.1d	5.3±1.3d	6.1±0.9d	0.1±0.1e	2.2±1.3de
Fermented at 14 °C														
BNP	3.71±0.03e	5.78±0.14c	26.7±0.4h	38.4±0.2c	3.8±0.1b	3.4±0.1fg	4.1±0.2c	7.6±0.6a	14.7±5.4bc	22.8±4.0abc	39.5±3.5c	61.6±9.8b	18.4±1.5bc	33.1±5.1a
BP	4.10±0.07d	8.52±0.04a	35.7±0.5d	46.0±0.3a	3.7±0.1bc	3.5±0.1ef	3.5±0.2cd	6.1±0.5b	13.8±3.4c	21.1±5.5abc	37.3±3.5c	63.2±12.6b	21.1±2.6bc	25.9±3.7ab
Fermented at 24 °C														
CNP	3.52±0.12e	5.64±0.02c	27.1±0.9h	39.6±0.3c	3.6±0.1cde	3.3±0.1g	1.7±0.1ef	2.0±0.7e	23.8±2.3ab	30.1±1.3a	70.2±11.1b	108.1±9.7a	26.4±3.1ab	26.1±6.8ab
CP	3.57±0.11e	7.12±0.19b	30.8±0.5f	43.5±0.4b	3.6±0.1cde	3.4±0.1fg	0.2±0.1g	1.9±0.8e	21.7±2.8abc	24.9±3.9a	72.1±9.1b	102.3±13.2a	12.1±5.7cd	22.9±5.5abc
Commercial samples														
COM1	7.54±0.16		40.9±0.3		3.4±0.1		3.4±0.2		1.1±0.3		14.2±0.9		1.8±0.2	
COM2	3.04±0.06		27.8±0.3		3.3±0.1		4.5±0.3		12.6±1.8		102.4±6.8		12.3±0.6	
Significance of variables and interactions														
Fermentation temperature (T)	*P*<0.001		*P*<0.001		*P*<0.001		*P*<0.001		*P*<0.001		*P*<0.001		*P*<0.001	
Use of starter (S)	*P*<0.001		*P*<0.001		*P*<0.001		*P*<0.001		*P*<0.001		*P*<0.001		*P*<0.001	
Pasteurization (P)	*P*<0.001		*P*<0.001		NS		*P*<0.001		NS		NS		*P*<0.01	
T*S	*P*<0.001		*P*<0.001		*P*<0.001		*P*<0.05		*P*<0.05		*P*<0.001		NS	
T*P	*P*<0.001		*P*<0.001		NS		*P*<0.001		NS		NS		*P*<0.05	
S*P	*P*<0.001		NS		NS		*P*<0.001		NS		NS		NS	
T*S*P	*P*<0.001		NS		*P*<0.05		*P*<0.001		NS		NS		*P*<0.05	

Different letters per each parameter indicate significant differences at $P < 0.05$; NS: not significant.
Trolox = 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid**.**

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Table 3. Main volatile carbonyl compounds and esters (peak area, TICs*10^6) detected in experimental samples of *borş* derived from two subsequent fermentations (1 and 2, the second in presence of starter) carried out at three different fermentation temperatures (4, 16 and 24 °C, indicated with the letters A, B, and C, respectively). Samples were either pasteurized (P) or not pasteurized (NP). Commercial samples (COM1 and COM2) were inserted for comparison.

