Fermentation of kefir grains in different brazilian raw cane sugar commercial brands

Fermentação de grãos kefir em diferentes marcas comerciais de açúcar mascavo brasileiro

DOI:10.34117/bjdv6n4-026
Recebimento dos originais: 01/03/2020
Aceitação para publicação: 01/04/2020

Pedro Paulo Lordelo Guimarães Tavares
Mestre em Ciência de Alimentos
Doutorando em Ciência de Alimentos pela Universidade Federal da Bahia
Endereço: Rua Barão de Jeremoabo, 147, Faculdade de Farmácia da UFBA, Ondina, Salvador - BA, 40170-115
E-mail: pp.lordelo@gmail.com

Renata Quartieri Nascimento
Mestre em Ciência de Alimentos
Doutoranda em Biotecnologia pela Universidade Federal da Bahia
Endereço: Rua Barão de Jeremoabo, 147, Faculdade de Farmácia da UFBA, Ondina, Salvador - BA, 40170-115
E-mail: rqnutri@gmail.com

Emanuele Araújo dos Anjos
Graduanda em Farmácia
Endereço: Rua Barão de Jeremoabo, 147, Faculdade de Farmácia da UFBA, Ondina, Salvador - BA, 40170-115
E-mail: maanuaaraujo@hotmail.com

Thâmilla Thalline Batista de Oliveira
Mestre em Ciência de Alimentos
Doutoranda em Ciência de Alimentos pela Universidade Federal da Bahia
Endereço: Rua Barão de Jeremoabo, 147, Faculdade de Farmácia da UFBA, Ondina, Salvador - BA, 40170-115
E-mail: thamillabatista@hotmail.com

Jéssica Santiago Falcão
Mestre em Ciência de Alimentos
Endereço: Rua Barão de Jeremoabo, 147, Faculdade de Farmácia da UFBA, Ondina, Salvador - BA, 40170-115
E-mail: jeu.santiago@gmail.com
ABSTRACT

Kefir is a probiotic fermented beverage resulted of the inoculation of kefir grains on different substrates. The raw cane sugar (also known as brown sugar) solution is one of the most common and most studied substrates. However, there is little evidence of the interference of the origin of the sugar in the grain fermentation intensity. Therefore, this work aims to evaluate the kinetics of the fermentation of kefir grains in brown sugar solution from five different commercial brands. The fermentative substrate was prepared with 10% brown sugar dissolved in deionized water, and 10% kefir grains were added. The fermentation process took place for 48 hours at room temperature. The analyzes performed were pH, titratable acidity, lactic acid bacteria and yeast count, all of them at time 0 and 48 (hours) of fermentation. Kefir grain biomass was weighed at times 0, 24 and 48 (hours). Native brand showed a more pronounced fermentation process, with greater growth in biomass, from 50.01 g to 122.03g, while Bella brand resulted in a substrate with small changes of pH and acidity and a good microbial growth.

Keywords: water kefir; fermented drink; brown sugar.

RESUMO

O kefir é uma bebida fermentada probiótica resultante da inoculação de grãos de kefir em diferentes substratos. A solução de açúcar mascavo é um dos substratos mais comuns e mais estudados. No entanto, há poucas evidências da interferência da origem do açúcar utilizado na intensidade da fermentação dos grãos. Portanto, este trabalho tem como objetivo avaliar a cinética da fermentação dos grãos de kefir em solução de açúcar mascavo de cinco marcas comerciais distintas. O substrato fermentativo foi elaborado na proporção de 10% de açúcar mascavo em água deionizada, e adicionados de 10% de grãos de kefir. O processo fermentativo ocorreu por um período de 48h a

Braz. J. of Develop., Curitiba, v. 6, n.4,p.17004 - 17012 apr. 2020. ISSN 2525-8761
temperatura ambiente. As análises realizadas foram pH, acidez, contagem de bactérias ácido-láticas e leveduras no tempo 0 (horas) e no tempo 48 (horas) de fermentação, além de pesagem da biomassa de grãos nos tempos 0, 24 e 48 (horas). A marca Native apresentou um processo fermentativo mais pronunciado, com maior crescimento de grãos de kefir (biomassa) de 50,01g para 122,03g, enquanto que a marca Bella resultou em um substrato com pequenas variações de acidez e pH e um bom crescimento microbiano.

