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Effect of kefir biomass on nutritional, microbiological, and sensory properties of mango-based popsicles

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Abstract

The purpose of the present work was to evaluate the effect of kefir biomass (grains) on the nutritional, microbiological, and sensory characteristics of mango-based popsicles. Mango pulp (1,000 mL), 5% of brown sugar, and 20% of kefir grains (triplicate) were mixed and fermented for 24 h at 28°C to create fermented kefir mango-based popsicle (FKMP); while only 1,000 mL of mango pulp was used for control popsicle (CP). Thereafter, the nutritional, microbial, and sensory analyses were performed in mango pulp, CP, and FKMP popsicles. Smith's salience index (SSI) results showed that the flavour attribute was highlighted in kefir mango-based popsicles, with mean scores ranging ($p < 0.05$) from 7.7 to 8.4 on a 9-point hedonic scale, indicating the participants' liking for the popsicles from moderate to extreme. Supplementation with kefir grains has increased the nutritional characteristics, mainly the protein content (0.38 g/100 g (CP) to 7.27 g/100 g (FKMP)). The PCR-DGGE analyses showed that bacteria such as *Lactobacillus, Lactococcus, Leuconostoc,* and *Acetobacter*, and yeasts such as *Saccharomyces, Kluyveromyces, Lachancea,* and *Kazachstania* were the microorganisms present. The present work is the first to report about kefir mango-based popsicle production, which allows for the consumption (by ingestion) of kefir biomass (grains) after the fermentation process. These results open new perspectives for the innovative application of kefir biomass in developing popsicles containing high protein content and kefir probiotic microorganisms.

Introduction

Demand for functional and probiotic food products is rapidly growing all over the world due to consumers' increased awareness regarding the impact of food on health (Salem *et al.,* 2015). 'Functional foods', 'probiotics', and 'nutraceuticals' are synonyms for foods with ingredients that can prevent and treat diseases (Magalhães *et al.,* 2011; Salem *et al.,* 2015; Cho *et al.,* 2018; Magalhães-Guedes *et al.,* 2018). Some authors have emphasised new foods that contain microorganisms referred to as probiotics (Wang *et al.,* 2017; Cho *et al.,* 2018; Martins *et al.,* 2018; Basavaiah *et al.,* 2019). Numerous health benefits have been claimed for probiotic microorganisms, and many products containing one or more groups of probiotic microorganisms are available worldwide (Vinderola *et al.,* 2005; Magalhães *et al.,* 2011; de Oliveira Leite *et al.,* 2013; Salem *et al.,* 2015; Cho *et al.,* 2018; Magalhães-Guedes *et al.,* 2018; Pankiewicz *et al.,* 2020).

Kefir grains are used in the production of functional and probiotic beverages such as the use of various substrates and different types of milks, such as cow, goat, sheep, coconut, rice, and soy milk (Magalhães *et al.,* 2011; Puerari *et al.,* 2012; Wang *et al.,* 2017, Viana *et al.,* 2017; Cho *et al.,* 2018; Magalhães-Guedes *et al.,* 2018; Amorim *et al.,* 2019). Kefir grains (biomass) are symbiotic mixed culture of yeasts and bacteria, in a matrix of proteins and polysaccharide 'kefiran' which are formed by the microorganisms (Magalhães *et al.,* 2010). Kefir grains are yellowish-white in colour, and resemble cauliflower blossoms (Corona *et al.,* 2016). A variety of microorganisms form kefir grains, with the *Lactobacillus* spp. being the most frequent

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(Magalhaes *et al.,* 2011). Other than that, lactic acid bacteria including *Lactococcus* and *Leuconostoc* are also common in kefir (Viana *et al.,* 2017; Magalhães-Guedes *et al.,* 2018). *Acetobacter* represents the acetic acid bacteria, and the yeasts in kefir are *Kluyveromyces, Candida,* and *Saccharomyces* (de Oliveira Leite *et al.,* 2013; Viana *et al.,* 2017). There are numerous benefits of consuming kefir beverages including antimicrobial (Rodrigues *et al.,* 2005), anti-inflammatory, antiallergic activity (Lee *et al.,* 2007), and antitumor (Vinderola *et al.,* 2005). Kefir may help bridge the gap between health benefits for the consumption of non-dairy foods and the benefits of probiotics without milk or dairy product consumption (Lee *et al.,* 2007; de Oliveira Leite *et al.,* 2013; Wang *et al.,* 2017; Cho *et al.,* 2018).

