MICROBIOLOGICAL ASPECT OF FERMENTATION OF *VIBURNUM OPULUS* L.

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Abstract

The theses are devoted to the study of viburnum fermentation process, the analysis of changes in the amount of yeast, the total number of microorganisms and lactic acid bacteria, as well as potential factors of influence on the studied groups of microorganisms. Changes in pH values, the number of microorganisms on different media, and potential adaptive properties of the microbiota were revealed.

Key words: *fermentation*, *Viburnum opulus*, *lactic acid bacteria, microbial interactions*, *gilaburu juice*.

Introduction. Fermented beverages based on plant raw materials are part of the traditional cuisine of many countries around the world. Depending on the geographical position of the country, the climate zone and, accordingly, the plants inherent in it, a significant variety of traditional fermented beverages is known [1]. Considering the popularity and prevalence, typical raw materials for traditional fermented beverages are cereals (millet, wheat, rice, barley, rye, oat), pseudocereals (buckwheat, amaranth, quinoa), legumes (soy, peas) [2]. However, uncharacteristic raw materials are sometimes encountered, such as black carrots, beets, grapes, cassava, pineapple, etc. [3].

Raw materials for various Turkish beverages are rather atypical, one of which is fermented viburnum juice (*Viburnum opulus*) or known in Turkish as *gilaburu* juice. Although the drink is made only in "artisanal" conditions, it attracts the attention of many scientists due to its useful therapeutic properties, for example, antispasmodic and analgesic effects. Currently, fermented gilaburu juice is considered a potential functional food product because it contains antioxidants (anthocyanins, ascorbic acid) and phenolic compounds [4].

Another researched aspect that may contribute to the functionality of the beverage is the presence of a significant amount of lactic acid bacteria in the finished drink, which can provide probiotic properties [5]. Despite the existence of studies dedicated to the isolation of lactic acid bacteria from the drink, little attention has been paid to the fermentation process itself and the study of the other part of the microbiota, which also has a potential impact on the beneficial and organoleptic properties of the finished drink.

Therefore, the aim of our work is a microbiological study of the fermentation process in the production of fermented gilaburu juice.

Materials and methods. Fermentation of viburnum (*V. opulus*) was carried out according to the traditional Turkish recipe, which involves soaking the berries in boiled water and keeping them at room temperature for 4 months [5]. In order to assess the dynamics of microbial changes during fermentation, samples of the fermentation medium were taken monthly. Serial dilutions of the samples were plated on agar media: Sabouraud (SA), nutrient agar (NA) and MRS, prepared according to [6]. The selected media allow for the analysis of the number of yeast cells, the total number of

microorganisms, as well as the number of lactic acid bacteria. To evaluate the impact of the fermentation medium factors, such as changes in acidity, on microbial growth or decline, pH measurements of the collected samples were conducted. As the control, the baseline value, i.e. the beginning of the experiment was chosen, which indicates changes in CFU/cm^3 and allows for the analysis of the microbial composition changes of the fermentation medium relative to the beginning.

The results were considered reliable if the α value > *0,95*. Statistically significant results are indicated on the constructed graphs for $p < 0,05$ – *; $p < 0,01$ – **; $p < 0,001$ – ***.

Results and discussion. A graph was created according to the obtained results, and it is shown in Figure 1.

