v. 3, n. 101, 2023

International Journal of Health Science

EFFECT OF PASTEURIZATION ON THE ACCEPTABILITY OF KEFIR FERMENTED IN WHOLE RED GRAPE JUICE

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Abstract: Water kefir is a fermented drink that has probiotic characteristics, but has a short shelf life due to the presence of viable microorganisms. Objective: To evaluate the effect of pasteurization as a conservation process on the sensorial, physicochemical and microbiological characteristics of a carbonated drink naturally fermented by kefir made with whole grape juice. Method: After production, the samples were analyzed (T1), then pasteurized and stored at -10°C for 40 days before new analyzes (T2). 50 individuals were recruited to carry out a sensory analysis test consisting of a triangular test and two affective tests: acceptance and preference. pH, fixed and volatile acidity, total dissolved solids (°Brix), alcohol content, lactic acid bacteria (LAB) and yeast counts were evaluated. Results: The pasteurization process caused a greater proportion of correct answers ($p<0.5$). For the acceptance test, no differences were found for the attributes evaluated (color, odor, flavor, sensation of refreshment and amount of gas in the product). The test revealed a preference for the pasteurized sample. A significant reduction in dissolved solids, an increase in alcohol content and yeast count was observed in unpasteurized samples ($p<0.5$). These effects were not observed in pasteurized samples, with a significant reduction in LAB and yeast counts ($p<0.5$). Conclusion: The sensory analysis revealed that the pasteurization of whole grape kefir did not affect its acceptance, but caused greater preference than the unpasteurized drink, with a difference between the products being noted. Pasteurization maintained most of the physicochemical characteristics, being responsible for avoiding an increase in yeast growth and the consequent alcoholic content of the product. Considering possible benefits of non-viable microorganisms (paraprobiotics), it is possible that pasteurization is a potential way to preserve the whole red grape flavored kefir fermented drink and increase its shelf life.

INTRODUCTION

PROBIOTICS, PREBIOTICS AND SYNBIOTICS

The word probiotic comes from the Greek and means “for life”. It was used in its early days as an antonym of antibiotic, as it was capable of stimulating the growth of beneficial bacteria (AKHTER et al., 2015). They are live microorganisms, which, if administered in adequate doses, provide health benefits to the host. These microorganisms are part of different genera and species, both bacteria and yeast (FLESCH; POZIOMYCK; DAMIN, 2014).

For probiotics to proliferate and exert their activity properly, they need to be cultivated correctly. The ideal nutrients or substrates for these microorganisms are called prebiotics (FLESCH; POZIOMYCK; DAMIN, 2014).

Prebiotics are substances that are not digested by the body and that selectively and beneficially affect the host. Its main function is to stimulate the metabolism of probiotic bacteria, altering the microbiota of the intestinal tract (AKHTER et al., 2015).

Examples of prebiotic substances are some sugars (absorbable or not), fibers, sugar alcohols and oligosaccharides (FLESCH; POZIOMYCK; DAMIN, 2014). Phenolic compounds such as flavonoids and tannins are also being related to this action in recent studies (BINNS et al., 2013).

When there is an association of one or more probiotics with one or more prebiotics, we can define this compound or food as symbiotic. In this case, the two must establish a synergistic relationship. The probiotic must be stimulated by the intestinal fermentation of the prebiotic or it must help to create a favorable intestinal environment, where the probiotic becomes more competitive (BINNS et al., 2013). An example of a food with these characteristics is kefir (FLESCH; POZIOMYCK; DAMIN, 2014).

There are several mechanisms that mediate the health benefits of viable beneficial bacterial cells. However, new terms such as "paraprobiotics" or "postbiotics" have emerged to denote that non-viable microbial cells, microbial fractions or cell lysates can also offer physiological benefits to the host by providing additional bioactivity. Postbiotics refer to products or by-products secreted by live bacteria or released after bacterial lysis, such as enzymes, peptides, polysaccharides, cell surface proteins that have anti-inflammatory, immunomodulatory, anti-obesogenic, anti-hypertensive, hypocholesterolemic, antiproliferative activities. and antioxidants. (AGUILAR-TOALÁA et al., 2018) On the other hand, paraprobiotics are non-viable probiotics with beneficial effects on the state of health and well-being, with application in food, being an excellent field of emerging research (ALMADA et al., 2016).