A popsicle or water ice cream is a variety of ice cream that consists of a frozen sweet block, which can be made from fruit juices or other sweet beverages. It is usually in a rectangular or cylindrical shape with a stick that runs vertically with a free extension of the solidified block at one of its ends, intended for tasting. Popsicles are globally appreciated (Balthazar *et al.,* 2017) especially in tropical countries with many beaches and ancient culture of consuming this refreshing food by both adults and kids. It is also easy to make and low cost, therefore, foregrounding the relevance of conciliating delicious food with the nutritional properties of kefir grains for consumers.

Popsicles prepared with fruit-based ingredients are considered nutritious due to their vitamin and mineral contents (da Silva *et al.,* 2020). Mango (*Mangifera indica* L. var. Rosa) is one of the most economically important tropical plants in Brazil (Pan and Zhang, 2018), and belongs to the Anacardiaceae family. The types of mango mostly consumed in Brazil are "Mango Rosa", "Mango Tommy", "Mango sword", and "Mango Palmer". The fruit contains an active constituent called mangiferin (Pan and Zhang, 2018) which could be used to treat respiratory and diabetic diseases. The fruit is also rich in phenolics, including phenolic acids (Pan and Zhang, 2018), flavonoids (Berardini *et al.,* 2004), and benzophenones (Berardini *et al.,* 2004). These compounds have demonstrated various bioactivities such as antioxidant, antidiabetic, anti-inflammatory, and immunomodulatory activities (Lin and Lee, 2014).

"Mango Rosa", also known as "Rosa da Bahia" or "Rosa de Pernambuco" is a species found easily in the Northeast region of Brazil. It is fibrous and very sweet, mostly used for juices and desserts, and is well accepted by the North-eastern consumers. It also contains nutritional values that are unique to it. These make it suitable for the production of popsicles.

Ripe mango fruits are a kind of crop waste that is rich in resources and may be used in mango-based popsicle processing. However, regular fruit popsicles still do not contain a considerable number of functional compounds and probiotic microorganisms (Martins *et al.,* 2018). Therefore, the objective of the present work was to evaluate the effect of the kefir biomass (grains) on the nutritional, microbial, and sensory characteristics of mango-based popsicles.

Materials and methods

The raw material used was Mango Rosa, sold in the Rio Vermelho fruit market in the city of Salvador, Bahia, Brazil. Mango fruits that are extremely mature and failed to meet the quality standards required for marketing were selected for the present work. These fruits are usually discarded as customers do not find them pleasing to the eye.

The identification of the maturity of the mango fruit is mainly based on the observation of the roughness and brightness of the skin, the formation of an "oval shoulder" in the peduncle region, the firmness of the flesh, and the colours of both the skin and the flesh (Subedi *et al.*, 2007). The selected fruits were extremely ripe, high skin roughness, low skin brightness, low flesh firmness, and an intensely pink skin.

Production of control popsicles and fermented kefir mango-based popsicles

The popsicle production was as follows: mango fruits that failed to meet the quality standards required for marketing were washed in clean water to remove residue. The selected fruits were extremely ripe, high skin roughness, low skin brightness, low flesh firmness, and an intensely pink skin, and were approximately 60 mm in diameter and 170 g each. The fruits were stored at 2°C to ensure the mango maintained its characteristics for making the popsicles.

The pulp was then extracted using an automatic de-pulping machine (ITAMETAL 0.5 DS, Itabuna, Bahia, Brazil), and a heat treatment was performed at 65°C for 45 min, and the mango pulp was frozen at -18°C for 24 h (Dias *et al.,* 2016). The must of the control popsicles was produced with 1,000 mL of mango pulp, while the fermented popsicle was made with 1,000 mL of mango pulp and previously tested proportions of 5% brown sugar and 20% kefir grains (triplicate). They were then divided into the Erlenmeyer flasks (2,000 mL) in triplicate.

The must was submitted to a kefir grain fermentation process for 24 h at 28°C. After fermentation process, the grains were mixed in a blender with a 2-L capacity and 600 W of power (Philips, Walita

RI 2044, Varginha, Minas Gerais, Brazil), with the fermented must for FKMP production. The control and fermented must were then subjected to rapid freezing (-18°C). After 5 min, wooden ice cream sticks were inserted, and the products were left to completely freeze. The CP and FKMP popsicles were then removed from the mould, individually packaged, and stored for 24 h at -10°C. For the nutritional and microbial analyses, samples were thawed ahead of time under refrigeration (0 - 4°C/4 h), and homogenised. For the sensory analyses, samples were served frozen.

