# IMPACT OF LOCALITY ON THE SENSORY PROFILE AND PHYSICOCHEMICAL CHARACTERISTICS OF KEFIR FROM BOSNIA AND HERZEGOVINA*

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## Abstract

Kefir is a fermented milk beverage originating from the Caucasus region. When compared to other fermented dairy products, kefir is unique due to the complex microbial flora in kefir grains, resulting in a combined fermentation process of the milk. Kefir grains are primarily composed of lactic acid bacteria, yeasts, and acetic acid bacteria. The microbial composition of kefir grains largely determines the formation of kefir's qualitative and technological properties, such as the physicochemical, biochemical, microbiological, and sensory characteristics of the ferment, as well as the growth rate of kefir grains (biomass).

The production area can influence the qualitative parameters. The aim of this study was to determine the influence of kefir grain origin on kefir quality. Kefir grains were collected from different regions of Bosnia and Herzegovina.

The results of the analysis of kefir produced from kefir grains from different locations showed a biomass increase above 5%, with pH values ranging from 4.1 to 4.6. The average sensory score of the analyzed kefirs (18.28) classified the kefir as extra class. The highest-rated samples were characterized by the following attributes: milky-sour, sharp, pleasant, with the typical flavor of kefir.

Keywords: *kefir, kefir grain, microbial composition, sensory analysis*

## INTRODUCTION

Kefir is a carbonated fermented milk beverage similar to yogurt, with its earliest production linked to the nomadic Ossetian and Karbadinian tribes in the Caucasus region (Samaržija, 2015). The word 'kefir' is of  Turkish origin, coming from 'keyif,' which means joy or satisfaction. However, kefir grains are often also called 'Grains of the Prophet,' as it is believed that the Prophet Muhammad entrusted kefir grains to the people in the mountains of the northern Caucasus (Lopitz-Otsoa *et al*., 2006; Gaware *et al*., 2011; Seifi 2016).

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Traditionally, kefir was made using kefir grains to ferment the milk, but today industrial starter cultures are primarily used. Kefir grains, which act as a natural starter culture, and can range from 0.2 to 3.0 cm in size. It is white to yellow in color, with an irregular round or elongated shape similar to cauliflower. It consists of proteins, a mixture of lactic acid bacteria, acetic acid bacteria, and yeasts bound together in a biofilm formed by a polysaccharide matrix (Loretan *et al.*, 2003; Shah, 2014; Dong *et al.*, 2018).
In kefir production, cow's milk is most commonly used as the medium, but sheep's, goat's, or even buffalo's milk can also be used (Cais-Sokolińska *et al.*, 2015). The symbiotic interaction is seen in the exchange of bioproducts, which act as energy sources or growth and survival factors for microbial species within the kefir grain. This results in the creation of a product with specific sensory, nutritional, and functional properties. The ratio and number of individual microbial species within the grain largely depend on its origin and storage conditions.
The first written records of kefir production and consumption in Bosnia and Herzegovina are linked to the Milkos dairy in Sarajevo, which operated as part of the UPI conglomerate. The technologists from this dairy brought kefir grains to Bosnia and Herzegovina during professional training in the USSR in 1960. For several years, kefir technology was refined under industrial conditions, and this method is still used in production today (Parijez, 1973). Over 65 years of kefir production, kefir grains have become popularized and widely adopted for home production.


Figure 1. Macroscopic view of kefir grain BH 22
(Source: Sakić-Dizdarević, 2021)

It has been found that the characteristics of kefir grains from Bosnia and Herzegovina are not sufficiently researched. The aim of this study was to examine the qualitative characteristics of kefir produced using kefir grains from different locations in Bosnia and Herzegovina.

## MATERIALS AND METHODS

To achieve the set objective, a three-month activation of kefir grains was conducted, during which their technological properties (titratable acidity, pH, and biomass) were examined, and the fermentation products were sensory evaluated.
For the experimental production of kefir, 26 different kefir grains from the collection of the Faculty of Agriculture and Food Sciences, University of Sarajevo, were used. The kefir grains were grouped into two categories: the SA group consisted of 13 kefir grains from the Sarajevo area, and the BH group consisted of 13 kefir grains from various regions of Bosnia and Herzegovina. During the 90-day reactivation period, kefir was produced from each individual grain (26 samples), with six pH measurements taken over this period, while the biomass (mass of kefir grains) was measured weekly. The sensory evaluation was conducted after 90 days.
The kefir grain, in a 1:10 ratio, was inoculated into sterile milk (ZZ with 2.8% milk fat) and incubated for 24 hours. The resulting mother culture (5%) was used for inoculation, and inoculated into fresh milk with gentle stirring and fermented for 20 hours. After fermentation, the ripening process occurred at a temperature of 8°C for 24 hours.

