Scientific Bulletin. Series F. Biotechnologies, Vol. XXVII, No. 2, 2023
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

CHARACTERIZING WATER KEFIR BEVERAGES WITH ANTIOXIDANT EFFECTS: PRELIMINARY ANALYSIS

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Abstract

Water kefir is a beverage that undergoes natural fermentation by inoculating water kefir grains in a mixture of filtered water, cane sugar, and fruits. The beverage has a mild level of carbonation, making it appropriate for individuals adhering to a vegan diet. Moreover, it is a viable substitute for individuals experiencing nutritional imbalances stemming from dysbiosis of their microbiota. This study aimed to examine the characteristics of water kefir produced from different fruit juices and evaluate the bioactive properties of the resultant beverages. The methods used were the antioxidant activity in vitro, the determination of major bioactive compounds, BRIX values, and a microbiological quantification of lactic acid bacteria, yeasts and actinobacteria. Using fruit juice to obtain water kefir was a beneficial and advantageous addition to functional drinks. The presence of certain compounds (phenolic compounds, e.g.) determined the potent antioxidant effects, proved by the in vitro method. The microbiological pattern of the beverages was correlated with the fermentative profile, BRIX levels, and functional characteristics.

Key words*: juice; antioxidant; fermentation; vegan; brix; bacteria.*

INTRODUCTION

Water kefir beverage (WKb) is a fermented drink gaining popularity due to its numerous health benefits and refreshing taste. Obtaining water kefir involves fermenting water kefir grains in a sugar-water solution. Water kefir grains are composed of a symbiotic consortium of bacteria and yeast that engage in the metabolic process of sugar fermentation. Water kefir grains are minute, translucent, gelatinous structures composed of a symbiotic consortium of bacteria and yeast. Water kefir is produced by fermenting sugar water with grains, producing a softly carbonated beverage with a mildly acidic and sweet taste. Water kefir grains possess unique attributes in contrast to milk kefir grains since they undergo fermentation in a water medium rather than milk, resulting in a beverage characterized by a tangy and creamy taste profile (https://blog.soin-et-nature.com/) The bacteria and yeast in water kefir grains work together to break down and metabolize sugars, creating a nutrient-rich beverage packed with live probiotics and beneficial enzymes (https://www.kombuchakamp.com).

Water kefir grains can generally be propagated and reused for many batches, making them a sustainable and cost-effective way to produce a healthy probiotic drink (Mazhar, 2021).

The fermentation process produces lactic and acetic acid and a range of vitamins, minerals, and probiotics that benefit human health. Water kefir is a fermented drink gaining popularity amongst health-conscious individuals due to its numerous potential health benefits. This refreshing beverage is packed with live probiotics and beneficial enzymes that help to improve gut health and boost the immune system. The probiotics in water kefir, created through fermentation, support the growth of healthy bacteria in the gut and help maintain good digestive health. This can lead to various benefits, including increased nutrient absorption and improved bowel function. Water kefir is also a great source of vitamins and minerals, including vitamin B12, K, calcium, and magnesium (https://www.wildfoods.co). These essential nutrients help to support overall health and well-being, including maintaining strong bones and teeth, promoting healthy blood clotting, and improving nerve and muscle function.

Moreover, water kefir exhibits anti-inflammatory and antioxidant characteristics, potentially mitigating inflammation and oxidative stress inside the human body. Consequently, this may protect against chronic ailments, including cancer, cardiovascular disease, and Alzheimer's disease (FoodPorty, 2023). Regularly drinking water kefir has been suggested to enhance gut health, bolster the immune system, support optimal bone and dental health, and protect against the development of chronic diseases. Nevertheless, further investigation is required to substantiate these prospective advantages (https://www.elperroflaco.com). This article aims to analyze the procedure involved in acquiring beverages using the water kefir fermentation technique, along with the evaluation of the resultant product.

MATERIALS AND METHODS

Water kefir beverages obtaining

WKb was prepared in 50 g of cane sugar (bio product) and 500 mL of mineral water (Laureys, 2017). The mixture was inoculated with 25 g water kefir grains (Fairment GmbH, Berlin). The water kefir grains were subjected to activation in a solution containing 5% cane sugar, which lasted for 48 hours. The beverage was acquired inside a container made of borosilicate glass, and the fermentation process was conducted in two stages at a temperature of 220°C, as seen in Figure 1.