KEFIR

The word kefir originates from the Slavic Keif which means "well-being" or "well-living". It is a symbiotic mixture of microorganisms, which has lactic acid bacteria and yeast in its composition, immobilized in a protein and polysaccharide matrix, functioning as a culture that develops with the addition of an appropriate substrate (LAUREYS; VUST, 2014).

Kefir grains whose substrate is milk are called "milk kefir". These have a yellowish color and irregular shape and size (ROCHA-GOMES et. al, 2018). The grains present in other substrates, the most common being brown sugar dissolved in water, are called "water kefir". They normally match the color of the culture solution on a transparent base, are less resistant and produce a slightly acidic drink (ROCHA-GOMES et. al, 2018).

There are descriptions of the effects of kefir as a probiotic, postbiotic and paraprobiotic. The kefir fermentation process generates a series of compounds that provide a characteristic flavor and aroma, as well as bioactive substances, responsible for nutraceutical properties, such as kefiran and dextran, short-chain fatty acids, bacteriocins, as well as independent effects on cell viability (BOURRIE et al., 2016; AHMED et al., 2013).

WATER KEFIR

Most research on kefir highlights the use of milk from different animals as a substrate for the grains. However, the use of milk as a substrate limits consumption for vegans, lactose intolerants and those allergic to dairy products (FIORDA et. al, 2017).

As an alternative to the use of milk, the substrate most commonly used for fermentation is brown sugar, but other non-dairy substrates, such as fruit juice, vegetables and molasses, have been widely used (FIORDA et. al, 2017).

Water kefir is mainly formed by lactic acid bacteria, acetic acid bacteria and yeast, living symbiotically (GULITZ, et. al; 2011). To obtain a fermented water kefir drink, the water kefir grains must go through a fermentation process, where a mixture of water, sugar or fruit juice is added to the grains (LAUREYS; VUST, 2014).

Traditionally, for the fermentation process, kefir grains are placed in a solution containing 8% sucrose, fruit (typically figs) and lemon slices. The process lasts around 2 to 4 days, at room temperature, under anaerobic conditions. After fermentation, the mixture is sieved to separate the drink from the grains, which can be used again for a new fermentation (ROSA, 2016; LAUREYS; VUYST, 2014). The drink obtained is a fermented drink with a yellowish color (or color characteristic of the substrate used), frothy, slightly alcoholic, with a slightly fruity, acidic and slightly sweet flavor (LAUREYS; VUYST, 2014).

A new proposal is the fermentation of fruit juice by water kefir, especially whole grape juice. The intake of grapes and their derivatives is associated with several beneficial actions for the body, making grapes considered a functional food. Among its derivatives, grape juice stands out due to the fact that it is a highly accessible drink (JUNIOR et. al., 2013; LEAL et. al., 2017), in addition to being an excellent source of vitamins and minerals and having high antioxidant capacity, attributed to the presence of phenolic compounds, such as flavonoids, which reduce the formation of free radicals, protecting against oxidative stress (CALDAS, 2015).

The prebiotic action related in recent studies to flavonoids, tannins and anthocyanins, is also an excellent property of grape juice, when we think about its association with beneficial bacteria (PRIOR, 2006; BINNS et al., 2013; LEITE et al., 2018).

OBJECTIVES

To evaluate the effect of the pasteurization process on the acceptability, preference and physicochemical and microbiological characteristics of a whole grape drink fermented by water kefir as a conservation process and consequently increasing its shelf life.