Microbiological evaluation - DNA extraction and PCR-DGGE analysis

The microbiological analysis of the kefir grains and popsicles (CP and FKMP) was carried out at the Molecular Biology Laboratory of the Federal University of Lavras - UFLA, Brazil. For the analysis, 1 g of kefir grains and 1 mL of CP and FKMP samples were used for DNA extraction (Magalhães *et al.,* 2010). The amplification was carried according to Puerari *et al.* (2012), using primers 338fgc and 518r spanning the V3 region of the 16S rDNA gene (for bacteria), and primers NS3 and YM951r (for yeasts). PCR products were analysed by PCR-DGGE using a Bio-Rad DCode Universal Mutation Detection System (Bio-Rad, Richmond, CA, USA) according to Puerari et al. (2012). PCR-DGGE bands sequences were compared to the GenBank database, available at http://www.ncbi.nlm.nih.gov/BLAST.

Analysis of microbial viability

The viability of yeasts, acetic acid bacteria, and lactic acid bacteria was analysed following the methodology described by da Silva *et al.* (2017). For the analysis, 1 mL mango-based kefir popsicles samples were diluted with 9 mL of sterile 0.1% peptone water which yielded 10^{-1} dilution. From this, subsequent serial dilutions were performed until 10^{-8} dilution was obtained. Yeast counting was performed by the Dichloran Rose Bengal Chloramphenicol (DRBC) agar medium. The plates were incubated for 5 d at 25°C. The counting of acetic acid bacteria was carried in the Glucose Yeast Maltose (GYM) agar medium. The plates were incubated at 35°C for 48 h. The counting of lactic acid bacteria was performed in Agar De Man Rogosa and Sharpe (MRS) agar medium. The plates were incubated at 35°C for 48 h. Plates that showed typical colony growth were selected for counting. The results were analysed and expressed in CFU/mL.

Nutritional measurement

The nutritional analyses of the mango pulp, CP, and FKMP samples were performed in triplicate following the methodology described by AOAC (2012). Lipids were analysed using the Soxhlet method. The humidity was determined by direct drying at 105°C (procedure 012/IV). For ash determination, the sample was initially dried, then the material was incinerated in a muffle at 550°C (procedure 018/IV). Total dietary fibre (TDF) was calculated using the enzymatic-gravimetric method. Protein contents were measured by the Soxhlet extraction system (TT 12/A, Gerhardt Ltd., Germany), and the Kjeldahl method (VAPO45, Gerhardt Ltd., Germany), respectively. Carbohydrates were assessed by the difference among other components. The pH value of the popsicles was measured at room temperature using a digital pH meter (Micronal, B474 model, Germany), and the soluble solids were determined using a digital refractometer (ATAGO PR-1000), and the results were expressed in the Brix degree. The total calorific value was calculated following RDC no. 360 from *Agência Nacional de Vigilância Sanitária* (ANVISA, 2003).

Sensory analysis

The CP and FKMP popsicles were evaluated in a sensory test by 100 untrained tasters (students and staff of the Federal University of Bahia, Brazil) ranging in age from 20 to 55 years old. The panellists were asked to indicate how much they liked or disliked each product on a 9-point hedonic scale (9 = like extremely; 1 = dislike extremely). Evaluations of the popsicles' appearance, colour, flavour, and texture attributes were conducted. These data were then submitted to the test of Smith's salience index (SSI) (Santos *et al.,* 2015) to calculate the best attribute. The sensory analysis project was approved by the Research Ethics Committee of the Nutrition School of the Federal University of Bahia, number 1.759.169.

Statistical analysis

All analyses were performed in triplicate. Data were expressed as descriptive measures (means ± standard deviations), and submitted to one-way analysis of variances (ANOVA), while the comparison of the means was done using the Tukey's test at a 95% confidence level (Nunes *et al.,* 2015). Data concerning sensory attributes (appearance, colour, flavour, and texture) were evaluated by Smith's salience coefficient using XLSTAT 2017.4 (Adinsoft, Paris, France).

Results and discussion

Nutritional characterisation of popsicles (CP and FKMP)

The kefir grains were suitable in the

fermentation process using mango must for the production of kefir mango-based popsicles. Many studies have found that mango is appreciated for making popsicles (Berardini *et al.,* 2004; Balthazar *et al.,* 2017). Previous studies have shown that mangoes demonstrate various bioactivities (Lin and Lee, 2014). The present work suggests that mango could serve as a good metabolic source for the development of healthy popsicles.