	Ethyl acetate		**Butyl acetate**		**Diacetyl**		**Acetaldehyde**		**Hexanal**		**Nonanal**	
	1	2	1	2	1	2	1	2	1	2	1	2
Fermented at 4 °C												
ANP	0.6±0.1f	2.5±0.7ef	0.2±0.1d	0.5±0.1d	8.7±2.0a	5.1±2.1bcd	0.4±0.1c	0.2±0.1cd	2.4±0.1b	1.1±0.1c	0.3±0.1bcd	0.6±0.1a
AP	0.3±0.1f	0.9±0.3f	0.1±0.1d	0.2±0.1d	5.9±2.3abc	7.5±1.1ab	0.2±0.1cd	1.1±0.1a	4.5±0.4a	1.0±0.1c	0.5±0.1ab	0.5±0.1ab
Fermented at 14 °C												
BNP	6.8±0.6cd	9.5±1.3bc	8.8±1.5c	15.1±2.3b	3.2±0.1cd	1.8±0.6d	0.7±0.2b	0.1±0.1cd	0.2±0.1d	0.4±0.1d	0.2±0.1cd	0.1±0.1d
BP	5.5±1.1de	9.2±1.4bcd	7.9±1.5c	17.5±1.1ab	3.3±0.2cd	1.9±0.3d	0.1±0.1cd	0.1±0.1cd	0.4±0.1d	0.5±0.1d	0.3±0.1bcd	0.2±0.1cd
Fermented at 24 °C												
CNP	13.5±3.6a	12.8±1.3ab	15.9±2.2b	22.1±4.7a	2.6±0.5cd	1.9±0.2d	0.1±0.1d	0.2±0.1cd	0.2±0.1d	0.4±0.1d	0.2±0.1cd	0.6±0.2a
CP	10.5±0.6abc	11.8±0.8ab	13.1±2.3bc	23.3±2.2a	1.8±0.5d	1.7±0.4d	0.2±0.1cd	0.1±0.1cd	0.4±0.1d	0.4±0.1d	0.4±0.1abc	0.3±0.1bcd
Commercial samples												
COM1	3.8±0.6		0.8±0.1		4.3±0.4		0.3±0.2		3.6±0.6		1.7±0.2	
COM2	11.3±1.2		6.7±0.6		1.2±0.1		0.7±0.2		1.1±0.3		0.8±0.2	
Significance of variables and interactions												
Fermentation temperature (T)	*P*<0.001		*P*<0.001		*P*<0.001		*P*<0.001		*P*<0.001		*P*<0.001	
Use of starter (S)	*P*<0.01		*P*<0.001		*P*<0.05		NS		*P*<0.001		*P*<0.05	
Pasteurization (P)	*P*<0.01		NS		NS		NS		*P*<0.001		NS	
T*S	*P*<0.05		*P*<0.001		NS		*P*<0.001		*P*<0.001		*P*<0.01	
T*P	NS		NS		NS		*P*<0.001		*P*<0.001		NS	
S*P	NS		NS		*P*<0.05		*P*<0.001		*P*<0.001		*P*<0.001	
T*S*P	NS		NS		*P*<0.05		*P*<0.001		*P*<0.001		*P*<0.01	

Different letters within columns indicate significant differences at 0.05 *P* value; NS: not significant; n.d.: not detected.

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Table 4. Intensity of odor notes detected in experimental samples of *borş* derived from two subsequent fermentations (1 and 2, the second in presence of starter) carried out at three different fermentation temperatures (4, 16 and 24 °C, indicated with the letters A, B, and C, respectively). Samples were either pasteurized (P) or not pasteurized (NP). Commercial samples (COM1 and COM2) were inserted for comparison.

	Bran		**Yogurt**		**Goat milk-Cheese**		**Pungent-sour**		**Ripe-Fermented fruit**		**Herbal-Spicy**	
	1	2	1	2	1	2	1	2	1	2	1	2
Fermented at 4 °C												
ANP	2.3±0.1a	2.6±0.2a	5.5±0.5ab	6.3±0.6a	0.2±0.1d	0.2±0.1d	0.0±0.0f	0.1±0.1f	0.2±0.1c	0.1±0.1c	0.0±0.0b	0.0±0.0b
AP	2.5±0.1a	2.5±0.2a	4.7±0.7b	5.6±0.3ab	0.1±0.1d	0.1±0.1d	0.2±0.1ef	1.0±0.5def	0.1±0.1c	0.2±0.1c	0.0±0.0b	0.1±0.1ab
Fermented at 14 °C												
BNP	0.1±0.1c	0.1±0.1c	2.3±0.3c	2.5±0.3c	3.3±0.4a	2.0±0.4bc	1.3±0.2cd	1.1±0.4cde	7.2±0.6a	7.4±0.6a	0.1±0.1ab	0.2±0.1a
BP	0.2±0.1c	0.2±0.1c	1.2±0.3d	2.4±0.6c	2.4±0.5b	2.5±0.4b	1.3±0.4cd	3.3±0.3b	7.8±0.7a	7.8±0.8a	0.2±0.1a	0.1±0.1ab
Fermented at 24 °C												
CNP	0.1±0.1c	2.5±0.2a	0.1±0.1e	0.2±0.1de	1.3±0.2c	3.3±0.2a	2.1±0.4c	3.2±0.5b	6.5±0.4a	7.1±0.9a	0.1±0.1ab	0.1±0.1ab
CP	0.1±0.1c	1.3±0.3b	0.2±0.1de	0.1±0.1e	0.1±0.1d	0.2±0.1d	3.2±0.3b	4.9±0.6a	6.8±0.4a	4.2±0.3b	0.0±0.0b	0.0±0.0b
Commercial samples												
COM1	2.5±0.2		0.1±0.1		0.0±0.0		0.2±0.1		1.3±0.3		4.3±0.2	
COM2	2.7±0.1		0.2±0.1		0.1±0.1		1.8±0.4		3.4±0.3		4.6±0.3	
Significance of variables and interactions												
Fermentation temperature (T)	*P*<0.001		*P*<0.001		*P*<0.001		*P*<0.001		*P*<0.001		*P*<0.001	
Use of starter (S)	*P*<0.001		*P*<0.001		*P*<0.001		NS		*P*<0.01		NS	
Pasteurization (P)	*P*<0.01		*P*<0.001		*P*<0.001		*P*<0.001		NS		NS	
T*S	*P*<0.001		*P*<0.05		NS		*P*<0.001		*P*<0.001		NS	
T*P	*P*<0.001		*P*<0.05		NS		*P*<0.05		*P*<0.05		*P*<0.05	
S*P	*P*<0.001		NS		*P*<0.001		*P*<0.01		*P*<0.001		NS	
T*S*P	*P*<0.001		NS		*P*<0.001		*P*<0.001		*P*<0.001		*P*<0.01	

