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Physicochemical, rheological, microbiological and sensory properties of newly developed coffee flavored kefir



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ARTICLE INFO	A B S T R A C T
<i>Keywords:</i> Fermented milk Kefir Antioxidant capacity Probiotic Temporal dominance of sensations	Kefir is a functional beverage and little known to consumers. This study aimed to develop and characterize the physicochemical, rheological, microbiological and sensory properties of coffee flavored kefir. Kefir formulations were prepared by adding different amounts of skim milk powder, instant coffee and refined sugar into the fermented milk. The optimal formulation was determined using the desirability function and subjected to Temporal Dominance of Sensations (TDS) test, acceptance and purchase intention. The responses of dry matter, ash, protein, carbohydrate, total energy value, pH, color, antioxidant capacity, acid acetic bacteria, mesophilic cocci, viscosity and TDS test showed significant differences ($p < 0.05$) between the formulations. However, water activity, lipid content, <i>Lactobacillus</i> spp. and yeast counts showed no significant differences ($p \ge 0.05$). All beverages showed non-Newtonian rheological behavior and high probiotic counts. The formulation with maximum coffee addition presented higher overall desirability. This sample was characterized by lower total energy value and higher antioxidant capacity and chroma. In the TDS test, the coffee flavor attribute was the dominant, followed by the bitter taste, cappuccino taste and residual taste. The optimal formulation presented good sensory acceptance and purchase intent.

1. Introduction

Kefir is a type of fermented milk result of the action of a series of specific microorganisms that compose it. Kefir grains are small and irregular and act when inoculated to the substrate at room temperature for approximately 24h (Dertli & Con, 2017; Purutoğlu et al., 2019; Teijeiro, Pérez, Antoni, & Golowczyc, 2018). It's consumption has become more popular in recent years as it is considered as a functional food due to its beneficial health effects such as antimicrobial, anticarcinogenic, anti-inflammatory, probiotic, prebiotic, wound healing, cholesterol lowering and lactose tolerance improvement (John & Deeseenthum, 2015; Sharifi et al., 2017).

Kefir microorganisms are present in a type of exopolysaccharide matrix called kefiran and act in synergy. Among the various microorganisms present in this matrix are Lactobacillus kefir, of genres Acetobacter, Lactococcus and Leuconostoc, Bifidobaterium sp., Lactobacillus casei and Streptococcus salivarius subsp. Thermophilus. These bacteria ferment lactose from milk and/or reconstituted milk as well as yeast Kluyveromyces marxianus. In addition, lactose non-fermenting yeasts such as Saccharomyces omnisporus, Saccharomyces

cerevisae and Saccharomyces exiguus also belong to this group of microorganisms (Dertli & Çon, 2017; Fiorda et al., 2016).

The production of this fermented milk occurs mainly from bovine milk (Bensmira, Nsabimana, & Jiang, 2010), but also can be made from milk from other mammals such as goat (Kaczyński, Cais-Sokolińska, & Rudzińska, 2018; Satir & Guzel-Seydim, 2015), sheep, mare (Cais-Sokolińska, Wójtowski, & Pikul, 2016) and buffalo (Gul, Atalar, Mortas, & Dervisoglu, 2018). However, in addition to milk, new studies have also developed water kefir (Koh et al., 2018; Laureys, Aerts, Vandamme, & De Vuyst, 2018). Besides, several food substances may be added to kefir formulation in order to add mainly desirable sensory characteristics to the product.

In turn, coffee is one of the most appreciated beverages worldwide by consumers. In some regions, coffee consumption is surpassed only by water consumption, given the appreciation of the sensory characteristics of the beverage and the beneficial effects caused by its ingestion (Hall et al., 2015; Moraes & Bolini, 2010; Nguyen et al., 2016). Given this, several dairy products have been made by adding coffee such as yogurt (Tan & Korel, 2007), dairy beverage (Li, Hayes, & Ziegler, 2014; Yoon et al., 2017) and dulce de leche (Guimarães, Leão, Pimenta,

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Ferreira, & Ferreira, 2012). According to Yoon et al., 2017, the palatability of coffee has been increasing and, as a result, various foods, including dairy products, are being produced with added coffee and have been marketed. Therefore, the aim of this study was to develop and characterize the physicochemical, rheological, microbiological and sensory properties of coffee flavored kefir and to determine an optimal formulation.