The nutritional measurements of kefir mango-based popsicles were characterised. The proximate composition (g/100 g) of the popsicles (CP and FKMP) was: total soluble solid (°Brix) (≈ 13 - 2), pH (6.0 - 4.1), ash (≈ 0.2 - 4.9), total lipid (≈ 0.2), total dietary fibre (1.6), protein (≈ 0.3 - 7.2), carbohydrate (≈ 17 - 5), and calorific value (Kcal/100 g) (≈ 74 - 49). The kefir biomass did not lead to significant changes ($p > 0.05$) in total lipid. The protein content in the FKMP presented a significant increase ($p < 0.05$) as compared to the CP and mango pulp (Table 1).

There was a decrease in pH and Brix values during 24 h of fermentation. This indicates that the fermentation process was followed by the consumption of sugars and the production of acids, thus demonstrates that kefir grains were able to ferment the mango pulp. The low pH (~ 4.1) and °Brix (~ 2) values present at the end of fermentation appear (in FKMP) to be responsible for the presence of lactic acid bacteria, acetic acid bacteria, and yeasts (Figure 1).

Kefir yeasts account for the consumption of sugars and the production of metabolites such as alcohols, esters, and aldehydes (Magalhães *et al.,* 2011; Magalhães-Guedes *et al.,* 2018, Basavaiah *et al.,* 2019). Lactic acid bacteria are mainly responsible for the production of lactic acids (Magalhães *et al.,* 2010; 2011; Magalhães-Guedes *et al.,* 2018), and acetic acid bacteria are responsible for the production of acetic acids (Magalhães *et al.,* 2010; 2011; Magalhães-Guedes *et al.,* 2018). The microbial diversity of kefir grains plays a role in kefir's aroma and its nutritional/functional and organoleptic characteristics (Magalhães *et al.,* 2010; 2011; de Oliveira Leite *et al.,* 2013; Cho *et al.,* 2018).

The protein and ash contents in the FKMP sample was significantly higher ($p < 0.05$) than in its CP counterpart. The protein content increased after 24 h of fermentation (Table 1), which might be correlated with the increase of microbial biomass during this process (Magalhães-Guedes *et al.,* 2018). The microorganisms in the kefir grains produce proteins during the fermentation process. Pankiewicz *et al.* (2020) reported in their studies that the use of the probiotic bacteria (*Lactobacillus rhamnosus* B 442) for milk fermentation caused the obtained ice cream had highest content of dry matter and protein.

The increase in protein content in the present work could be due to the fact that these proteins are part of the chemical constitution of the biomass (grains) of kefir, and also dispersed in the fermentation medium (Magalhães-Guedes *et al.,* 2018). This resulted in the secretion of protein molecules contributing to the nutritional value of the kefir popsicles (de Oliveira Leite *et al.,* 2013). An important fact is due to the production of a protein popsicle without adding cost to the final product because the kefir grains are distributed free among the Brazilian population. Protein is of great importance due to its various biological properties such as

Table 1. Nutritional measurement of control popsicles (CP) and fermented kefir mango-based popsicles (final popsicle - FKMP).

Parameters	Mango pulp	CP	FKMP
Total soluble solid (°Brix)	13.30 ± 0.01^a	13.30 ± 0.01^a	2.21 ± 0.04^b
pH	6.28 ± 0.01^a	6.28 ± 0.01^a	4.10 ± 0.01^b
Ash (g/100 g)	0.25 ± 0.05^a	0.25 ± 0.05^a	4.92 ± 0.04^b
Total lipid (g/100 g)	0.24 ± 0.01^a	0.24 ± 0.01^a	0.25 ± 0.01^a
Total dietary fibre (TDF) (g/100 g)	1.6 ± 0.01^a	1.6 ± 0.01^a	1.6 ± 0.01^a
Protein (g/100 g)	0.38 ± 0.02^a	0.38 ± 0.02^a	7.27 ± 0.01^b
Carbohydrate (g/100 mL)	17.75 ± 0.09^a	17.75 ± 0.09^a	5.02 ± 0.07^b
Calorific value (Kcal/100 g)	74.83 ± 0.09^a	74.83 ± 0.09^a	49.16 ± 0.06^b

Data are means ± standard deviations. Means with the same letter in the same row do not differ significantly ($p < 0.05$) based on Tukey's test. Control popsicles (CP) = mango-based popsicle; fermented kefir mango-based popsicle (FKMP) = final popsicle.