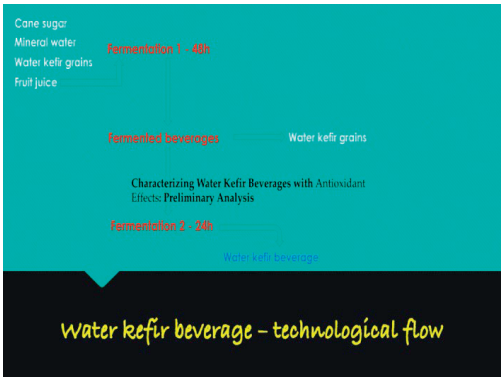

Figure 1. Water kefir obtaining - technological flow

Following the first fermentation stage, filtering isolated the water kefir grains from the liquid. Subsequently, they were immersed in water containing 5% cane sugar for at least 24 hours before their utilization in a subsequent fermentation process (Laureys, 2017).

Fermented drinks were obtained from the following mixtures:

1. WKR - Water and freshly squeezed and filtered pomegranate juice (ratio 4:1);
2. WKT - Cold peach tea (1 bag/250 mL water), 5% cane sugar;
3. WKC - 800 mL water and 200 mL fresh multi-fruit juice, 3% cane sugar;
4. WKPG - 600 mL water and 400 mL pomegranate nectar.

The fermentation process was conducted in a state of calmness, devoid of any agitation, and monitored regularly with the collection of samples every 12 hours (Cevik et al., 2019). Control 1 means the water kefir fermented at the end of the first fermentation phase with apple concentrate; Control 2 means water kefir fermented at the end of the second fermentation phase with apple concentrate; Control 3 means with apple concentrate water kefir fermented at the end of the second fermentation phase with lemon juice concentrates (Laureys & De Vuyst, 2014).

Microbiological analysis of the beverages

The viability of lactic acid bacteria was determined using MRS. The seeded plates were placed under anaerobic conditions at 300C. The yeasts were determined using YPG, and the plates were incubated at 300C. The presence of bacteria of the genus Acetobacter was highlighted by using the following culture medium (g/500 mL, Glucose - 10 g, Peptone - 2.5 g, Yeast extract - 2.5 g, Na2HPO4 - 1.35 g, Citric acid - 0.75 g, agar - 1g, pH 7.5). The plates were also incubated at 300C (https://2018.igem.org). Microbiological examination was conducted after the completion of each stage inside the technical procedure (Gulhan, 2023).

Physico-chemical analyses

The pH value was measured with a portable pH electrode, ATC.

The Brix value of the solution was measured using a portable refractometer (Okamura et al., 2022) and was correlated with the soluble sugar

contents in fermented beverages (https://www.vinolab.hr).

To quantify the overall phenolic content, the Folin-Ciocalteu test was used. The spectrophotometer was used to measure the absorbance of the combination at a wavelength of 760 nm. The obtained data were then quantified as micrograms of gallic acid equivalents per milligram of the sample (Periya et al., 2021). (µg GAE/mg) (Molole et al., 2022).

The DPPH scavenging activity assay measures the antioxidant capacity and reflects the specific antioxidant compounds present in the sample. The absorbance of the mixture was read at 517 nm using a spectrophotometer. The percentage of DPPH scavenging activity was determined by comparing the absorbance value of the sample to a control containing only DPPH solution and ethanol (Baliyan et al., 2022).

RESULTS AND DISCUSSIONS

The pH level is a crucial factor in the fermentation process of water kefir. The pH of a solution is a quantitative measure of its acidity or alkalinity, which significantly impacts the development and metabolic processes of microorganisms participating in fermentation (Laureys et al., 2017). The pH values directly depend on the formula used in the fermentation process. A typical behavior was presented by the WKR sample, determining a decrease after 24 hours of fermentation ($p \leq 0.001$). WKC showed an atypical behavior, with a sudden decrease, and then the value showed higher values by 0.4 units (Figure 2).

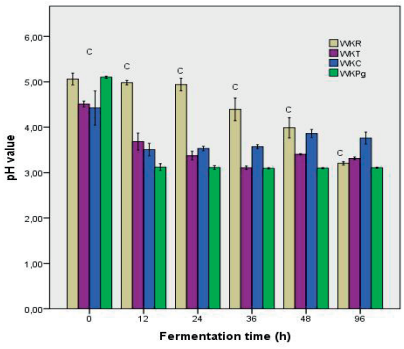

Figure 2. pH values of water kefir beverages conforming to technological flow

The rest of the samples had relatively constant evolutions that were assimilated to the fermentative capacity of the response of the inoculum to the carbon source.

A negative correlation was observed between the pH and Brix levels in the process of water kefir fermentation. During fermentation, the pH undergoes a decline as a result of the synthesis of organic acids, while the Brix value experiences a reduction owing to the use of sugars by microbes (Gulhan, 2023) (Figure 3).