Palavras-chave: kefir de água; bebida fermentada; açúcar mascavo.

1 INTRODUCTION

Kefir is a probiotic fermented drink originated from Russia, made by the inoculation of kefir grains in a nutritious substrate, one of the most common being the brown sugar solution (Leite et al., 2013). Kefir grains can be defined as an aggregation of yeasts, lactic acid bacteria and acetic acid bacteria (Magalhães et al., 2010) that coexist in symbiosis and are surrounded by a polysaccharide matrix, called kefiran.

During the fermentation process, there is a production of lactic, acetic and gluconic acids, alcohol and carbon dioxide, which provide unique organoleptic properties of kefir, as well as an increase in microorganism count. Kefir grains can be donated between people, featuring a collaborative and free consumption of kefir (Magalhães, 2008). The intensity of the fermentation process can vary depending on conditions such as temperature, grain origin and type of substrate used for fermentation. These factors could be assessed through a series of analyzes, such as lactic acid bacteria and yeasts count, titratable acidity, pH, among others (Santos, 2015).

Kefir grains may have a different microbial population, depending on their origin. Different microorganisms have been identified in kefir grains from different regions (Weschenfelder et al., 2011). The factors that interfere on this distinct microbial composition seems to be mainly related to climate and substrate used (Oliveira, 2003). However, in general, bacteria and yeasts of the genus Lactobacillus and Saccharomyces, respectively, tend to be the most prevalent.

Several scientific studies evaluated the fermentation products of water kefir (Chen et al., 2008; Jianzhong et al., 2009; Weschenfelder et al., 2011), however there is a lack in the analysis of the influence of brown sugar from different origins in the fermentation process. Thus, this work aimed to evaluate the fermentation kinetics of water kefir grains inoculated in brown sugar solution of five different commercial brands.
2 MATERIALS AND METHODS

2.1 MATERIALS

The proposed work is an exploratory study of experimental nature carried out at the Faculty of Pharmacy of the Federal University of Bahia, during the period of April, 2018. The materials used were: water kefir grains obtained from the Laboratory of Probiotics of the UFRB (Federal University of Recôncavo Baiano) and brown sugar samples from five different commercial brands: Native, Mãe Terra, Bella, Tia Sônia and Jasmine, obtained at Salvador local commerce.

2.1 METHODS

2.2.1 SUBSTRATE PREPARATION AND FERMENTATION

Five substrates were prepared, each containing 400mL deionized water and 50g of brown sugar of their respective brand. 50g of water kefir grains were added to the substrates, which represented a volume of 10% of the total content. The fermentation took place over a period of 48 hours at room temperature in the Sensory Analysis Laboratory of the Faculty of Pharmacy (UFBA – Federal University of Bahia).

2.2.2 Fermentation Kinetics

To evaluate the fermentation process, the following analyzes were carried out: pH with digital pH meter (KASVI, K39-1014B model); titratable acidity with sodium hydroxide solution, according to the methodology proposed by the Adolfo Lutz Institute (2008); lactic acid bacteria count using MRS medium supplemented with 100mg/L of cycloheximide; yeasts count using Sabouraud Agar medium supplemented with 50mg/L of chloramphenicol. For both microorganisms counts, serial dilutions of the samples of $10^3$, $10^4$ and $10^5$ were performed, of which 100μl were inoculated and spread in the plates, according to APHA (2002). All of the analyzes were performed at the beginning (time 0 hour) and at the end (time 48 hours) of fermentation. Finally, the kefir grains were weighed on an analytical balance (Sartorius, model TE 2014S ) at times 0 hours, 24 hours and 48 hours.

2.2.3 Statistical Treatment

The following statistical analyzes were performed in the STATISTICA 7.0 software: ANOVA and Tukey test were performed to verify differences between the five samples at the same analyzed time. T test was performed to verify differences between the same sample in the two distinct times analyzed. Regarding the grain biomass, another Tukey test was performed, instead
of the T test, to evaluate differences between analysis times, since there was an additional analyzed period (24 hours).