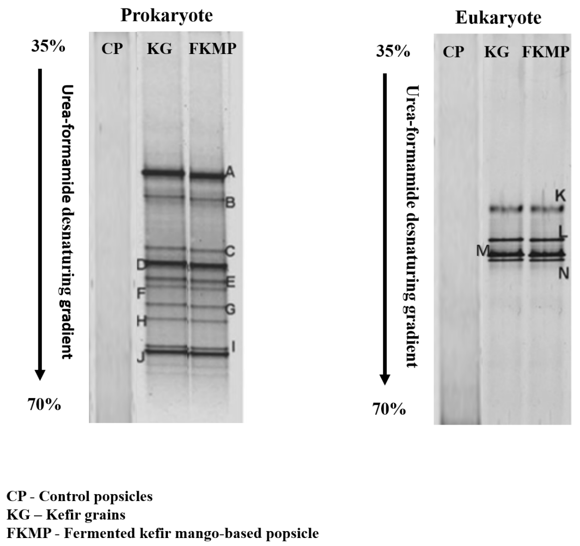

Figure 1. Profiles of microbial communities from kefir grains (biomass) (KG) in mango-based control popsicles (CP) and final fermented kefir mango-based popsicles (FKMP). Identified prokaryotic groups included Banda A: *Lactobacillus paracasei* (accession number - AB368902.1), Banda B: *L. kefiri* (accession number - AB3626680.1), Banda C: *L. parabuchneri* (accession number - AB368914.1), Banda D: *L. casei* (accession number - EU626005.1), Banda E: *L. paracasei* subsp. *paracasei* (accession number - NR025880.1), Banda F: *L. paracasei* subsp. *tolerans* (accession number - AB181950.1), Banda G: *L. buchneri* (accession number - FJ867641.1), Banda H: *Lactococcus lactis* (accession number - EU194346.1), Banda I: *Leuconostoc citreum* (accession number - FJ378896.1), and Banda J: *Acetobacter lovaniensis* (accession number - AB308060.1). Eukaryotic groups were Banda K: *Kluyveromyces lactis* (accession number - AJ229069.1), Banda L: *Saccharomyces cerevisiae* (accession number - EU649673.1), Banda M: *Kazachstania aerobia* (accession number - AY582126.1), and Banda N: *Lachancea meyersii* (accession number - AY645661.1).

immunomodulatory, antimicrobial, antiviral, anticancer, antiulcer activities, and cardiovascular system benefits (Davoodi *et al.,* 2016). The differences in the protein and ash levels of the CP and FKMP samples occurred due to the addition of kefir biomass (grains) to the FKMP popsicles (Magalhães-Guedes *et al.,* 2018; Martins *et al.,* 2018). It is possible to combine kefir grains and concentrated fruit juice (specifically mango) for the production of protein-rich popsicles as a suitable processing condition for potential industrial applications. The Recommended Daily Allowance (DDR) for protein consumption in the diet is currently fixed at 50 g per day for a normal adult, and its value to be calculated is about 0.8 g per kilogram of body weight (de Oliveira Leite *et al.,* 2013), and 2.5 - 3.0 g of fibre in the food portion (ANVISA, 2012). Therefore, the popsicle produced in the present work has significance dietary value, since, with a 50 g popsicle, approximately 3.5 g of protein can be ingested.

The kefir biomass (grains) is also a good source of nutrients with functional properties such as vitamins and minerals (Corona *et al.,* 2016). Biomass is produced by microorganisms during the fermentation process. After fermentation, the biomass is harvested and may be used as a protein source or subjected to new fermentative processing (Corona *et al.,* 2016; Cho *et al.,* 2018). In the present work, the kefir biomass was maintained in the final product to increase the protein value in the FKMP popsicles with an initial value of 0.38 g/100 g (CP) and a final value of 7.27 g/100 g (FKMP).

Kefir has proteins that are partially digested, and in this respect, the human body easily utilises them (Corona *et al.,* 2016). The presence of high protein content in FKMP popsicles can provide the commercial appeal of functional food since these potent compounds act to reduce the risk of non-communicable diseases (Davoodi *et al.,* 2016; Li *et al.,* 2019). The functional potential of the FKMP popsicles can contribute to the delivery of functional bioactive compounds with recognised health benefits in humans.

