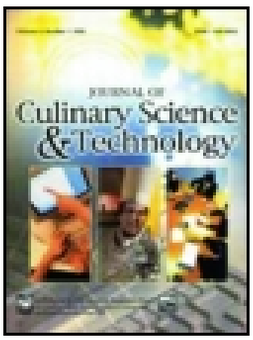

Journal of Culinary Science & Technology

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/wcsc20

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To cite this article: Poliana Guiomar De Almeida Brasiel , Thaís Costa de Almeida , Kácia Mateus , Antônio Fernandes de Carvalho , Sheila Cristina Potente Dutra Luquetti & Maria Do Carmo Gouveia Peluzio (2020): Maintenance of Probiotic Characteristics of Dry Kefir: Is It Possible?, Journal of Culinary Science & Technology

To link to this article: https://doi.org/10.1080/15428052.2020.1862010

Published online: 23 Dec 2020.

JOURNAL OF CULINARY SCIENCE & TECHNOLOGY
https://doi.org/10.1080/15428052.2020.1862010

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Maintenance of Probiotic Characteristics of Dry Kefir: Is It Possible?

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ABSTRACT

Kefir is a natural probiotic containing a complex mixture of bacteria and yeast associated in a matrix composed of protein and polysaccharide, and to it are attributed several beneficial properties to health. In this study, we have investigated the effect of kefir drying on the microbiological counts of lactic acid bacteria and yeasts. The viable bacteria and yeast counts in dry kefir were reduced when compared to fresh kefir 6.46 log cfu/g and 4.14 log cfu/g, respectively.. Thus, it was possible to maintain sufficient stability of microbes in the powdered product, indicating that industrial processing may contribute to the viability and survival of probiotic bacteria. Despite the technological challenges, the spray drying of kefir seems to be a promising method for the use of this probiotic complex on a larger scale.

ARTICLE HISTORY
Received 19 December 2019
Revised 4 November 2020
Accepted 6 December 2020

KEYWORDS
Kefir powder; spray drying; probiotics; fermented foods; health benefits

Introduction

The kefir is a natural probiotic containing a complex and specific mixture of bacteria and yeast association – lactose-fermenting and non-lactose-fermenting – in a protein-polysaccharide matrix (Farnworth, 2005; Machado et al., 2013).

The fermented milk containing lactic acid bacteria, the so-called probiotics, including kefir, emerged as an alternative therapy due to the growing interest for well-being and a healthy lifestyle. The fermented beverage kefir presents many health benefits, including antibacterial, anticarcinogenic, immunomodulation, and hepatic and gastrointestinal disease effects (Bengoa, Iraporda, & Garrote, 2018; Bellikci-Koyu et al., 2019; George et al., 2018; Golli-Bennour et al., 2019; Ritchie & Romanuk, 2012).

Kefir grains are the key to kefir production, fermented milk, and its final product has a different microbiological profile from the grains. Due to the production aspects and difficulty of standardization in the home environment, kefir drying can represent a tool for its large-scale production, safety, and

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greater access to the population. There is a growing interest arising in the inclusion of dried foods with viable probiotics to provide a health benefit to consumers (Farnworth, 2005; Kosin & Rakshit, 2006; Sánchez, Hernández, Auleda, & Raventós, 2011).

The dehydration of kefir and the production of instant powders may provide a solution to extend the market values of this drink. However, the loss of viable cell number is an important factor of fermented milk quality (Morelli & Capurso, 2012). Thus, drying techniques to obtain dehydrated probiotic organisms in a viable state have proven to be useful; and although lyophilization has been the most widely used, spray drying is less expensive and is more energy-efficient as well (Ananta et al., 2004; Atalar & Dervisoglu, 2015; Teijeiro, Pérez, L De Antoni, & Golowczyc, 2018).

Therefore, the objectives of this work were to evaluate the viability of lactic acid bacteria and yeasts in kefir powder when submitted to a drying process and to compare its microbiological characteristics to fresh kefir; as well as the microbiological count of fresh kefir kept under refrigeration. The purpose is to guide the development of a product of easy use, with a greater shelf life and maintenance of the beneficial properties attributed to kefir.

