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RESEARCHES REGARDING THE MICROBIOTA OF THE HOMEMADE BORS, AS HEALTHY SOURCE

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Key words: *traditional borş, wheat bran, microbiota*

INTRODUCTION

The present study was carried out in two directions which had as common purpose the obtaining of information about the traditional product borş, as well as a qualitative and quantitative evaluation of its microbiota.

Borş is a traditional Romanian product, a liquid obtained from the fermentation of a mixture of cereals in which wheat bran predominates. This product prepared at home or in the industrial system is used to give a sour taste to traditional soups or is consumed as a soft drink (Grosu-Tudor, S et al., 2019; Radu, A. R, 2014).

The fermentation process has the effect of lowering the level of carbohydrates and indigestible polysaccharides and improves the shelf life and texture, increases the antioxidant activity and contributes to the taste and aroma of the final product.

Today, many of the fermentation-based food and beverage products are manufactured at home or in small businesses, often using the traditional fermentation process (Wouters, D., 2013; Feng, Y., et al., 2019).

There is little information about the Romanian borş, which are found mainly in travel and gastronomy guides.

From a scientific point of view, the product has not been thoroughly analyzed so far.

However, people who prepare and consume borş have long believed that in addition to the pleasant taste, it can also have beneficial effects on human health, especially due to the high level of vitamins and minerals.

Borş is a good tonic, which is used for the beneficial effects observed in respiratory diseases, digestive problems (indigestion, regurgitation), biliary and liver diseases and even in the treatment of cancer (Radu, A. R., 2014; Gille, D., 2018).

Homemade borş, as grandmother used to do, has many benefits for the body compared to commercial products that may contain preservatives, flavor enhancers and various food additives, which alter its nutritional quality (Wouters, D., et al., 2013; Basinskiene, L., et al., 2020).

The microbial ecology of many products and beverages made by cereals fermentation (rice, wheat, corn or sorghum) is quite complex and not yet fully known, but most likely mixed cultures of yeast, bacteria and filamentous mold are involved. Yeasts act mainly on carbohydrates while bacteria present proteolytic activity (Wouters, D., et al., 2013; Blandino, A et al., 2013; Basinskiene, L., et al., 2020; Anal, A.K., 2019).

The wheat bran resulting from the process of milling wheat is considered a by-product with a low commercial value and is most often used in baked products to increase the level of insoluble fiber or is used to animal feeding.

In terms of nutritional value, bran has been described as an interesting raw material not only due to its high dietary fiber content, but also due to the presence of other biologically active compounds such as vitamins B and E, alkylresorcinols, lignans, phenolic acids, phytosterols, tocopherols and folates (Petrova, P., et al., 2020; Anal, A.K., 2019; Erkmen, O., 2022).

The addition of wheat bran in products is very limited due to technological conditions and sensitive properties (it is bitter).

Several strategies are applied today to improve these sensory qualities, the most promising being fermentation.

For example, the main component used in the production of Romanian borş is wheat bran, to which hot water and part of the previously fermented portion (huşte) are added (Blandino, A et al., 2013; Erkmen, O., 2022).

Fermentation can last between 2-3 days and is done in wooden or glass vessels, the liquid could be replaced daily with warm water to get more fermented product.

Borş is an acidic liquid used not only in Romanian cuisine to give a sour taste to a variety of traditional soups known as borş or soup, but also as a soft drink. Another Romanian drink made by fermenting cereal bran in which millet predominates is braga, a sweet and sour drink (Radu, A. R., 2014).

The microbiota developed after fermentation of the mixture of cereals, herbs and huşte has a different composition at different times prior to

fermentation and borş obtaining (Grosu-Tudor, S., 2019; Petrova, P., 2020).

MATERIALS AND METHODS

Preparation methods of homemade borş

A theoretical research was made by analyzing the specialized literature and the gastronomic profile sites and the most frequently accessed recipes for preparing homemade borş were quantified.

A target group of 50 people, aged between 15 and 60, from Secuieni, Bacau County, was interviewed through a questionnaire. The questionnaire, as a research method, involves the use of a set of open or closed questions, designed so as to record in writing the opinions of the respondents to a topic, answers that will be statistically analyzed (Chelcea, S.,2007).

For the subject of our study, 4 closed questions and 1 open question were asked. These were statistically interpreted.