2. Material and methods

2.1. Material

UHT milk (3% Fat) (Porto Alegre S/A, Ponte Nova/MG, Brazil), skim milk powder (Nutril, Indulac LTDA, Contagem/MG, Brazil), instant coffee (Três Corações Alimentos S/A, Natal/RN, Brazil) and refined sugar (Camil Alimentos S/A, Barra Bonita/SP, Brazil) were purchased in Lavras/MG, Brazil.

2.2. Kefir beverage production

Kefir was prepared by inoculating 5% of kefir grains in the mixture containing UHT milk and skim milk powder. The amounts of skim milk powder added in UHT milk (w/w) were 0% (F2, F3 and F6), 4.33% (F7), 6.5% (F4 and F5) and 13% (F1). The mixture was incubated in a thermostatically controlled incubator at 25 ± 1 °C for 24h. After fermentation, the kefir grains were separated and washed with deionized water. The fermented milk was kept refrigerated at 4 ± 1 °C for 24h. After this time, instant coffee and sugar were added (Table 1).

2.3. Physicochemical characterization

Kefir formulations were analyzed for dry extract, protein, lipids, ash and pH according to the methodology of the Association of Official Analytical Chemists (AOAC, 2005). Dry extract was determined by gravimetric method at 105 °C to constant weight. Protein content was determined by the Kjeldahl method and estimated by the nitrogen conversion factor of 6.38. Lipid content was determined by soxhlet extraction with petroleum ether. Ash was determined by muffle incineration at 550 °C. The pH of the beverage was measured using a digital pH meter. Carbohydrate content was determined by the difference between dry extract and the sum of the total percentage of protein, lipid and ash. The total energy value was estimated by the conversion factors for protein and carbohydrate (4 kcal/g) and lipid (9 kcal/g) content.

Water activity was determined using water activity meter (Aqualab SERIES 4 TE) at 25 °C. Color analysis was performed by direct reading on colorimeter (Konica Minolta, Spectrophotometer model CM-5), using color scale CIELab, with illuminant D65 and 10° viewing angle. The parameters obtained were L* (luminosity) and a* (red and green intensity) and b* (yellow and blue intensity) and the chroma (C*) and hue (H°) values were calculated by equations (1) and (2), respectively. C* represents color saturation. H° values vary from 0°/360° (pure red), 90° (pure yellow), 180° (pure green) to 270° (pure blue) (Lee, Wu, & Siow, 2013).

Table 1

Composition of kefir formulations.

Formulations	Instant coffee (%)	Sugar (%)	Fermented milk (%)
F1	0.10	6.00	93.90
F2	2.00	6.00	92.00
F3	0.10	12.00	87.90
F4	1.05	6.00	92.95
F5	0.10	9.00	90.90
F6	1.05	9.00	89.95
F7	0.70	6.00	93.30

$$C^* = \sqrt{a^{*^2} + b^{*^2}}$$
(1)

$$\mathrm{H}^{\mathrm{o}} = \mathrm{tan}^{-1} \left(\frac{\mathrm{b}^{*}}{\mathrm{a}^{*}} \right) \tag{2}$$

2.4. Antioxidant capacity

2.4.1. Extract preparation

The extract was prepared according to the methodology described by Rufino et al. (2006, 2007). For this, 10 g of the sample was added to test tubes along with 40 mL of methanol (50% v/v) at room temperature for 1 h. The tubes were centrifuged (25406.55 g) for 15 min and the supernatant was collected. Afterward, 40 mL of acetone (70% v/v) were added to the residue at room temperature for 1h and centrifuged (25406.55 g) for 15 min. The supernatant was removed and added to the first. The final volume was completed to 100 mL with deionized water. The extract was used to determine the antioxidant capacity by capturing the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl) and by the iron reduction method (FRAP).

2.4.2. DPPH assay

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical assay method was performed according to Rufino et al. (2007). A methanolic DPPH solution (0.06 mmol/L) was prepared. 0.1 mL aliquots of each extract dilution were added from 3.9 mL DPPH solution (0.06 mmol/L) in test tube. The tubes were homogenized, kept protected from light, and the reaction time was measured by spectrophotometer every minute until stabilization using a wavelength of 515 nm. The stabilization time was 30 min for all samples. Methanol was used as blank. Results were expressed as EC_{50} (g sample/g DPPH).