Materials and methods

Kefir preparation

Kefir grains originating from Universidade Federal de Viçosa (UFV), Minas Gerais, Brazil were used for fermentation. Kefir was prepared using commercial pasteurized whole milk as a substrate (Benfica®, MG, Brazil) in a proportion of 10 g of grains inoculated in 100 mL of milk and incubated at 25°C for 24 h in a glass container. The grains were then removed by filtration through a plastic sieve (pore size 5.6 mm, D × H 200 mm × 50 mm).

Spray drying

The kefir was homogenized (Marconi, Piracicaba, Brazil) before spray drying. The samples were spray dried using a laboratory-scale spray-dryer (Mini Spray Dryer B-290; Büchi, Flawil, Switzerland) at a constant air inlet temperature of 134°C and outlet air temperature of 66°C, whit pump rate of 35%. The powder was collected in a single cyclone separator.

Enumeration of microorganisms

To determine the concentration of viable microorganisms in kefir, appropriate dilutions in saline solution (0.85% NaCl) were carried out and plate counts were performed on de Man, Rogosa and Sharpe (MRS) agar (Merck,

Darmstadt, Germany) for lactic acid bacteria (LAB) and acidified Potato Glucose Agar (Fluka) for yeast. The results were expressed as colony-forming units (cfu)/g for the dried kefir and cfu/ml for the fermented product.

The kefir and powder were mixed for 1 min in a vortex mixer, maintained at room temperature for 30 min and then serially diluted in saline solution. LAB was enumerated on MRS agar by the drop count technique, incubated at a temperature of 37°C for 48 hours. Yeasts were grown in Potato Glucose Agar with tartaric acid (10%) to lower the pH of this medium and inhibiting bacterial growth under aerobic conditions at 25°C for five days. The Normative Instructions from the Ministry of Agriculture, Livestock and Food Supply (Brasil, 2003) were followed. The analyses were carried in the Experimental Nutrition Laboratory of the Department of Nutrition, Federal University of Juiz de Fora, Minas Gerais, Brazil. To investigate survival rates and their evolution during storage time, we performed microbiological counts of fresh kefir and the dry kefir, kept at room temperature and under refrigeration (4–8°C). The kefir production method and the drying process described above are shown in Figure 1.

pH analysis

The pH was measured by a pH electrode (Gehaka, PG1800; São Paulo, SP, Brazil) connected to an ion analyzer. The electrode was calibrated at the start of each assay by buffer solutions with pH 4.0 and 7.0 as standard.

Statistical analysis

All tests were performed in quadruplicate. Data were analyzed by statistical program GraphPad Prism 5 (GraphPad Software, Inc., La Jolla, CA, USA) and expressed as means ± standard error of the mean (SEM). One-way ANOVA followed by Newman-Keuls posttest determined the differences between the days of kefir storage under refrigeration, and the difference in microbiological count between fresh and dry kefir was analyzed by Student's unpaired *t*-test. Results were considered significant when $p < .05$.

Results and discussion

The results showed that kefir powder maintained at room temperature presented a marked decrease in the number of LAB after one week of storage, the transition from 6.82 log cfu/g (at time zero) to 5.64 log cfu/g. After 14 days no LAB was observed.

The LAB count on fresh kefir was 8.90 log cfu/ml, and 6.34 log cfu/ml for yeast, this result is similar to that found in other studies and meets the one that was proposed by the CODEX Standard for Fermented Milks (World Health