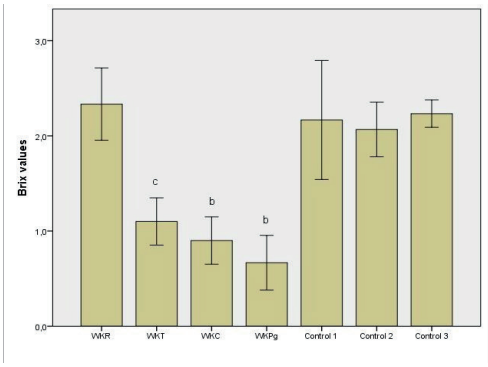

Figure 3. Brix values of water kefir beverages conforming to technological flow (Control 3 vs. samples; n = 3)

This means that as the water kefir becomes more acidic (lower pH), the beverage's sweetness decreases (lower Brix value). The correlation between pH and Brix value might exhibit variability contingent upon the distinct parameters of fermentation, the composition of microorganisms involved, and the length of the fermentation process (Safak et al., 2023). It was very clearly represented in Figure 4 in the case of Control 1 and Control 2 variations. The classic fermentation mixture (Control 3) was stable and influenced only by the initial quantity of cane sugar. The type of inoculum has no additional influence because it was the same in all samples.

The level of phenolic compounds in water kefir depends on several factors, including the ingredients used, fermentation conditions, and the specific strains of bacteria and yeast in the inoculum. The phenolic compounds in water kefir primarily come from the ingredients used during fermentation (Şafak et al., 2023). These compounds can be released into the beverage during fermentation due to enzymatic activity,

microbial metabolism, and other biochemical reactions (Figures 3 and 5).

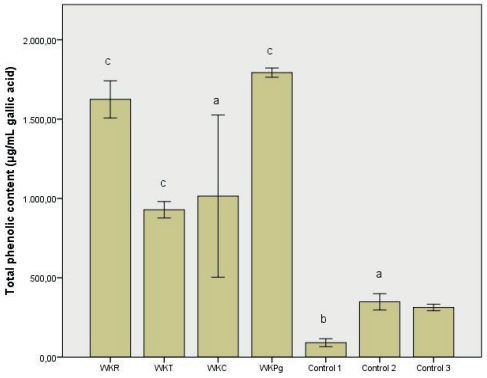

Figure 4. Total phenolic content of water kefir beverages conforming to technological flow
(Control 3 vs. samples; n = 3)

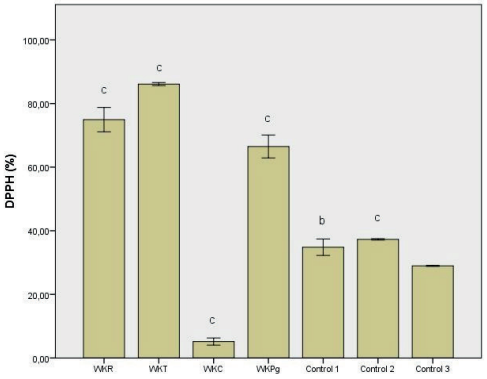

Figure 5. DPPH scavenging activity (%) of water kefir beverages conforming to technological flow
(Control 3 vs. samples; n = 3)

The key microbiological components in water kefir include water kefir grains, yeast, and bacteria. The yeast in water kefir grains plays a crucial role in fermentation. Yeasts consume sugars in the sweetened water (Figure 4) and produce alcohol and carbon dioxide (Laureys et al., 2014). As the fermentation process progresses, the alcohol produced by yeast is further metabolized by bacteria into various compounds, including lactic acid. The lactic acid bacteria (LAB) present in water kefir grains were primarily responsible for lactic acid production and values of the pH (Figure 2).
Figures 6-8 show a pattern specific to each formula compared to the control (Control 3). Controls 1 and 2 demonstrated a balanced ratio of the number of strains analyzed. WKT was shown to support a high value of acetic bacteria, demonstrating better options than this one. Pomegranate juice (Figure 6) determined a value similar to the control in the number of strains of Lactobacillus and WKC strains.

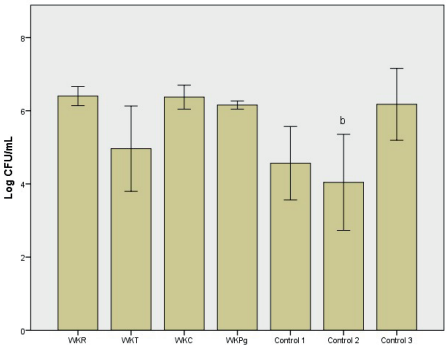

Figure 6. Log CFU/mL for lactic acid bacteria from water kefir beverages conforming to technological flow
(Control 3 vs. samples; n = 3)