*Determination of kefir pH value*
The pH value was measured using a pH meter (Methrom 632, Switzerland) with an electrode for liquid media (WTW SenTix 41, Germany), which was previously calibrated with standard buffers at pH=4 and pH=7.

*Determination of kefir grain biomass*
The increase in kefir grain biomass was measured gravimetrically. After 24 hours of fermenting the grains in milk at a ratio of 1:10, the grains were separated from the fermented medium using a strainer, rinsed with water, and weighed on an analytical scale. The mass was recorded daily, and biomass increase was calculated according to the formula (Guzel-Seydim *et al*., 2021).

$$\text{Biomass increase (\%)} = \frac{W2-W1}{W1} \text{ X } 100$$

In the formula, W1 represents the initial mass of the kefir grain, and W2 is the mass measured 24 hours after fermentation.

*Sensory analysis of kefir*
The sensory analysis of individual kefir samples produced using 26 kefir grains was conducted by a panel of 5 trained evaluators. A scoring system with a maximum of 20 points was used, assessing the sensory characteristics of appearance (maximum 1 point), color (maximum 1 point), consistency (maximum 4 points), odour (maximum 2 points), and taste (maximum 12 points). In addition, the kefir was evaluated qualitatively, with descriptions of its individual characteristics.

## RESULTS AND DISUSSION

*Determination of kefir pH value*

Figure 2. shows the average pH values of kefir samples from the SA group over six measurements during the 90-day kefir grain reactivation period.

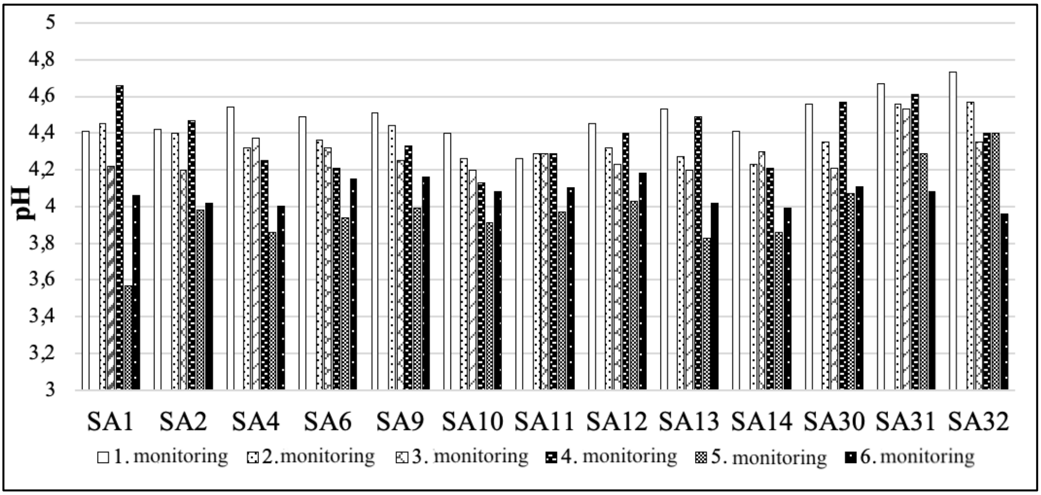


Figure 2. Average pH values of kefir samples from the SA group

The pH values of kefir samples produced from SA group grains ranged from 4.10 to 4.50, with the lowest average value found in sample SA10 and the highest in SA31. A lower pH value in kefir grains indicates an increased presence of lactic acid bacteria. Sulmiyati *et al.* (2019) state that lactic acid bacteria convert lactose into lactic acid through metabolic activity, resulting in a reduction of the medium's pH value. Figure 3 shows the average pH values of kefir samples from the BH group over six measurements during the 90-day kefir grain reactivation period.