Microbiological evaluation of kefir grains and popsicles (CP and FKMP)

Currently, several investigations have focused on the beneficial effects of probiotic agents and their possible roles in preventing and treating various diseases (de Oliveira Leite *et al.,* 2013; Cho *et al.,* 2018). Kefir belongs to the probiotic group, and is popular in several countries. Its grains contain various prebiotics nutritional metabolites and probiotic microorganisms such as lactic acid bacteria, acetic acid bacteria, and yeasts, coupled together with casein, proteins, polysaccharides, and various vitamins (de Oliveira Leite *et al.,* 2013; Wang *et al.,* 2017). Kefir has specific characteristics (such as taste and aroma) that are typically attributed to the presence of a complex microbial population (de Oliveira Leite *et al.,* 2013; Cho *et al.,* 2018). In the literature, several health-promoting properties have been associated with kefir consumption (de Oliveira Leite *et al.,* 2013; Cho *et al.,* 2018). The beneficial action of kefir can be attributed to the probiotic microbial population present in the grains and nutritional metabolites released by the fermentative process (de Oliveira Leite *et al.,* 2013; Wang *et al.,* 2017; Cho *et al.,* 2018; Magalhães-Guedes *et al.,* 2018). Therefore, the identification of these microbial species is extremely important.

Traditional identification processes are only partially selective and exclude species of the total microbial community in the analysed samples (Corona *et al.,* 2016), therefore, the use of molecular biology is necessary (Magalhães *et al.,* 2010; 2011; Cho *et al.,* 2018). PCR-DGGE analysis was used to determine the total microbial composition of kefir biomass (grains), and CP and FKMP popsicles. The CP popsicle did not present microbiota in its constitution (Figure 1). Only the kefir grains and the FKMP presented microbial diversity (Figures 1 and 2). The sequence results showed between 99 and 100% identity with the sequences retrieved from GenBank accession numbers. PCR-DGGE bands concerning the kefir grains and FKMP were clearly identified as the following microbial groups:

Prokaryotic group:

i. Banda A: *Lactobacillus paracasei* (accession number - AB368902.1);
ii. Banda B: *L. kefiri* (accession number - AB3626680.1);
iii. Banda C: *L. parabuchneri* (accession number - AB368914.1);
iv. Banda D: *L. casei* (accession number - EU626005.1);
v. Banda E: *L. paracasei* subsp. *paracasei* (accession number - NR025880.1);
vi. Banda F: *L. paracasei* subsp. *tolerans* (accession number - AB181950.1);
vii. Banda G: *L. buchneri* (accession number - FJ867641.1);
viii. Banda H: *Lactococcus lactis* (accession number - EU194346.1);
ix. Banda I: *Leuconostoc citreum* (accession number - FJ378896.1);
x. Banda J: *Acetobacter lovaniensis* (accession number - AB308060.1).

Eukaryotic group:

i. Banda K: *Kluyveromyces lactis* (accession number - AJ229069.1);
ii. Banda L: *Saccharomyces cerevisiae* (accession number - EU649673.1);
iii. Banda M: *Kazachstania aerobia* (accession number - AY582126.1);
iv. Banda N: *Lachancea meyersii* (accession number - AY645661.1).

The main bacterial genus was *Lactobacillus* (Figures 1 and 2). These bacteria have been previously found in kefir grains/beverages, and are characterised as probiotic microorganisms (Corona *et al.,* 2016; Wang *et al.,* 2017; Cho *et al.,* 2018).

Various species of yeasts and bacteria present in different kefir grains/beverages have been reported by numerous authors (Magalhães *et al.,* 2010; 2011; de Oliveira Leite *et al.,* 2013); and lactic acid bacteria, acetic acid bacteria, and yeasts co-exist in kefir grains (Magalhães *et al.,* 2010; Viana *et al.,* 2017; Magalhães-Guedes *et al.,* 2018).

The present work indicated that the kefir biomass and FKMP popsicles contain a diverse spectrum of lactic acid bacteria including *Lactobacillus, Lactococcus,* and *Leuconostoc*. One important bacterium found in the present work was *Lactobacillus kefiri*, and it is capable of producing vitamins in kefir fermentation process (Corona *et al.,* 2016; Cho *et al.,* 2018).