3 RESULTS AND DISCUSSION

Figure 1 below indicates the behavior of kefir microorganisms during the 48h fermentation process. Regarding lactic acid bacteria, it can be seen that Native brand showed the greatest growth, with 5.92 log\(^{10}\)CFU/ml at the beginning and 7.79 log\(^{10}\)CFU/ml, in relation to others, indicating that Native brown sugar can produce a more nutritious substrate for bacteria such as *Lactobacillus casei* and *paracasei*, predominant strains of bacteria in water kefir grains (Laureys & De Vuyst, 2017). The fact that Native sugar is of organic production may have influenced this result since according to Faria (2012), organic sugars tend to be richer in nutrients, such as iron and calcium. On the other hand, Jasmin brand possessed the lowest growth of this group of bacteria at the end of the fermentation, with values from 5.75 to 6.60 log\(^{10}\)CFU/ml, which may suggest a lower nutritive potential. Magalhães (2008) found results similar to this study, when analyzing lactic acid bacteria of water kefir.

Figure 1 – Counts of lactic acid bacteria (1) and yeasts (2) at the beginning and end of the fermentation by water kefir grains.

![Graphs showing bacterial and yeast counts over time](image)

Lower case letters indicate that there is no significant difference between samples at the same time analyzed, according to the Tukey test (considering \(p < 0.05\)). Equal capital letters indicate that there is no statistical difference for the same sample at the different analyzed times, according to the T test (considering \(p < 0.05\)).

Brown sugar brands: (■) Native; (●) Mãe Terra; (▲) Bella; (▼) Tia Sônia; (♦) Jasmine.

For the yeast count, the result was similar: Native had a value of 6.24 log\(^{10}\)CFU/mL, therefore, showed higher values at the end of fermentation, while Jasmine, with 5.49 log\(^{10}\)CFU/mL resulted in the lowest average for these microorganisms. The yeasts most present in water kefir
grains are of the genus *Saccharomyces*, responsible for the fermentation of sucrose to produce various metabolites, such as ethanol, CO₂ and vitamins and are responsible for the unique flavor of kefir, so they are essential during the fermentation process (Laureys & De Vuyst, 2014).

Figure 2 represents the pH (1) and acidity (2) values of the substrates at the beginning and end of fermentation. As expected, these parameters showed opposite behaviors. The present microorganisms in kefir grain are responsible for the production of various acids such as acetic and lactic acid, which in turn result in the pH decrease of the substrate. These factors would be desirable, since they can reduce the deterioration and microbiological contamination of pathogens in kefir (Magalhães et al., 2010).

The Native brand showed a higher pH of 5.66 in the beginning of the fermentation and the smallest value of 3.52 at the end of fermentation, indicating that there could be more intense metabolism of kefir grain microorganisms and higher acidification in the medium. This information is corroborated by the result of the titratable acidity, since it had a higher acidity evaluated, of 4.37 g citric acid/100mL at the end of fermentation. On the other hand, Mãe Terra and Bella showed higher pH values, of 3.77 and 3.67 respectively, and lower acidity, of 1.90 and 1.53 respectively, at the end of the fermentation, indicating a lower intensity of action by the microorganisms of the kefir. In a study on the fermentation kinetics of water kefir, Laureys & De Vuyst (2014) found similar results in relation to pH, corroborating the data from this study.

![Figure 2](image)

Lower case letters indicate that there is no significant difference between samples at the same time analyzed, according to the Tukey test (considering p <0.05). Equal capital letters indicate that there is no statistical difference for the same sample at the different analyzed times, according to the T test (considering p <0.05).

Brown sugar brands: (■) Native; (●) Mãe Terra; (▲) Bella; (▼) Tia Sônia; (♦) Jasmine.
Figure 3 indicates the behavior of the biomass of kefir grains during the 48h fermentation. In general, the increase in the biomass of kefir grains from all samples was much higher than that described by other authors (Freitas et al., 2016; Santos, 2015), indicating that the sugars used had excellent nutritional potential for kefir. Native, specifically, was the one that resulted in the largest biomass of grains at the end of the 48h fermentation, with a value of 50.01 g (initial) to 122.03 g (final). On the other hand, Bella sugar obtained a lower biomass of kefir, with 98.25g at the end. The biomass growth of kefir grains is affected by a number of factors, such as temperature and presence of nutrients (Santos, 2015). As the experiment was carried out at room temperature, this may have been a stimulus for kefir grains, since the city of Salvador-BA has high average temperatures (25-28°C) during the period of April.