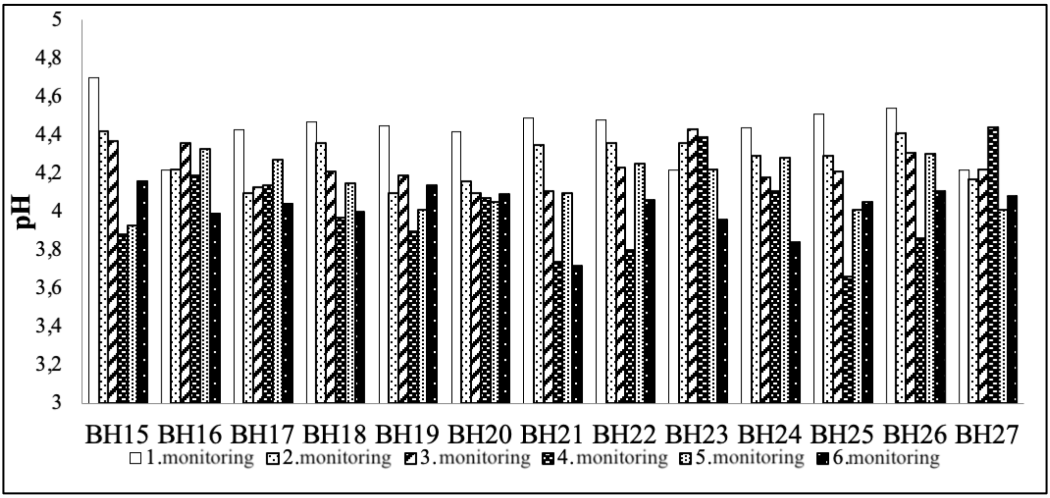


**Figure 3.** Average pH values of kefir samples from the BH group

The pH values of kefir samples produced from BH group grains ranged from 4.00 to 4.30. Most kefir samples had an average pH value around 4.20, with sample BH21 having the lowest (4.09) and samples BH23 and BH26 the highest (4.26). A lower pH value in kefir is desirable for certain micronutrients. For example, a lower pH in fermented milk enhances calcium absorption, as this mineral is present in ionic form in such a medium (Hui *et al*., 2007).
Based on the analysis results, it can be concluded that a three-month reactivation period was necessary for the grains to fully activate, thereby enriching the grain microbiota, primarily with lactic acid bacteria.

*Determination of kefir grain biomass*
The average biomass growth values of the 26 kefir grains (SA and BH) monitored over the three-month period are shown in figure 4. The highest average biomass increase was observed in sample BH16 (13.87%), while the lowest was in SA4 (1.40%). According to Libudzisz & Piatkiewicz (1990), the average daily biomass increase for kefir grains ranges from 5 to 7%.

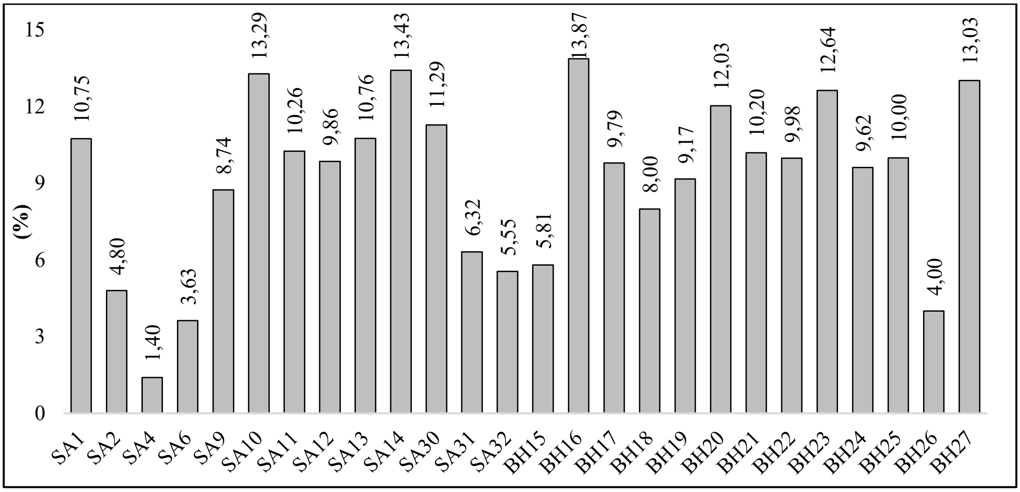


Figure 4. Average biomass growth values of tested kefir grains from groups SA and BH

The biomass increase is driven by the growth of microorganisms that use milk or other media as a food source. Additionally, frequent inoculation leads to the synthesis of kefir grain components. In contrast to these findings, the kefir grains SA2, SA4, and SA6 showed an average biomass increase below 5% (figure 4). This result may be due to damage to the grains before the reactivation process, which can impoverish or completely degrade the microbiota.