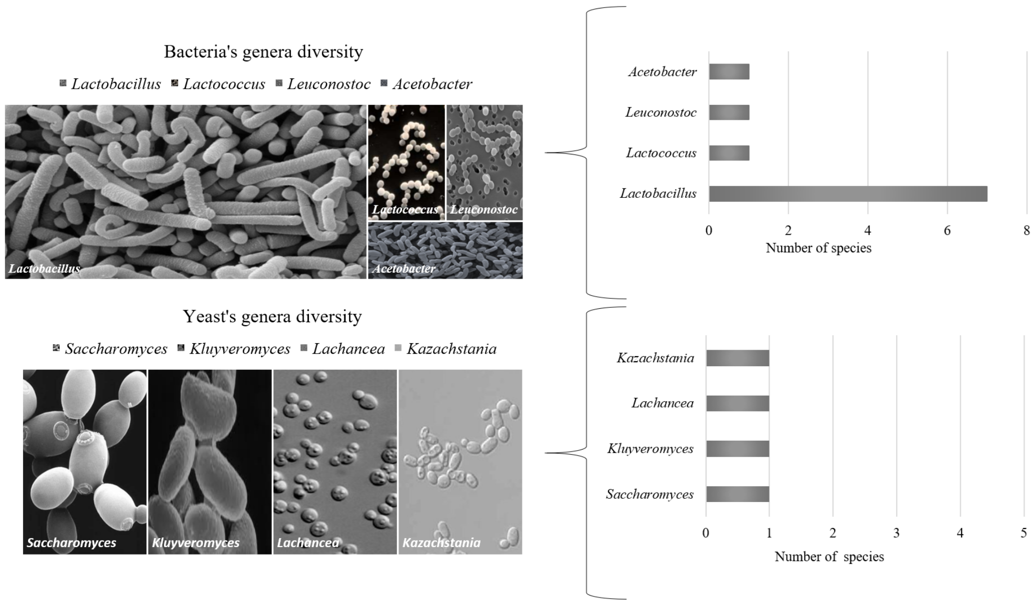

Figure 2. Diversity of the microbial genera present in kefir grains and fermented kefir mango-based popsicles (FKMP).

The acetic acid species *Acetobacter lovaniensis* was also identified, and the *Acetobacter* genus has also been described in kefir grains and has suitable probiotic properties (Viana *et al.,* 2017; Cho *et al.,* 2018). Additionally, the yeast *Kluyveromyces lactis* was identified in the kefir grains together with *Saccharomyces cerevisiae, Kazachstania aerobia,* and *Lachancea meyersii* (Figure 1). These yeasts represented the most commonly identified yeast isolates in kefir grains, and also possess suitable probiotic properties (Cho *et al.,* 2018). After fermentation, the FKMP microbiota remained unchanged. The popsicles did not present undesirable and/or pathogenic microorganisms in their composition.

Consumers' growing interest in functional products has led to the incorporation of probiotic microorganisms into food products resulting in functional food. Some studies have demonstrated that it is possible to produce popsicle-type frozen desserts using different ratios of microbial mixtures. This factor may serve as an excellent option for healthy diet incorporation of probiotic microorganisms (Salem *et al.,* 2015).

For the possible probiotic potential of kefir mango-based popsicles, the microbial population needs to remain viable in the final product (Salem *et al.,* 2015). Thus, this analysis was performed on mango-based kefir popsicles. In the analysis of microbial viability, the presence of 1.0×10^6 CFU/mL of yeasts, 1.0×10^{10} CFU/mL of lactic acid bacteria, and 1.0×10^8 CFU/mL of acetic acid bacteria was observed in the kefir mango-based popsicles sample. This fact is due to the characteristic of high acidity that the beverage has due to the fermentation process performed by the lactic and acetic, which are prevalent in kefir (Magalhães *et al.,* 2010; Viana *et al.,* 2017; Magalhães-Guedes *et al.,* 2018). It is also important to highlight the symbiotic relationship between yeasts and acetic bacteria that occurs due to the production of ethanol by the yeasts and the consequent consumption of this by the acetic acid bacteria, causing acidification of the medium. While the growth of bacteria is stimulated by the production of growth factors (vitamins) and nitrogen compounds soluble in yeasts (Magalhães *et al.,* 2010; Viana *et al.,* 2017; Magalhães-Guedes *et al.,* 2018). Due to the results, we can consider the kefir mango-based popsicles with the characteristic of "food with probiotic potential" (Salem *et al.,* 2015).

Sensory evaluation of popsicles (CP and FKMP)

The results of the sensory acceptance of CP and FKMP popsicles are presented in Figure 3. The sensory attributes scored between 7.6 and 8.4 on a 9-point hedonic scale, indicating panellists' liking for the popsicles from moderate to extreme. Significant differences ($p < 0.05$) were observed between the CP and FKMP popsicles' texture, colour, and flavour. Flavour presented particularly great value variation,