2.4.3. FRAP assay

The determination of antioxidant capacityby the iron reduction method (FRAP) was performed according to the methodology described by Rufino et al. (2006). Initially, 90 μ L of each extract dilution, 2.7 mL of FRAP (consisting of TPTZ, FeCl₃ and acetate buffer) and 270 μ L of deionized water were added to the test tube and homogenized. Then the mixture was kept in dark at 37 °C for 30 min. Absorbance readings were taken on a spectrophotometer at 595 nm and FRAP reagent was used as blank. Antioxidant capacity was quantified by the FRAP method using standard ferrous sulfate curve (Fe₂SO₄) (500–2000 μ mol/L) and the results were expressed as μ mol/L ferrous sulfate/g of sample.

2.5. Rheological behavior

Product viscosity was evaluated according to Giarola, Pereira, and de Resende (2015) with adaptations. A DVIII Ultra concentric cylinder rotational viscometer (Brookfield Engineering Laboratories, Stoughton, USA) with a 13R/RP sample adapter (19.05 mm in diameter and 64.77 mm deep) and an SC4-34 (9.39 mm diameter and 24.23 mm long) coaxial shear sensor was used. The samples were subjected to an increased deformation rate ramp, varying linearly from 0.03 to 30.83 (s⁻¹) for 9 min, 12 readings being taken at 18 °C. Consistency index (k) and power law index (n) were determined using the power law model to interpret the relationship between shear stress and shear rate. Rheological measurements were taken in three independent trials was adjusted using Rheocalc software (version V.3.1, Brookfield Engineering Laboratories, Stoughton, EUA).

2.6. Microbiological analysis

The enumeration of microorganisms was performed by serial dilutions in peptone water (0.1%). Inoculation was performed by surface spreading in different culture media, according to procedures described by Garofalo et al. (2015), with some modifications. Lactic acid bacteria

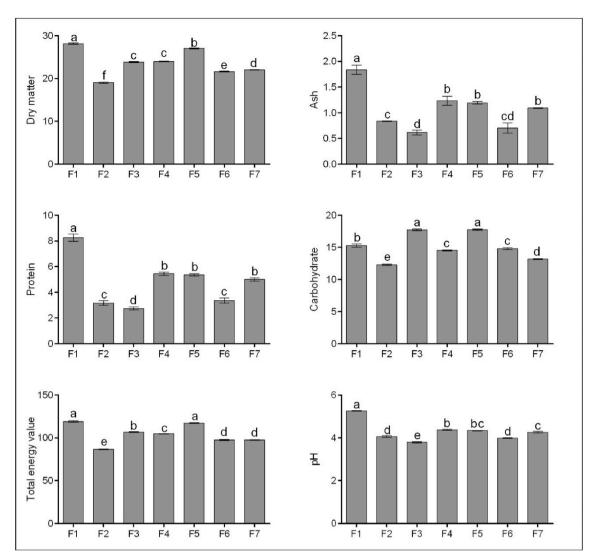


Fig. 1. Composition (%), total energy value (kcal/100 g) and pH values of kefir formulations (F). Results are expressed as mean \pm standard deviation, three repetitions. Different letters above columns indicate significant difference when compared to each other by Tukey test (p < 0.05).

(*Lactobacillus* spp.) counts were performed on MRS agar at 37 °C under anaerobiosis. Mesophilic cocci on M17 agar at 25 °C under aerobiosis. Acetic acid bacteria (AAB) in GY medium (50 g/L glucose, 10 g/L yeast extract, 20 g/L agar) at 28 °C under aerobic conditions. All media above were supplemented with 4 mL/L nystatin to inhibit yeast growth. Yeast counting was performed in YEPD or YEPG medium (10 g/L yeast extract, 10 g/L peptone, 20 g/L glucose, 20 g/L agar), supplemented with chloramphenicol (100 mg/L) at 28 °C, under aerobiosis. The enumeration of bacteria and yeast was performed after 3 days of incubation. Viable counts results were expressed as log averages of colony forming units per mL of sample (log CFU/mL).

2.7. Sensory analysis

Temporal Dominance of Sensations test (TDS) was performed by 122 untrained participants (60 women and 62 men), aged 18–60 years. Then, the product acceptance and purchase intention test were performed. The sample was served in a disposable plastic cup (50 mL) coded with random three-digit numbers at refrigeration temperature (4 °C). 30 mL of kefir were served in individual white light booths.

Attributes for the TDS test were previously defined in focus group (data not shown). To perform the TDS, the tasters were instructed to place the sample in the mouth (2s delay time) and immediately start the analysis, recording the dominant attributes for 35s.