MATERIAL AND METHODS

The sensory analysis was carried out in the experimental kitchen of Universidade Presbiteriana Mackenzie, with fifty (50) untrained tasters, of both sexes, aged at least 18 years. The environment where the analyzes were carried out had uniform lighting, room temperature and did not allow contact between tasters, so that these factors did not interfere with the evaluation.

The project was approved by the ethics committee of Universidade Presbiteriana Mackenzie (CAAE - 48483015.7.0000.0084). The tasters signed a Free and Informed Consent form (TCLE) (Appendix 1) explaining the objective of the study, exemption from risks and other clarifications, delivered together with the analysis form where the evaluations were completed (Appendix 2).

The carbonated whole grape kefir samples were supplied, already properly prepared, by the production company: ``Tekóporã Comércio de Alimentos e Probiotics Ltda`` in 250 mL glass bottles and were kept under refrigeration (4 to 10ºC). The pasteurization conditions were not reported due to industrial confidentiality. Each taster received around 10 ml of each of the drinks with the coding for each identified sample and received the necessary guidance for each test carried out. The samples used for sensory analysis were pasteurized and unpasteurized samples, after 40 days of production and storage in a refrigerator.

SENSORY ANALYSIS

The methods chosen for evaluating the samples were:

- Discriminative analytical difference test using the triangular method;
- Affective acceptance test using a hedonic scale;
- Affective acceptance test using an ideal scale;
- Affective preference test by paired comparison.

To carry out the triangular test, each taster initially received 3 (three) coded samples of the drinks: 2 (two) corresponding to the unpasteurized drink, 1 (one) sample of the pasteurized drink and 1 (one) glass of water to cleanse the palate between samples. The tasters were instructed to evaluate the samples in their general aspects and identify which of the samples was different in their perception.

After the first stage of the analysis, the evaluators received another 2 (two) coded samples: 1 (one) sample of the unpasteurized drink, 1 (one) sample of the pasteurized drink and 1 (one) glass of water to cleanse the palate between the tastings.

An acceptability test was carried out, where tasters were instructed to evaluate the aspects of: color, odor, flavor and sensation of refreshment, using a hedonic scale of nine (9) points: (9) I liked it extremely (8) I liked it a lot (7) I liked it moderately (6) I liked it a little (5) I neither liked nor disliked it (4) I disliked it a little (3) I disliked it moderately (2) I disliked it a lot (1) I disliked it extremely.

The quantity of gas aspect was evaluated using an ideal five (5) point scale: (2+) much more gas than I like (1+) more gas than I like, (0) the way I like it (1 -) less gas than I like (2-) much less gas than I like.

At the end of the tastings, the tasters carried out a paired preference test, in which they indicated which of the samples they liked the most and made comments about the products consumed.

PHYSICOCHEMICAL AND MICROBIOLOGICAL CHARACTERIZATION

To verify which changes, in terms of composition, were generated in pasteurized and unpasteurized beverages, physical-chemical and microbiological tests were carried out on the samples, in accordance with their already standardized industrial protocols. The unpasteurized sample was analyzed immediately after its production (T1) and 40 days after its production, together with the pasteurized sample (T2). The analyzes carried out were as follows: pH using a bench pH meter (Tecnal®), °Brix (%) using a bench refractometer (Tecnal®), total acidity (meq/L) was performed by titration, acidity volatile content (ueq/L) by steam drag followed by titration, fixed acidity (meq/L) by calculation, alcoholic degree (m/v) by steam drag followed by density measurement in a pycnometer, all according to Lutz (2008). The count of lactic acid bacteria and yeasts (log CFU/mL) was carried out after serial dilution of the samples in a sterile environment and inoculation (pour plate) in MRS agar in anaerobic conditions for 48h and YGC in aerobic conditions for 7 days, respectively, both 30°C.