Different letters per each parameter indicate significant differences at $P < 0.05$; NS: not significant.

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Figure captions

Fig. 1. Experimental design and sampling points during of *borş* production. The letters "A", "B", and "C" indicate three fermentation temperatures: 4, 14, and 24 °C; the numbers "1" and "2" are referred to first and second fermentation (the latter in the presence of starter); the letters "NP" and "P" indicate "not pasteurized" and "pasteurized", respectively.

Fig. 2. Results of principal component analysis (PCA) carried out on physico-chemical data and aroma of experimental samples of *borş* derived from two subsequent fermentations (1 and 2, the second in presence of starter) carried out at three of fermentation temperatures (4, 14 and 24 °C, indicated as A, B, and C, respectively). Samples were either pasteurized (P) or not pasteurized (NP). COM1 and COM2 are two commercial *borş* samples.

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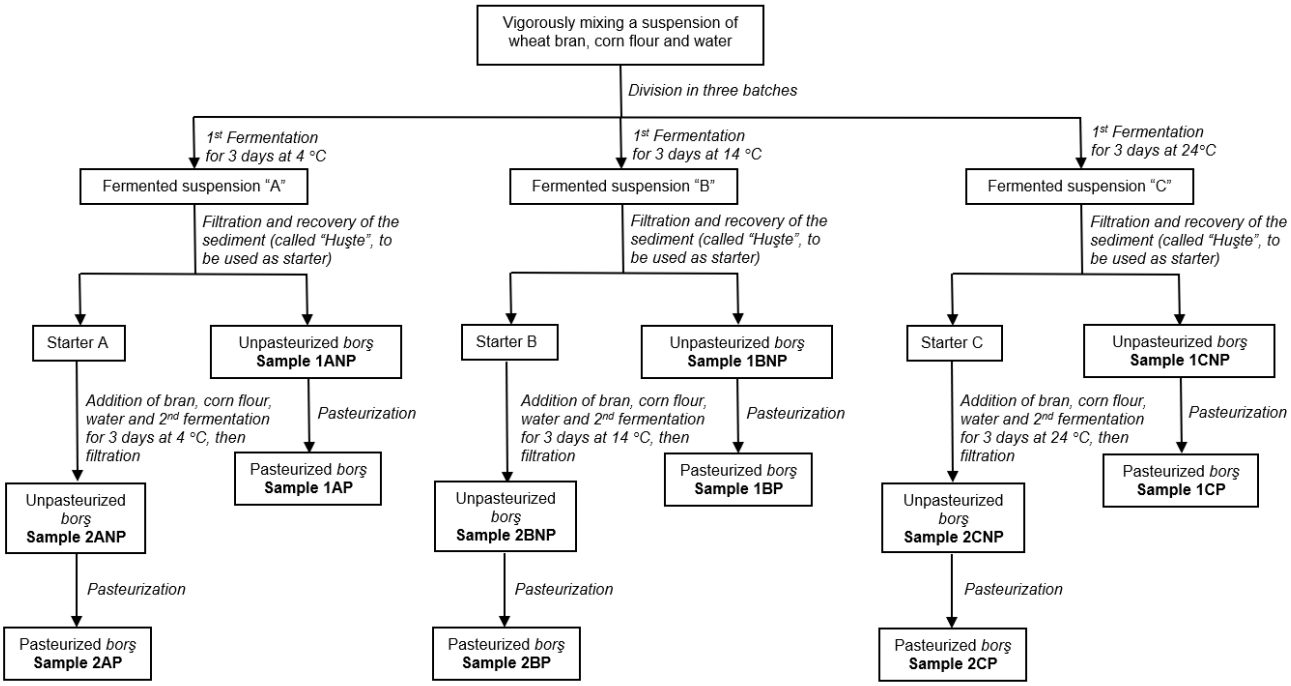

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