The biomass of kefir grains, as an essential technological parameter, showed a consistent increase depending on the intensity of the fermentation process repetitions, while simultaneously establishing a stable ratio of the main microorganisms in the grain, namely lactic acid bacteria and yeasts (Gorek & Tramsek, 2007). This characteristic is particularly significant in industrial kefir production, as it enables continuous production and accelerates the process of obtaining the final product (Rosa *et al*., 2017). Biomass increase is also an important parameter for the potential use of kefir grains for other applications, such as polysaccharide production (Gorsek & Tramsek, 2008). A certain number of analyzed kefir grains from Bosnia and Herzegovina demonstrated technological potential (biomass production).

The analysis of kefir grain growth dynamics (biomass) based on the locations BH and SA is shown in figure 5.

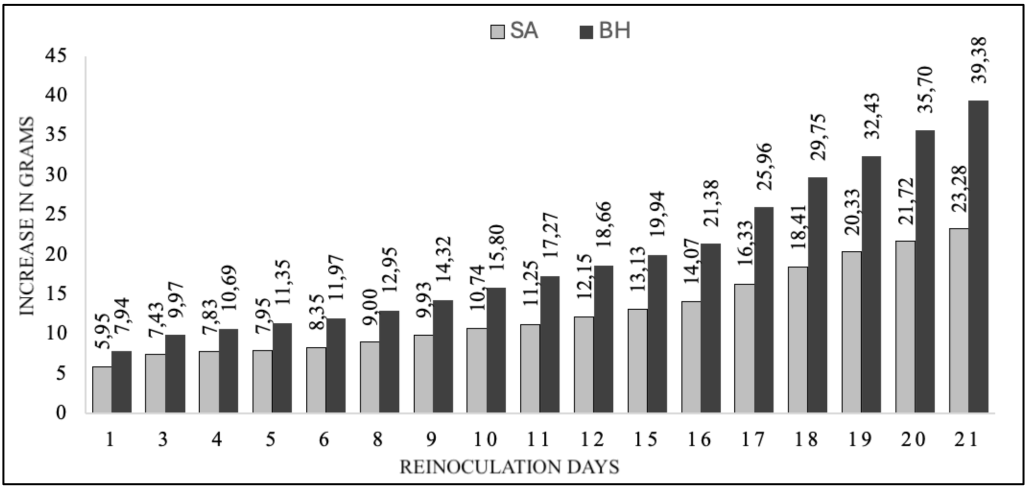


Figure 5. Biomass dynamics of kefir grains from groups SA and BH over a 90-day period

The BH kefir grains showed more efficient biomass increase compared to the SA group, except for grain BH26, which had a biomass increase below 5%.

*Sensory analysis of kefir grains*

The results of the sensory analysis of kefir produced from kefir grains BH and SA are shown in graph 5.

The highest overall scores were observed in samples SA10 (19.50), BH17/BH26 (19.50), with characteristics such as pleasant, clean, milky-sour, and kefir-like. The main components contributing to the pleasant aroma of kefir include alcohols (ethanol), ketones (acetoin and 2-3 butanedione), esters (ethyl acetate), aldehydes (acetaldehyde), and acids (lactic acid) (Farag *et al*., 2020). Lactic acid bacteria play a crucial role in the creation of aromatic components in kefir, while yeast activity is also significant for alcohol production. In their study, Walsh *et al*. (2016) highlight *Lactobacillus kefiranofaciens* among the notable lactic acid bacteria that impact the pleasant aroma. Additionally, other bacteria significant for aroma creation include *Lactobacillus acidophilus, Levilactobacillus brevis, Lactobacillus helveticus, Lacticaseibacillus casei, Lactococcus cremoris, Lactococcus lactis, and Leuconostoc mesenteroides* (Farag *et al*., 2020).