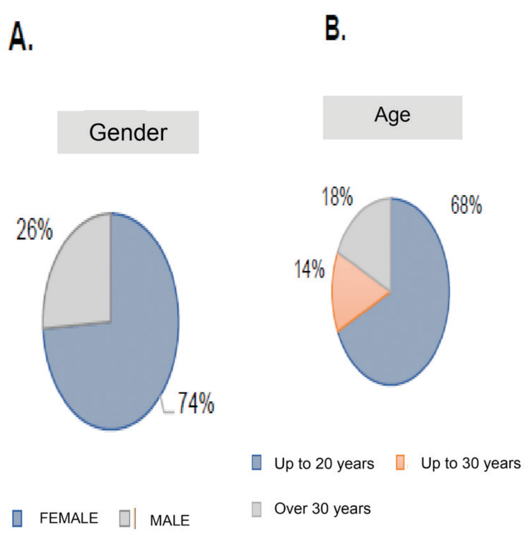
STATISTICAL ANALYSIS

The collected data were computed and subjected to interpretation and statistical analysis, in the Microsoft Office Excel software, using the Student's T Test, considering $p > 0.05$ statistically significant.

RESULTS AND DISCUSSION

SENSORY ANALYSIS

The sensory analysis was composed of fifty (50) providers, the majority of whom were female (Figure 1 A) and aged up to twenty (20) years (Figure 1 B), and, among the tasters, 46% had never consumed no type of carbonated probiotic drink (Figure 1 C).



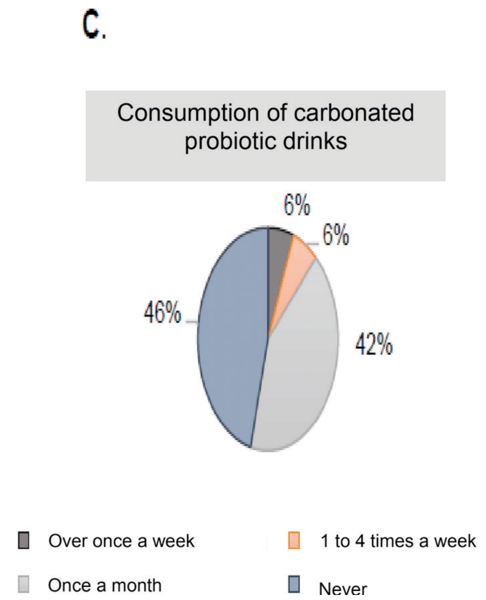


Figure 1: basic information about the tasters participating in the sensory analysis.

In the triangular test, 24 correct answers were obtained from the panelists. The minimum number of correct answers, to establish a significant difference between the samples, in an analysis with 50 respondents and a significance level of 5% is 23 correct answers (LUTZ, 2008). Therefore, according to the discriminative analytical test of triangular difference, pasteurized and unpasteurized beverages are statistically different at a significance level of 3 to 4%.

In the acceptability test, all the acceptance parameters evaluated (color, odor, flavor and sensation of refreshment), in both samples (pasteurized and unpasteurized), were found between 6.9 and 7.6, which corresponds to the scale hedonic evaluation range of "I liked it moderately" and "I liked it a lot". Table 1 shows the means obtained for each parameter evaluated. The presence of identical letters indicates that the samples do not differ from each other (p>0.05), with the means being considered statistically similar.

Parameters	Unpasteurized sample	Pasteurized sample	*p*
Color	7,58^{A}	7,44 ^{A}	*0,61*
Smell	7,26 ^{A}	7,48 ^{A}	0,42
Savor	7,00 ^{A}	7,00 ^{A}	1,00
Refreshment	6,96 ^{A}	6,90 ^{A}	0,82

Table 1: average acceptance values of unpasteurized and pasteurized whole red grape flavored kefir fermented drink samples.

The assessment of the amount of gas (Figure 2) was carried out using an ideal scale. In both samples, participants considered that the amount of gas was ideal (0). Considering only the choices above and below the ideal, the unpasteurized sample tended to score above the ideal: "much more gas than I like" and "more gas than I like" (2+ and 1+) and the pasteurized did not show a trend towards either end. Therefore, the amount of gas was also considered mostly ideal for both drinks, in the ideal scale acceptance test.