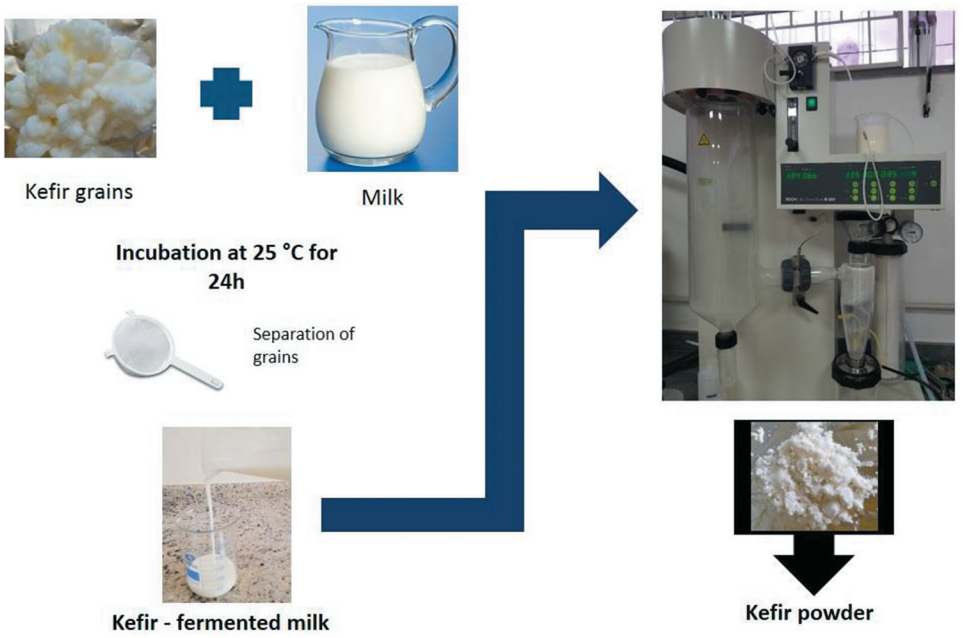

Figure 1. Scheme of the production of kefir milk. The kefir grains were weighed, added to the milk in a sterilized glass container in the proportion of 1:10 (m/v) and incubated in aerobic medium for 24 hours at 25°C ± 2°C. After the fermentation, the fermented milk was separated from the grains with the aid of a sieve. The kefir produced was used for the spray drying process.

Organization/Food and Agriculture Organization of the United Nations, 2011). Refrigeration was effective in stability and maintenance of this amount. Values of 8.90–8.57 log cfu/ml were found in the kefir for 35 days (5 weeks) under evaluation (Figure 2). Despite the significant reduction, the counts established maintained a minimum probiotic value. The complex constitution of LAB (10^8–10^9), yeasts (10^5–10^6), and acetic acid bacteria (10^5–10^6) in kefir grains, differ from the final product although influences its composition. The different microbial composition of the kefir grains depends on the region of origin, time of use, the substrate used and the manipulation techniques (Farnworth, 2005; Garrote, Abraham, & Antoni, 2001). Table 1 shows the evolution of LAB in kefir (fresh and dry) over days.

The pH values of kefir samples were determined through the 5 weeks, varying between 4.10 and 3.75, as presented in Figure 3. Cultivation substrate, grain/milk ratio, microbiological composition, and storage time significantly influence pH values. Food products with pH between 3.5 and 4.5, such as fermented milks, present buffering capacity, as they produce alkaline residues, responsible for the increase of the pH of the gastrointestinal tract. This increase confers a protective effect on the viability of *Lactobacillus* and *Bifidobacterium* sensitive to acids present in this tract. This result corroborates with the present work, in which due to its low pH (≤ 4.1), the main strains

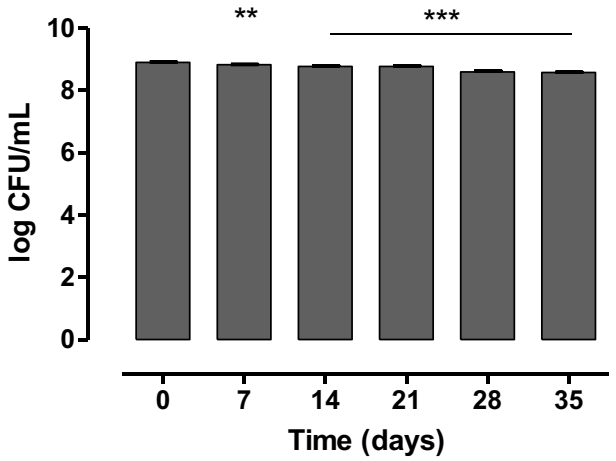

Figure 2. Microbial counts of lactic acid bacteria from kefir elaborated with whole cow milk, during the entire period storage under refrigeration. Results are expressed as mean ± SEM and were analyzed by one-way ANOVA, followed by Newman-Keuls test. The ** and *** symbols indicate that the difference is significant ($p < .01$ and $p < .001$; respectively) when compared to time zero.

Table 1. Cell counts of lactic acid bacteria in kefir and its powder during the storage period under refrigeration.