Preparation and monitoring of borş samples in the laboratory

After evaluating the first stage of the study, the preparation and monitoring of 9 experimental variants of borş were realized. The variants were characterized from organoleptic point of view and the developed microbiota was analyzed. In the preparation of the experimental borş variants, the quantities of wheat bran and huşte used in the experiment were varied, as well as the water type (see Table 1, Fig. 1).

After weighing the ingredients and mixing them with water, the product is mixed and left at 30-35 °C. The used water and the aromatic plants were brought to boiling point and then allowed to cool to 45 °C. Then it was poured over the corn flour with wheat bran and huşte mixture.

The initial measurements and observations for each sample were made after 12 hours of mixing the ingredients, the time required to accommodate and to begin the metabolic processes due to the microbiota developed in each sample. The final measurements were made after one week.

The evaluation of each sample included: organoleptic analysis, measurement of physico-chemical parameters: pH, dry matter content, carbohydrate content, microbial biomass analysis, determination of cell number using the Thoma chamber, Gram staining and transfer to culture media for evaluation of cultural aspects (Cappuccino, J. G., 2020; Li, E., et al., 2010).

The microbiota developed in each experimental variant was quantitatively evaluated by: biomass (mg), determination of cell number using the Thoma chamber (cells/mL) and the microorganisms identified in each sample were morphologically and culturally evaluated, and Gram stains were made. To determine the microbial biomass by wet weight (mg), it was proceed as follows: 5 mL were collected and centrifuged from each sample (apparatus EBA 200 Hettich) for 3 min at 15000 rpm (Li, E., et al., 2010; Erkmen, O., 2022).

The evaluation of carbohydrates amount (° Brix) and of the dry matter (SU %) were done with the help of the ATC portable refractometer.

Determining the number of cells was realized with the help of CE Thoma ISOLAB camera. The microscope used was Euromex Holand BS 1152-EPL optical microscope and the calculations were made with the formula:

$$N \text{ (cells/ mL)} = n \times 10^4 \times F \quad (1)$$

where: n - number of cells from square

F - dilution factor

Gram staining, used as diagnostic purposes (Fig. 2) has the widest use in microbiology, being the first step in the determination of bacteria. Then etching with Lugol's solution, then bleaching with 9 : 1 acetone alcohol and recoloring with a basic red dye, fuchsia Pfeiffer. The result of this analysis is the separation of bacterial strains into: Gram positive (G +), Gram negative (G-) or Gram variable (Cappuccino, J. G., et al., 2020; Erkmen, O., 2022).

Table 1. Experimental variants prepared in the laboratory

Sample	Corn flour (g)	Wheat bran (g)	"Huşte" (g)	Distilled water (mL)	Fountain water (mL)	"Aquatique" mineral water (mL)
V1-a	50	50	50	300	-	-
V1-b	50	100	100	-	300	-
V1-c	50	150	150	-	-	300
V2-a	50	100	100	300	-	-
V2-b	50	100	150	-	300	-
V2-c	50	150	150	-	-	300
V3-a	50	150	150	300	-	-
V3-b	50	100	100	-	300	-
V3-c	50	150	150	-	-	300
M-control	-	-	150	300	-	-

Figure 1. Materials and experimental borş variants obtained in the laboratory

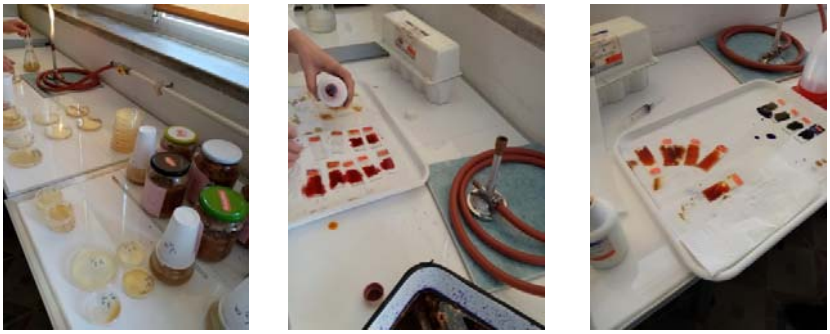


Figure 2. Gram staining of the tested samples

RESULTS AND DISCUSSIONS

Five recipes for preparing homemade borş were identified as the most frequently accessed from different web pages and specialized literature.