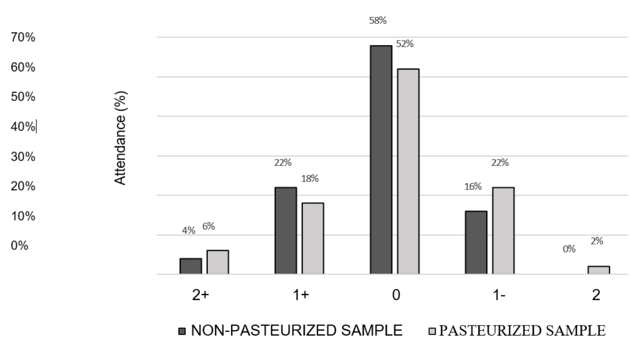


Figure 2: Frequency distribution in the assessment of the amount of gas by ideal scale of samples of unpasteurized and pasteurized whole red grape flavored kefir fermented drink.

The sensory analysis revealed that there is a statistically proven difference between pasteurized and unpasteurized drinks, but this difference does not affect the acceptability of the drink by the consumer and does not generate rejection, which shows that the pasteurization process did not affect the sensorial quality of the product. In the preference test by paired comparison, the pasteurized sample was preferred by the tasters in the evaluation of all aspects, being indicated as the sample that pleased the most by 27 (54%) of the fifty (50) tasters.

The study by Losada and Torres (2018) evaluated the sensorial change in beer pasteurization. This study allowed us to conclude that the pasteurization process effectively alters the sensorial attributes of the beer, but that such modifications do not seem to affect the tasters' choice of pasteurized beer or unpasteurized beer.

In the study by Ezequiel (2010), the influence of heat treatment on the physical-chemical and sensorial characteristics of a red wine subject to pasteurization treatment and another not subject to pasteurization treatment was studied. At the sensorial level, few differences were found between the two modalities, with higher quality parameters in pasteurized wine and others in wine not subject to treatment. In other words, in general, in both studies, pasteurization did not bring negative aspects to the products, as in the present study.

PHYSICOCHEMICAL AND MICROBIOLOGICAL ANALYSIS

The results of the physical-chemical analysis of the unpasteurized and pasteurized samples are shown in Table 2. It is possible to observe that both, in T2, did not have a significant change in pH and did not have changes regarding total acidity and fixed acidity compared to T1. The soluble solids content decreased in T2 of the unpasteurized sample, indicating metabolic activity with sugar consumption, but was maintained in the pasteurized sample. Volatile acidity increased in both samples. The alcohol content of the unpasteurized sample increased significantly in T2, while the pasteurized sample maintained its alcohol content in T2.

In parallel, the results of the microbiological tests (Table 2) show that the yeast count (log CFU/mL) increased in the order of 10^1 in T2, in the unpasteurized sample. In the pasteurized sample, the yeast count decreased by approximately 104 times. The lactic acid bacteria count (log CFU/mL) showed that from T1 to T2 the amount of lactic acid bacteria slightly decreased, demonstrating spontaneous death of lactic acid bacteria. In the pasteurized sample, the decrease in T2 was in the order 10^2. These results clearly demonstrate that the observed sugar consumption was used for alcoholic fermentation in the unpasteurized sample, even when stored under refrigeration, which led to an increase in yeast counts. While the sample was pasteurized, the initial alcohol content was maintained, demonstrating the efficiency of the thermal processing used to preserve the product.

The whole red grape kefir fermented drink, in addition to all the probiotic properties of its composition, has the additional properties of grape, which is an excellent source of vitamins and minerals, in addition to containing flavonoids, tannins and anthocyanins that have prebiotic action (PRIOR, 2006; BINNS et al, 2013; LEITE et al., 2018).