Samples/Days	0	7	14	21	28	35
Fresh kefir (log cfu/mL)	8.90 ± 0.01	8.83 ± 0.007**	8.77 ± 0.01***	8.77 ± 0.01***	8.60 ± 0.02***	8.57 ± 0.01***
Kefir powder (log cfu/g)	6.82 ± 0.02	6.29 ± 0.04*	6.38 ± 0.05	6.59 ± 0.04	6.32 ± 0.07	6.29 ± 0.02*

Results are expressed as mean ± SEM and were analyzed by one-way ANOVA, followed by the Newman-Keuls test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; compared to time zero).

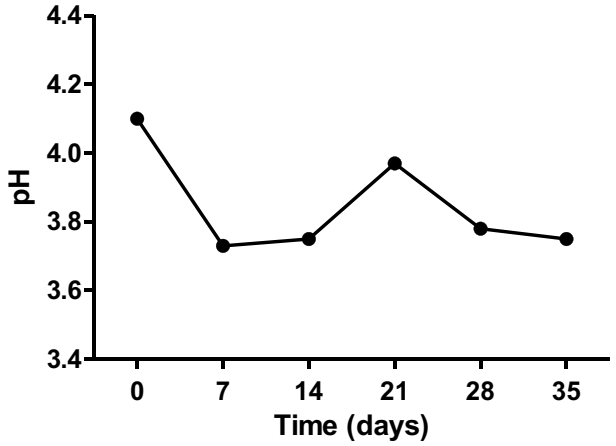

Figure 3. pH values from kefir elaborated with whole cow milk, during the entire period storage under refrigeration (five weeks).

found in kefir, maintain their viability and resist to the reduced pH of the gastrointestinal tract (Farnworth, 2008).

Proper acidity ensures inhibition of the development of pathogenic and deteriorating microorganisms that may alter the product during its shelf life. Other study did not find a significant difference in pH along the kefir storage in different samples, but observed a strong drop in pH during fermentation itself, in the presence of the grains, which can be justified by the degradation of lactose, resulting from the action of the bacteria present in the grains (Irigoyen, Arana, Castiella, Torre, & Ibánez, 2005).

When the dry kefir was evaluated, after the spray drying process, the LAB values had a variation of 6.82–6.30 log cfu/g (Figure 4). Compared to the values of fermented milk, there was a significant decrease of 0.52 log cfu in kefir powder. When analyzing yeast viability, populations also differed between beverage and dry kefir (Figure 5).

Spray drying can produce stable powders of some strains of bacteria and yeast; however, with the high temperatures involved in this process, the species require a certain level of thermal tolerance. Also, the degree of survival or destruction of bacteria during spray drying depends on the temperature/time binomial used (Chávez & Ledeboer, 2007; Paéz et al., 2012). Unlike other studies that evaluated a kefir isolate (Golowczyc, Gerez, Silva Analı, L De Antoni, & Teixeira, 2011; Golowczyc, Silva, Abraham, De Antoni, & Teixeira, 2010), we evaluated the whole fermented milk, which is a great challenge because it is a complex task involving several strains of bacteria and yeasts. Figure 6 shows the comparison between the LAB means of kefir and the powder after the production.

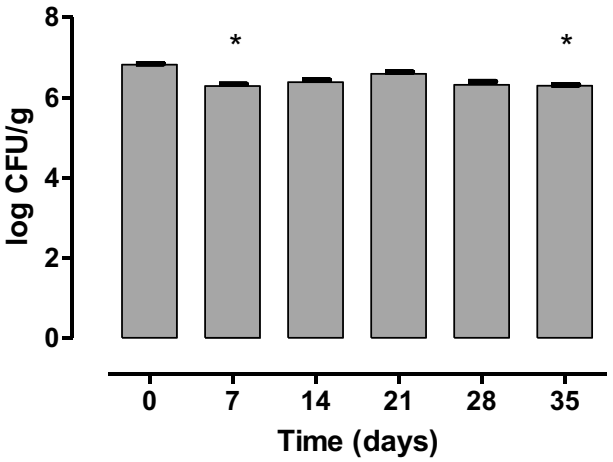

Figure 4. Survival rate for lactic acid bacteria after spray drying. Results are expressed as mean ± SEM and were analyzed by one-way ANOVA, followed by Newman-Keuls test. The * symbol indicates a significant difference ($p < .05$) during the storage period compared with the initial counts (time zero).