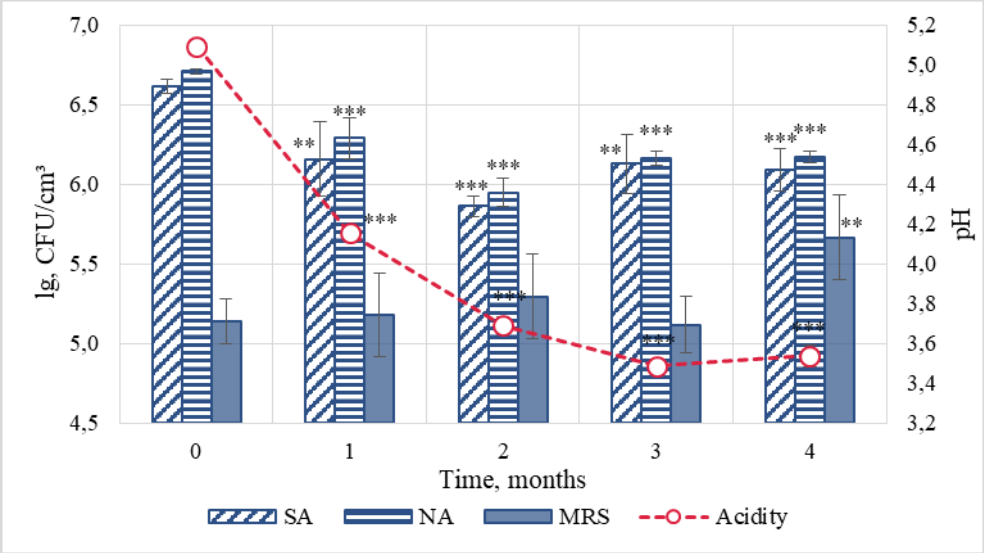

Fig. 1. Changes in the number of microorganisms and acidity during viburnum fermentation

According to Figure 1, it can be observed that there is a significant amount of yeast in the fermentation medium, up to (6.61 ± 0.04) CFU/cm^3, as well as the total number of microorganisms – (6.71 ± 0.01) CFU/cm^3 at the beginning of the fermentation, i.e. during the initial soaking of berries. The pH baseline value is (5.09 ± 0.01), which is lower than the pH of water (6.50 ± 0.01), and may be associated with the acidity of the berries. Over the next two months, a gradual decrease in the number of microorganisms is observed on the SA and NA media, which may be due to the lack of nutrients in the fermentation medium, as well as unfavorable pH values, which decreased to (4.15 ± 0.01) and to (3.69 ± 0.04) after the first and second month of fermentation, respectively.

In the studies [5, 7], it is reported that there is a significant number and species diversity of lactic acid bacteria on viburnum berries themselves and in the finished drink. However, according to the obtained data, no statistically significant changes or microbial growth were observed in the first 2 months of fermentation on the MRS medium. A slight increase from (5.13 ± 0.14) CFU/cm^3 at the baseline value to (5.30 ± 0.27) CFU/cm^3 was recorded. The decrease in the pH value itself and, accordingly, the number of microorganisms detected on other media may well be related to the

presence and slow growth of lactic acid bacteria in the fermentation medium and the synthesis of lactic acid.

Although there was a tendency for a decrease in the number of yeast cells and the total number of microorganisms during the first two months of fermentation, in the third month, on the contrary, a slight increase was observed to (6.13 ± 0.19) CFU/cm^3 and (6.16 ± 0.05) CFU/cm^3 for SA and NA media, respectively. Such an increase may be attributed to the adaptation of the microbiota to pH and the gradual release of nutritional components from viburnum berries. There were no statistically significant changes in the growth of lactic acid bacteria in the third month, but a further decrease in pH was detected to (3.49 ± 0.02), indicating their activity and further acidification of the fermentation medium.

The sample taken in the fourth month did not indicate significant changes in pH, the value of which gradually "levels out" to (3.54 ± 0.05). An increase in the number of yeast cells and the total number of microorganisms was not observed; these indicators at the end of the fermentation were (6.09 ± 0.13) CFU/cm^3 and (6.17 ± 0.03) CFU/cm^3, respectively, which may indicate the gradual adaptation of microorganisms and their ability to survive in the fermentation medium. An increase in the number of lactic acid bacteria on the MRS was also observed, up to (5.66 ± 0.27) CFU/cm^3, which is statistically significant compared to the baseline value.

Conclusions. The obtained results may indicate the presence of close intermicrobial interactions during the fermentation process of viburnum, as well as the resilience and the adaptability of all microorganisms to the fermentation environment. The ability of lactic acid bacteria to survive at low pH has been revealed; acidification of the environment may indicate the presence of acid-producing strains, opening up the possibility of further isolation and study of these microorganisms.

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