Depending on the number of accesses, the following recipes were highlighted:

- recipe 1- https://www.bucataras.ro/retete/cum-se-umple-borsul-83506.html,
- recipe 2-https://www.libertatea.ro/lifestyle/cum-se-face-borsul-13301,
- recipe 3- https://www.lalena.ro/reteta/985/Bors-acru-de-casa,
- recipe 4-https://savoriurbane.com/cum-se-umple-borsul-de-casa-reteta-traditionala-veche,
- recipe 5- https://pofta-buna.com/bors-de-putina-cum-se-umple-reteta-de-bors-cu-huste.

The recipes marked with numbers 6 and 7 collected and used in the area of Chiticeni, Saucesti commune, Bacau and those found in the bibliography mentioned above, have as common denominator the use of the following ingredients: wheat bran, corn flour, huşte, aromatic plants (larch, thyme, basil, cherry springs). Preparation conditions of the product were: boiled water and keeping the product at about 30 °C.

Recipe No. 6

The local recipe contains: 500 g of wheat bran, 300 g of corn flour, 900 g of huşte, 5 liters of water, 3 sprigs of cherry, a bunch of larch leaves and a sprig of thyme.

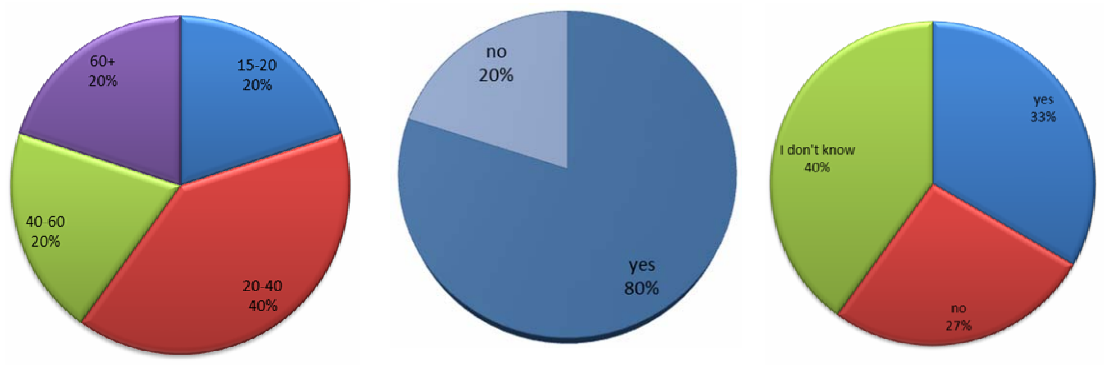
Preparation: The corn flour, wheat bran and huşte were mixed with a bit of warm water in a bowl. The sprigs of cherry, thyme sprigs and larch leaves were added in 5 liters of water, and boiled. Then, this product was added in the above-obtained cereal composition. The obtained product was well mixed with a wooden spoon, and then it was covered and left to warm, at temperatures above 30 ^{o}C. After 3 days in the warm environment, in which it is mixed several times a day, the product is left to clear and then drained from the huşte. The homemade borş is thus obtained.

Recipe No. 7

The local recipe contains the following ingredients: 500 g wheat bran, 300 g corn flour, 900 g huşte, 4 liters of water, 3 sprigs of cherry, 2 strands of basil, a few leaves of *Melissa* L.

Preparation: The corn flour, wheat bran and huşte were mixed with a bit of warm water in a bowl. The sprigs of cherry, strands of basil and a few leaves of *Melissa* L. were added in 4 liters of water and boiled. Then, this product was added in the above-obtained cereal composition. The other operations are identical to those described in Recipe no. 6.

The questionnaire was applied in Chiticeni locality, Saucesti commune, Bacau, on 50 respondents with ages between 15 and over 60 years old. 10% of men and 90% of women took part in the survey. It has been shown that there is no scientific knowledge of the nutritional qualities of borş traditional product, but it is used in the preparation of traditional food (Fig. 3). The product is prepared at home, especially by people over 40 years old (61%), young people preferring to buy it (39%).



a **b** **c**

Figure 3. Respondents at the questionnaire (a), Using of borş in food preparation (b), Knowing the qualities of the borş product (c)

Organoleptic and physico - chemical characterization of borş samples

The assessment of the organoleptic qualities was done by tasting and grading on a scale of appreciation from 1 - 5 for taste, from 1 - 3 for appearance, and for color, the intensity was appreciated (Table 2 and Table 3).