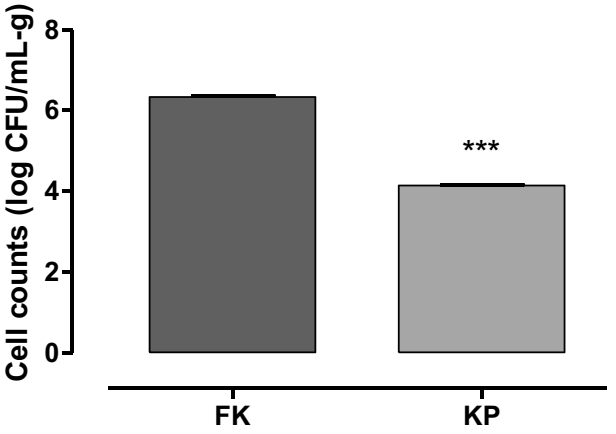

Figure 5. Average of the microbial counts of yeasts from kefir, before (dark gray) and after (light gray) spray drying process. Results are expressed as mean ± SEM and was analyzed by *t*-test (unpaired) (*** $p < .001$). FK – Fresh kefir; KP – Kefir powder.

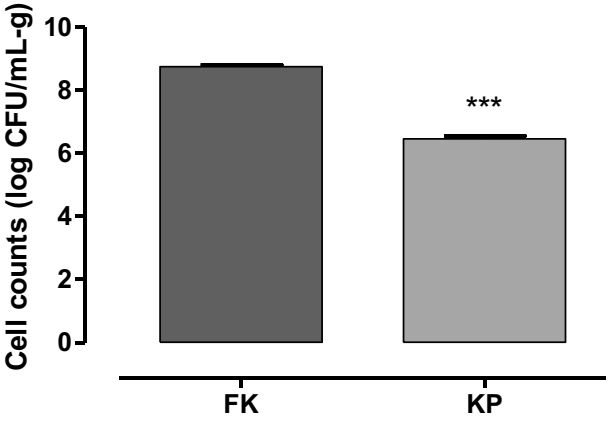

Figure 6. Average of the microbial counts of lactic acid bacteria from kefir, before (dark gray) and after (light gray) spray drying process. Results are expressed as mean ± SEM and was analyzed by *t*-test (unpaired) (*** $p < .001$). FK – Fresh kefir; KP – Kefir powder.

Viability during storage is the result of diverse variables that include the initial number of microorganisms, water activity, storage conditions and packaging (Chávez & Ledeboer, 2007). These issues must be considered to optimize the long-term survival of probiotics dried. Our results showed that the storage temperature was a critical parameter that affected the survival of microorganisms and only under refrigeration it was possible to maintain the viability of the powdered kefir. Although the values showed a significant decrease in LAB and yeast counts compared to the fresh kefir (−26.0% and −34.5%, respectively), the values found meet the minimum counts established with a small amount of dry powder (approximately 1.25 g/day).

As a result of the microbiological composition and chemistry, the kefir can be considered as a complex probiotic, which has in its composition living microorganisms that are capable of improving the intestinal microbial balance producing effects beneficial to the health of the individual. Its beneficial health properties have been reported in several studies and driven the development of products that preserve these characteristics (Bell, Ferrão, Pimentel, Pintado, & Fernandes, 2018; Bengoa, Iraporda, & Garrote, 2018; Reid, Jass, Tom Sebulsky, & Mccormick, 2003; Santos, 2015).

Conclusions

During the refrigerated storage period, the microbiological counts remained stable, and although they had have occurred the point changes of the values remained satisfactory, in compliance with regulations. In relation to the physical-chemical analysis, the same occurred with the pH. Yeasts were more sensitive to the drying method and parameters used. The results indicated that industrial processing may contribute to the viability and improvement of probiotic bacteria survival, despite the technological challenges, the drying of kefir by spray drying seems to be a promising method for the use of this probiotic complex on a larger scale.

Acknowledgments

We would like to thank "Fundação de Amparo à Pesquisa do Estado de Minas Gerais" (FAPEMIG- CDS - APQ-01332-16).

Funding

This work was supported by the Fundação de Amparo à Pesquisa do Estado de Minas Gerais [APQ-01332-16].

Declaration of interest statement

The authors declare that there are no conflicts of interest.

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