In the acceptance test, tasters were asked to evaluate how much they liked or disliked the sample in relation to the attributes of appearance, aroma, taste, texture and overall appearance using a 9-point structured hedonic scale (1 - extremely disliked at 9 - extremely liked). For purchase intent, it was assessed whether or not tasters would be potential buyers of the product on the market using a 5-point structured scale (1-certainly would not buy at 5-certainly would buy) (Stone & Sidel, 1985). In addition, the participants informed through a questionnaire if they knew and had already consumed traditional kefir.

2.8. Statistical analysis

The experiment was carried out in a completely randomized design with three replications. Analysis of variance (ANOVA) was used to compare the different kefir formulations, followed by the Tukey test. Statistical analyses were performed using GraphPad Prism 5 software, with a 5% probability level.

The desirability function was employed to simultaneously optimize the response variables (Derringer & Suich, 1980) according to the desirable characteristics in kefir. In the present study, antioxidant capacity by the DPPH method and caloric value were minimized (Eq. (3)). Antioxidant capacity by the FRAP method, protein, acetic acid bacteria

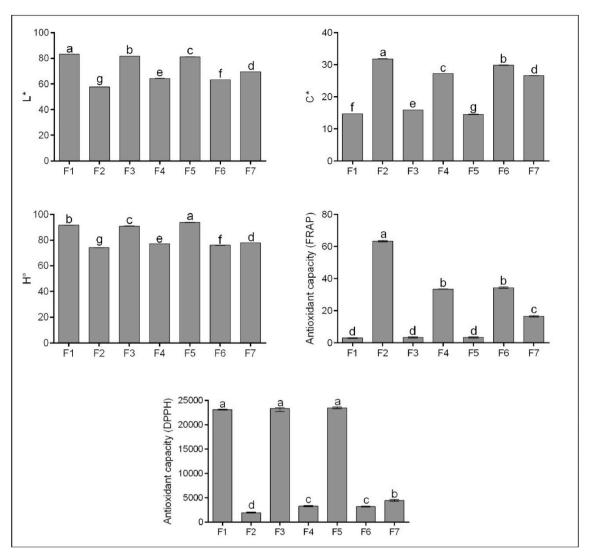


Fig. 2. Colorimetric parameters and antioxidant capacity by DPPH EC_{50} (g sample/g DPPH) and FRAP (µmol/L ferrous sulfate/g sample) of kefir formulations (F). Results are expressed as mean \pm standard deviation, three repetitions. Different letters above columns indicate significant difference when compared to each other by Tukey test (p < 0.05).

(AAB) and mesophilic cocci were maximized (Eq. (4)).

$$\begin{split} d_{i} &= \begin{cases} 1, & \text{if } \hat{y}_{i} \leq T_{i} \\ \left(\frac{U_{i} - \hat{y}_{i}}{U_{i} - T_{i}}\right)^{r_{i}}, & \text{if } T_{i} < \hat{y}_{i} < U \\ 0, & \text{if } \hat{y}_{i} > U \end{cases} \tag{3} \\ d_{i} &= \begin{cases} 0, & \text{if } \hat{y}_{i} \leq L_{i} \\ \left(\frac{\hat{y}_{i} - L_{i}}{T_{i} - L_{i}}\right)^{r_{i}}, & \text{if } L_{i} < \hat{y}_{i} < T_{i} \\ 1, & \text{if } \hat{y}_{i} \geq T_{i} \end{cases} \tag{4}$$

where d_i is the value of individual desirabilities; T_i is the desired ideal value; U_i is the maximum desired value; L_i is the minimum desired value.

After obtaining the individual desirability values, the overall desirability (D) was calculated (Eq. (5)).

$$D = \sqrt[N]{\left(\prod_{i=1}^{N} d_{i}\right)}$$
(5)

where N is the individual desirability number.

TDS curves were calculated according to the methodology described by Pineau et al. (2009) using the SensoMaker software (Nunes & Pinheiro, 2013). Calculations were performed based on the confidence interval of a binomial ratio, based on a normal approximation. Three parameters for each dominant sensation were also computed using the TDS curves (DRmax. - Maximum dominance rate, TDRmax. - Time when maximum dominance occurs and Plateau - Attribute duration, time interval where dominance rate is 90% or more of DRmax.) (Pineau et al., 2009).