Table 2. Initial organoleptic evaluation of borş experimental variants

Sample Initial measurements	Taste	Appearance	Color
M-control	5	1	yellow
V1-a	1	1	yellow
V1-b	2	2	yellow
V1-c	3	2	yellow
V2-a	2	1	yellow
V2-b	2	2	yellow
V2-c	4	3	yellow
V3-a	2	2	yellow
V3-b	3	2	yellow
V3-c	5	3	yellow

Scoring interval: taste: 1-5; appearance: 1-3.

It was found after tasting that the taste and appearance of the product varies depending on: the type and quality of water used and the amount of wheat bran.

Thus the most sour was the variant - *V3-c* (Aquatique water, 150 g wheat bran and 150 g huşte); less sour - variants *V1-a* and *V1-b* (distilled water and well water with a 50 / 100 g wheat bran and 50/100 g huşte).

Direct counting with the help of Thoma camera is an indirect method of assessing the microbiota, a method that has the advantage of observing their morphology, size and grouping, but being a probable method, to confirm the results it is necessary to corroborate with cultural analysis methods (Cappuccino, J. G., et al., 2020).

Table 3. Final organoleptic evaluation of borş experimental variants

Sample Final measurements	Taste	Appearance	Color
M-control	5	1	yellow*
V1-a	2	1	yellow
V1-b	2	2	yellow +
V1-c	4	2	yellow
V2-a	3	2	yellow*
V2-b	3	2	yellow
V2-c	5	3	yellow
V3-a	3	2	yellow**
V3-b	4	2	yellow*
V3-c	5	3	yellow ++

Note: taste: 1-5; appearance: 1-3; color: dark yellow (*), color intensity (+)

In Table 4 are detailed the aspects observed in the case of each analyzed experimental variants.

As can be seen, the most numerous microbiota variant is *V3-c*, followed by *V1-c*, *V2-b* and *V1-a*, the least numerous being *V3-a*.

Regarding the morphology and the way of cells grouping, it could be found that the cocobacilli form predominates, followed by the bacillary and the round shape with dimensions of microns order (Table 4).

The lowest initial biomass was identified in the case of *V2-a* variant, and the highest in the case of *V3-b*. The lowest final biomass was identified in the case of *V3-c* variant and the highest for *V2-a*.

This is due to the active process of bacterial multiplication.

Table 4. Evaluation of the microbiota of borş experimental variants (count with Thoma camera)

Experimental variants	Number of cells after 6 days	Observations
M-control	$3.2x10^4$	Majority coccobacillus, few isolated cocci; small sizes, moving bacteria
V1-a	$3.5x10^4$	Majority coccobacillus, few isolated cocci; small sizes, moving bacteria
V1-b	$2.5x10^4$	Majority coccobacillus, few diplococci, rarely tetrads; small sizes, moving bacteria
V1-c	$3.5x10^4$	Majority cocci (isolated and diplococci), few bacilli; small sizes, 80% immobile bacteria
V2-a	$3x10^5$	Majority bacilli, few isolated cocci; small sizes, approx. 15% immobile bacteria
V2-b	$3.5x10^4$	Majority bacilli, few isolated cocci; small sizes, immobile bacteria (aprox. 70-80%)
V2-c	$2.5x10^4$	Isolated cocci, rarely diplococci; small sizes, immobile bacteria
V3-a	2.2×10^4	Majority coccobacillus, few isolated cocci; small sizes, moving bacteria
V3-b	$3x10^4$	Majority coccobacillus, few isolated cocci; small sizes lactobacilli, moving bacteria
V3-c	$3.6x10^4$	Majority coccobacillus, few diplococcic, lactobacilli; small sizes, moving bacteria