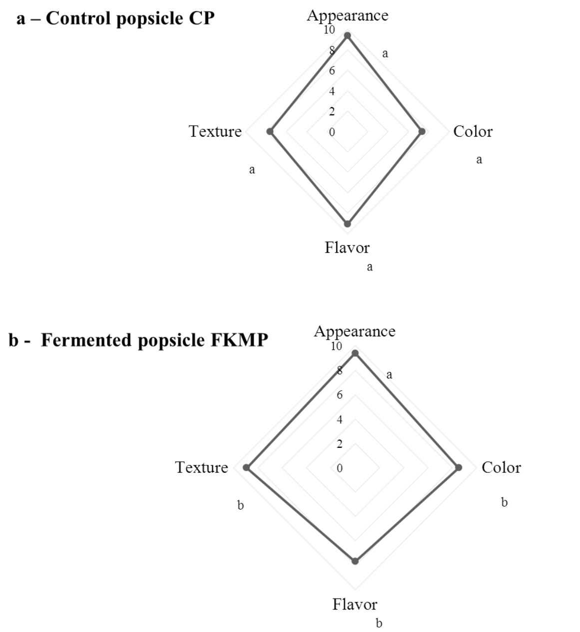

Figure 3. Sensory evaluation of mango-based control popsicles (CP) (a), and final fermented kefir mango-based popsicles (FKMP) (b).

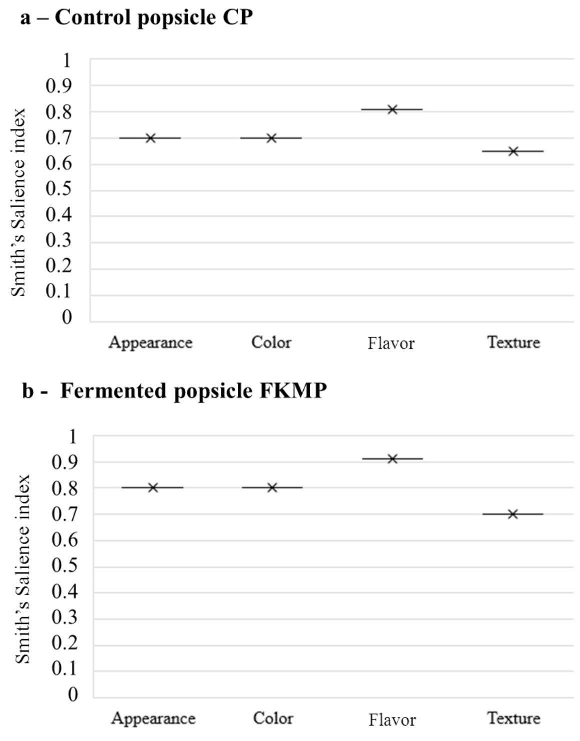

Figure 4. Smith's salience index (SSI) values showing a variation in descriptors that characterise the sample set of mango-based control popsicles (CP) (a), and final fermented kefir mango-based popsicles (FKMP) (b), including appearance, colour, flavour, and texture.

with mean scores ranging ($p < 0.05$) from 7.7 to 8.4 for CP and FKMP popsicles, respectively. However, values did not vary in relation to the popsicles' overall appearance.

The flavour attribute was highlighted in both popsicle types based on Smith's salience index (SSI) test (Figure 4). The use of the SSI test enabled the identification of the most important sensory attribute of kefir mango-based popsicles, which was the flavour.

The relevance of each sensory attribute is defined by the frequency of citation by consumers. Therefore, a quantitative parameter to express this factor is required (Santos *et al.,* 2015). The results of the present work will be relevant to the fermented foods industries once the combination of kefir and fruit pulp has been proven effective in popsicle production.

Conclusion

The fermented kefir mango-based popsicle produced in the present work was a good protein food source, and could serve as an excellent vehicle for healthy incorporation of probiotic microorganisms. Supplementation with kefir biomass (grains) has been found to exert a significant effect on the flavour and nutritional characteristics of popsicles, mainly their protein content (0.38 (CP) to 7.27 g/100 g (FKMP)). Our results indicated that bacterial genera such as *Lactobacillus, Lactococcus,* and *Acetobacter*, and yeast genera such as *Saccharomyces, Kluyveromyces, Lachancea,* and *Kazachstania* were the microorganisms present in kefir grains and kefir mango-based popsicles. The present work is the first to report on kefir mango-based popsicle production, which enables the consumption (by ingestion) of the kefir biomass (grains) after the fermentation process. The sensory acceptance of the popsicles ranged between 7.6 and 8.4 on a 9-point hedonic scale. This indicates excellent sensory acceptance of the popsicles produced. The use of kefir biomass offers a promising tool for the innovation and diversification of fruit-based popsicles with functional properties. Further experiments should be performed to characterise the functional properties of FKMP popsicles *in vitro* and *in vivo*.

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