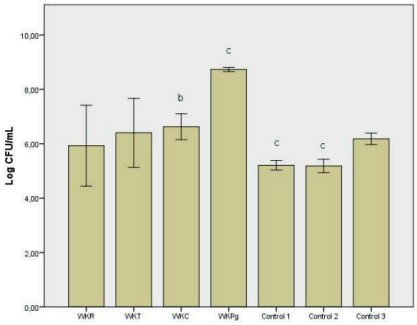

Figure 7. Log CFU/mL for yeast from water kefir beverages conforming to technological flow
(Control 3 vs. samples; n = 3)

Water kefir is a fermented drink gaining popularity due to its refreshing taste and potential health benefits. Our study variation of water kefir involves using fruit juice as the sugar solution. This method adds flavor and nutrients to the drink, making it a tasty and healthy alternative to sugary sodas or juices (Randazzo et al., 2016). During fermentation, the water kefir grains consume the sugar in the juice, producing a tangy, fizzy drink loaded with beneficial probiotics. This aspect was especially available for pomegranate nectar, especially. The longer the fermentation time,

the stronger the flavor could diminish, and the carbonation degrees the sweet taste (Sabokbar & Khodaiyan, 2016).

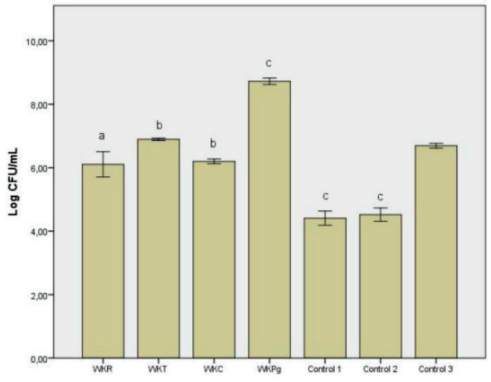

Figure 8. Log CFU/mL for *Acetobacter* from water kefir beverages conforming to technological flow (Control 3 vs. samples; n = 3)

During fermentation, the pH of the water kefir mixture gradually decreases due to the accumulation of organic acids. This decrease in pH is essential for creating an acidic environment that favors the growth of beneficial bacteria and yeasts while inhibiting the growth of potentially harmful microorganisms (Kim et al., 2016). The optimal pH range obtained for WKR and WKPG for water kefir fermentation typically exceeds 3.0.

The kefir grains and the microorganisms they contain exhibit optimal growth and demonstrate efficient fermentation processes within this specified range (Moretti et al., 2022). The acidic nature of water kefir, characterized by a low pH, acts as a growth inhibitor for spoilage bacteria and pathogens. This property plays a crucial role in preserving the quality and ensuring the safety of water kefir. (Pihurov et al., 2023).

Examining the correlation between the antioxidant content and the microbial composition of water kefir is a complex aspect of this study. The microbial makeup of water kefir is distinguished by a diverse array of microorganisms, including lactic acid bacteria (LAB), acetic acid bacteria, yeasts, and a variety of other microbial species (Peluzio et al., 2021). The composition and abundance of microorganisms in kefir might vary according to variables such as the specific kefir grains used and the fermenting conditions Laureys & De Vuyst, 2014).

The microbial processes occurring during fermentation can impact the synthesis and concentration of antioxidants (Vieira et al., 2021).

As an illustration, lactic acid bacteria (LAB) can generate specific antioxidant molecules through fermentation, augmenting the resultant product's overall antioxidant potential (Feng and Wang, 2020). The fluctuation in antioxidant concentrations across different batches is a significant obstacle in considering water kefir as a reliable and constant provider of antioxidants (da Silva Vale et al., 2023).

Although water kefir it should not be seen as a substitute for a properly balanced diet. Moreover, it should be noted that there might be variations in individual reactions to antioxidants (Moretti et al., 2022).

Furthermore, consuming excessive water, kefir, or other fermented drinks could have unfavorable consequences for some individuals, such as experiencing gastrointestinal pain due to probiotics (Peluzio et al., 2021).

Acknowledging that the possible health advantages associated with antioxidants in water kefir may exhibit a different prominence than those found in fruits and vegetables, which serve as abundant reservoirs of these molecules (Constantin et al., 2023).

CONCLUSIONS

Incorporating fruit juice in producing water kefir is a pleasant and beneficial inclusion to one's regular regimen. The preparation based on different fruit juices was accessible, while its consumption presents many benefits. In addition to providing a delightful and palatable experience, this product offers a substantial concentration of probiotics, for WKPg. Moreover, it should be noted that there might be variations in rest of the samples. The inclusion of water kefir in a complete nutritional strategy warrants careful consideration, as it has been reported to possess antioxidant benefits in the context of WKR, WKT, and WKPg. The final samples exhibit the strongest association between the benefits of probiotics and their antioxidant characteristics.

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