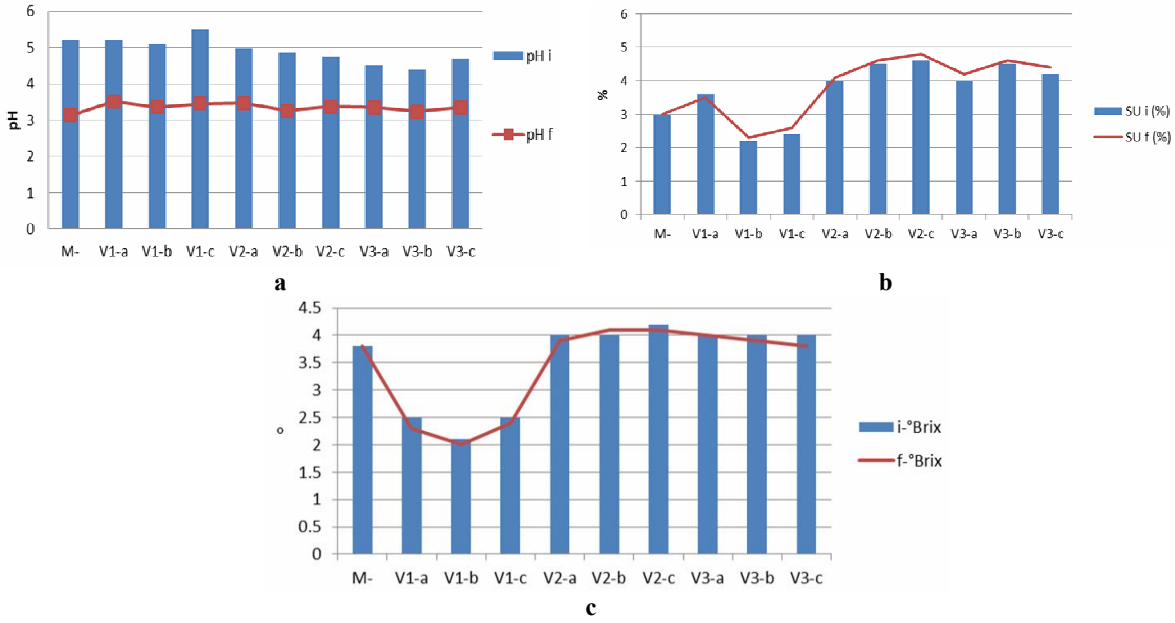


Figure 4. Physico-chemical measurements of borş experimental variants:
pH (a); dry matter (%) (b); °Brix (c)

CONCLUSIONS

The analysis of the questionnaire results of the media materials regarding the traditional borş product, illustrates an increased interest of the population for its using and preparation. This interest is supported by the health and nutritional value of this product.

Analysis of experimental variants of borş prepared in the laboratory from: different types of water (Aquatique mineral water, distilled water and spring water from own household) along with various proportions of huşte, corn flour and wheat bran showed that there were variations compared to the control sample.

Physico-chemical measurements and organoleptic analyzes performed on the final samples showed a slight increase in the acidity of the experimental variants of borş compared to the initial measurements (Fig. 4-a,b,c).

The decrease in pH was manifested in all borş experimental samples as the microbiota develops especially in the variants with more huşte and wheat bran and stabilized after 72 hours around the average 3.14.

The quantitative evaluation of the microbiota was evident in the case of the final measurements, which was observed by the direct counts under the Thoma camera microscope and the observations made on the fresh and Gram stained preparations, an increase in the number of lactobacilli (*V3-c* and *V3-b* variants).

ABSTRACT

The aim of this study is the quantitative and qualitative evaluation of the homemade bors microbiota, a microbiota developed after the fermentation of the mixture of cereals, herbs and huste. Bors, a specific product for traditional Romanian gastronomy, is considered a real therapeutic remedy. It is a product rich in organic acids, minerals, vitamins and enzymes, resulting from the fermentation process of bran.

The microbiota that helps fermentation generates B vitamins, D vitamin, enzymes, minerals in easily assimilable forms (calcium, magnesium, phosphorus), and vital trace oligoelements for health: selenium, but especially chromium with important benefits in regulating blood sugar and lowering cholesterol. Homemade bors (as grandmother used to do) has many benefits for the body compared to commercial products that may contain preservatives, flavor enhancers and various food additives, which alter its nutritional quality.

The objectives of this research were: identification in the literature of studies and researches on this product; identification of conditions and ways of preparation of homemade bors, realization of experimental variants of bors in laboratory conditions: 9 experimental variants and monitoring of their physico-chemical, organoleptic parameters and evaluation of the microbiota developed in each experimental variant. A questionnaire was applied regarding the preparation and consumption of this product.

The obtained results showed a quantitative differentiation of the microbiota, of the organoleptic qualities and a variation of the physico-chemical parameters of the bors samples, depending on the quantities of the huste, flour, corn and wheat bran and the water properties used in the work.

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