Lower case letters indicate that there is no significant difference between samples at the same time analyzed, according to the Tukey test (considering p <0.05). Equal capital letters indicate that there is no statistical difference for the same sample at the different analyzed times, according to the Tukey test (considering p <0.05).

Brown sugar brands: (■) Native; ( ●) Mãe Terra; ( ▲) Bella; ( ▼) Tia Sônia; (♦) Jasmine.

4 CONCLUSIONS

In general terms, all the brown sugar commercial brands evaluated showed good potential as a fermentative substrate for kefir grains. Native brand resulted in a beverage with stronger fermentation and a higher content of microorganisms and may be explained by the fact that this
sugar is organic and could have better nutritional composition. On the other hand, Bella brand resulted in a drink with good values of lactic acid bacteria and yeasts and a more discrete fermentation, as perceived by the results of pH and acidity. In relation to the final production of kefir biomass, Native seems to be more suitable.

ACKNOWLEDGEMENTS

The researchers would like to thank the Graduate Program in Food Science of the Faculty of Pharmacy – Federal University of Bahia (UFBA) for providing laboratory and reagents in order to carry out the analyzes.

REFERENCES

APHA. (2002). *Compendium of methods for the microbiological examination of foods*. APHA, Washington.

Chen, H. C.; Wang, S. Y.; & Chen, M. J. (2008). Microbiological study of lactic acid bacteria in kefir grains by culture-dependent and culture-independent methods. *Food Microbiology*, 25, 492-501.

Faria, D.A.M. (2012). *Estudo nutricional e sensorial de açúcares cristal, refinado, demerara e mascavo orgânicos e convencionais*. (Master Dissertation). 2012. 74p. São Carlos: Universidade Federal de São Carlos.

Freitas, B.S.M., Souza, J.L.F., Santos, M.S., Campos, J.H.G., Neto, A.R., & Egea, M.B. (2016). *Cinética de reprodução da biomassa do quefir*. V Congresso Estadual de Iniciação Científica e Tecnológica do IF Goiano.

Adolfo Lutz Institute. (2008). *Métodos Físico-Químicos para Análise de Alimentos*. 1 ed digital. São Paulo. Available at: <http://www.crq4.org.br/sms/files/file/analisedealimentosial_2008.pdf>. Accessed in: 05.04.2018.

Jianzhong, Z. et al. (2009). Analysis of the microflora in Tibetan kefir grains using denaturing gradient gel electrophoresis. *Food Microbiology*, 26, 770-775.

Laureys, D.L., & De Vuyst, L. (2014). Microbial species diversity, community dynamics, and metabolite kinetics of water kefir fermentation. *Applied and Environmental Microbiology*. 8(80).

Laureys, D., De Vuyst, L. (2017). The water kefir grain inoculum determines the characteristics of the resulting water kefir fermentation process. *Journal of Applied Microbiology*. 122(3), 719-732.
Leite, A. M. O., Miguel, M. A. L., Peixoto, R. S., Rosado, A. S., Silva, J. T., & Paschoalin, V. M. F. (2013). Microbiological, technological and therapeutic properties of kefir: a natural probiotic beverage. Brazilian Journal of Microbiology. 44(2), 341-349.

Magalhães, K.T.M. (2008). Caracterização microbiológica e química de quefir de leite e água com açúcar mascavo (Master Dissertation). 2008. 108p. Lavras: Universidade Federal de Lavras.

Magalhães, K. T., Pereira, G. V. M., Campos, C. R., Dragone, G., & Schwan, R. F. (2010). Brazilian kefir: structure microbial communities and chemical composition. Brazilian Journal of Microbiology. São Paulo.

Oliveira, R. B. S. (2003). Análise microbiológica do kefir em grão, suspensão, liofilizado e adicionado a ração de coelhos (Master Dissertation). 2003. 59 p. Afenas: Universidade de Alfenas.

Santos, F. L. (2015). Kefir – Propriedades funcionais e gastronômicas. Cruz das Almas/Bahia: UFRB, 123p.

Weschenfelder, S., Pereira, G. M., Carvalho, H. H. C., & Wiest, J. M. (2011). Caracterização físico-química e sensorial de kefir tradicional e derivados. Arquivos Brasileiros de Medicina Veterinária e Zootecnia. Porto Alegre, 63(2), 473-480.