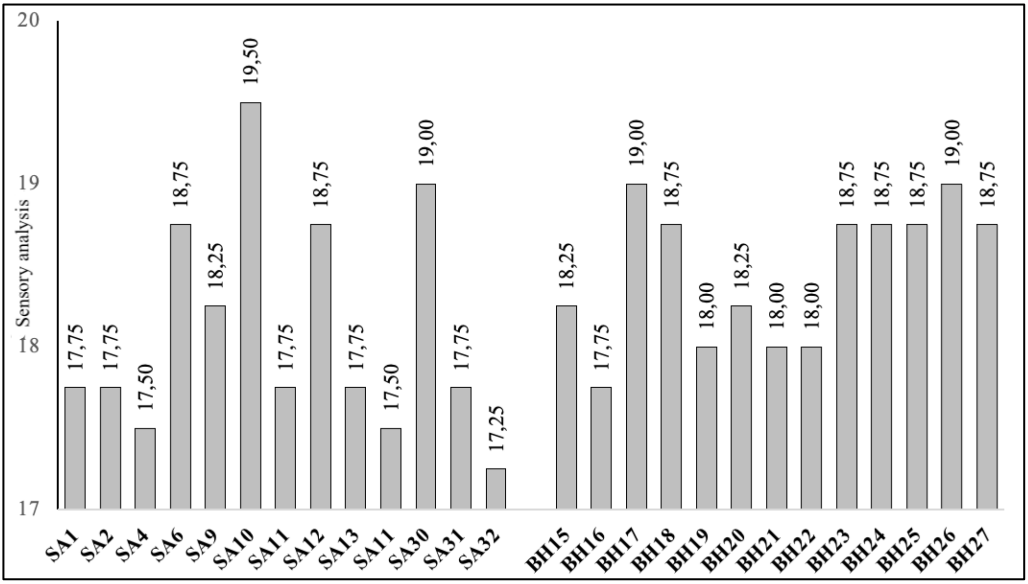


Figure 6. Average sensory score of kefir produced from 26 kefir grains

The samples with the lowest scores were SA32 (17.25) and BH16 (17.75), with the following characteristics: intensely sour, unusual yeast and mold flavor, bitter, with an atypical taste. The pronounced sour taste may be attributed to acetic acid, which is produced as a result of the metabolic activity of heterofermentative lactic acid bacteria or acetic bacteria (Magalhães *et al*., 2011; Irigoyen *et al*., 2012). Some acids that may negatively affect the overall aroma of kefir include octanoic and n-decanoic acids, which contribute to a soapy, pungent, waxy, and rancid aroma (Farag *et al*., 2020). Diacetyl is one component that, if present in excessively high concentrations, can lead to the formation of an atypical, sharp odor in kefir (Ott *et al*., 1999).

## CONCLUSIONS

Kefir is considered a functional product due to its diverse microbial flora. It was found that kefir produced from SA kefir grains had a slightly higher pH compared to BH. The highest average biomass increase was recorded in samples SA14 and BH16, indicating technological potential. BH kefir samples had a higher overall sensory score (between 18 and 19) compared to SA samples. A significant number of tested kefir grains showed good technological potential, suggesting that future efforts should focus on standardizing this product and studying its functional characteristics. Most kefir grains were collected from the Sarajevo Canton area, indicating a long tradition of use and production of this product.

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## UTJECAJ LOKALITETA NA SENZORNI PROFIL I FIZIČKO-HEMIJSKE OSOBINE KEFIRA SA PODRUČJA BOSNE I HERCEGOVINE

### Sažetak

Kefir je fermentirani mliječni napitak koji potiče sa područja Kavkaza. U odnosu na ostale fermentirane mliječne proizvode razlikuje se po kompleksnoj mikrobnoj flori kefirnog zrna koja dovode do kombinovane fermentacije mlijeka. Sastav kefirnog zrna čine dominantno bakterija mliječne kiseline, kvasaci i sirćetne bakterija. Mikrobni sastav kefirnog zrna u znatnoj mjeri određuje formiranje kvalitativnih i tehnološki osobina kefira kao što su fizičko-hemijske, biohemijskih, mikrobiološke i senzorne osobine fermenta, kao i brzinu rasta kefirnih zrna (biomasa).

Područje proizvodnje može utjecati na kvalitativne parametre. Cilj ovog rada bio je da se odredi utjecaj lokaliteta kefirnog zrna na kvalitetu kefira. Kefirna zrna prikupljena su sa različitih područja Bosne i Hercegovine.

Rezultati analiza kefira proizvedenog od kefirnih zrna sa različitih lokaliteta pokazali su porast biomase iznad 5%, pH vrijednost varirala je u intervalu od 4,1 do 4,6. Prosječna senzorna ocjena analiziranih kefira (18,28) kategorisala je kefir u ekstra klasu. Najbolje ocijenjene uzorke karakterisale su sljedeće osobine: mliječno-kiseo, rezak, prijatan sa tipičanim okusom po kefiru.

Ključne riječi: *kefir, kefirno zrno, mikrobiološki sastav, senzorna analiza*