One of the biggest problems faced regarding its commercialization is its short shelf life, due to the microorganisms being in their viable form and continuing to multiply and carry out their fermentative processes (FIORDA et. al, 2017). An alternative found to increase its shelf life was the implementation of a conservation method, as a way of controlling the natural process of deterioration, caused mainly by the excessive amount of yeast, which favors the formation of gases and imparts alcoholic characteristics to the drink (BRINQUES, 2015; FIORDA et. al, 2017).

Pasteurization of the whole red grape

Parameters	T1 (Initial) Unpasteurized sample	T2 (40 days) Unpasteurized sample	T2 (40 days) Pasteurized sample
Brix (%)	13,4 A	12,4 B	13,6 A
pH	3,0 A	3,1 A	3,0 A
Total acidity (meq/L)	0,3 A	0,3 A	0,3 A
Volatile acidity (µeq/L)	4,9 A	7,7 B	7,0 C
Fixed acidity (meq/L)	0,3 A	0,3 A	0,3 A
Alcoholic degree (m/v)	0,1 A	1,3 B	0,1 A
Contagem de bactérias láticas (log UFC/mL)	6,4 A	5,2 B	3,2 C
Yeast count (log UFC/mL)	6,5 A	7,3 B	3,00 C

Table 2: physical-chemical and microbiological analysis of the samples, at their initial time (T1) and 40 days after the pasteurization process (T2).

kefir fermented drink was carried out as a way to control or reduce the microbial rate. Considering that the rate of destruction of microorganisms is exponential at temperature, the pasteurization method does not eliminate all microorganisms present in the drink, but acts by delaying or preventing the growth of most microorganisms (BRINQUES, 2015; CHEN; ROSENTHAL, 2015).

Another important aspect, which justifies the use of a microbial conservation method in a probiotic drink, whose properties are related to viable microorganisms, is that recent studies have been describing beneficial properties of inactivated probiotics, known as para-probiotics. Para-probiotics can be defined as "non-viable" microbial cells that, when consumed, provide health benefits. Just like probiotics, they contribute to modulating the immune system and act by inhibiting intestinal pathogens, but their mechanisms of action have not yet been completely elucidated. The advantages of para-probiotics for industry and consumers are that they are easier to handle, can be used in products exposed to high temperatures, allow for longer shelf life and are easier to store and transport (ALMADA,2017).

Calatayud et al. (2021) used a combination of in vitro tools that mimic colonic fermentation and intestinal digestion to study the effect of different levels of pasteurized and unpasteurized water kefir on intestinal microbiota, epithelial barrier function, and immunomodulation. Water kefir increased the beneficial production of short-chain fatty acids, reduced fermentative proteolytic compounds, and increased the abundance of the genus Bifidobacterium. The observed benefits were increased by pasteurization. Pasteurized products also improved inflammation-induced intestinal epithelial barrier disruption. These data endorse the potential health benefits of grape kefir and demonstrate that pasteurization, carried out to extend the shelf life and stability of the product, although it inactivates part of the probiotic microorganisms, also enhances these benefits, probably by generating a para-probiotic product.

CONCLUSION

The pasteurization of whole grape kefir did not affect the perception of color, odor, flavor, sensation of refreshment and amount of gas of the product, but it caused greater preference than the unpasteurized drink. The sensory analysis revealed that a difference was perceived between the pasteurized and unpasteurized beverage, but that it did not affect the acceptability of the beverage by the consumer and did not generate rejection, which shows that the pasteurization process did not affect the sensorial quality of the product.

Pasteurization maintained most of the physicochemical characteristics, being responsible for avoiding an increase in yeast growth and the consequent alcoholic content of the product.

After physical-chemical and microbiological analyses, which demonstrated that pasteurization, despite reducing the microbial rate, does not completely eliminate the microorganisms present in the drink, and with the information acquired about the benefits of para-probiotics, a way can be considered potential to preserve the whole red grape flavored kefir fermented drink and increase its shelf life.

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