3. Results and discussion

3.1. Physicochemical characterization

The contents of dry extract, protein, carbohydrate, ash and total energy value (Fig. 1) were influenced (p < 0.05) by the composition of the formulations, presenting values between 18.90 and 28.28%, 2.74 and 8.86%, 12.28 and 17.76%, 0.61 and 1.84% and 86.66 and 119.20 kcal/100 g, respectively. Lipid content and water activity showed no significant difference (p \geq 0.05) for the different formulations, with an average value of 2.76% and 0.980, respectively. Silva, Santos, Santana, Silva, & Conceição, 2018 evaluated the composition of bovine milk kefir, obtaining protein, lipid, ash, carbohydrate and caloric values of, respectively, 2.9 g/100 g; 2.5 g/100 g; 0.7 g/100 g; 13.9 g/100 g and 89.7 kcal/100 g, similar to those found in the present study.

Table 2

Rheological parameters of kefir formulations.

Formulations	k	n	\mathbb{R}^2
F1	5.009	0.287	0.986
F2	1.608	0.355	0.997
F3	1.690	0.034	0.999
F4	2.815	0.325	0.994
F5	2.812	0.302	0.990
F6	1.556	0.348	0.997
F7	2.070	0.326	0.994

Consistency Index (k); Flow behavior index (n); Determination coefficient (R²).

The highest values of dry extract and ash (Fig. 1) were found for formulation F1, mainly due to the higher addition of solids from milk powder. In addition, this formulation had a higher protein content (p < 0.05), since milk powder has approximately 34% protein (informed by the manufacturer). F3 presented the lowest value of this response due to the lower addition of protein sources. According to John and Deeseenthum (2015) and Sharifi et al. (2017), kefir has a protein content of at least 2.7%. Kaczyński et al. (2018) found protein content of 3.19%, similar to that found in this work for formulations without the addition of milk powder (F2, F3 and F6). Carbohydrate content increased (p < 0.05) mainly with increasing proportion of sugar and milk powder (F3 and F5). F2 presented the lowest total energy value, since it has low protein and carbohydrate contents.

The pH values of the formulations ranged from 3.76 to 5.29 (Fig. 1), with the highest values observed (p < 0.05) for the formulations containing the highest amount of milk powder (F1, F4, F5 and F7). In milk powder, the presence of phosphate salts plays a buffering activity, leading to a slow pH reduction during fermentation and making it higher at the end of the process (Kim, Oh, & Imm, 2018). On the other hand, it was found that the formulation containing higher sugar content (F3) reached the lowest pH value of the beverage, as observed by Silva et al. (2018). During the fermentation process, carbohydrates are consumed by homo and heterofermentative lactic bacteria, as well as yeast, culminating in various organic acids and other metabolites. The pH values can be taken as indirect indicators of microbial growth (Costa, Alencar, Santos Leandro, Mendonça, & Ferreira, 2018). Thus, it can be inferred that the F3 formulation provided a more favorable medium for the development of kefir grain microbial populations during fermentation, because, according to Silva et al. (2018), the high sucrose content contributes to microbial growth, reducing the pH value of the beverages.

The colorimetric parameters (L*, C* and H°) showed significant difference (p < 0.05) between the formulations (Fig. 2). The

luminosity values (L*) ranged from 57.65 to 83.21. In general, the addition of instant coffee and milk powder resulted in formulations with the lowest and highest L* values, respectively and therefore, F1 had the highest value. The hue (H°) of the formulations ranged from 74.18 to 93.87 (Fig. 2), while the chroma (C*), which indicates color saturation, ranged from 14.68 to 31.85. The increase of instant coffee resulted in the decrease and increase of the tonality (H°) and chroma (C*) values of the formulations, respectively. This fact may be explained by the higher concentration of melanoidins from coffee addition, making the color of the formulation more intense.

3.2. Antioxidant capacity

The FRAP method expresses the antioxidant capacity per sample mass. However, the determination of antioxidant capacity by DPPH method expresses the sample mass required to reduce a DPPH mass. Thus, the high antioxidant capacity occurs with low value of ratio of the sample mass to DPPH (Rufino et al., 2006, 2007).

Regarding the antioxidant capacity (Fig. 2), it was verified that the formulation with higher concentration of instant coffee (F2) resulted in the increase of antioxidant capacity, since it presented greater reduction of Fe³⁺ to Fe²⁺ ions, as well as higher ability to eliminate DPPH radicals. On the other hand, formulations with lower percentage of instant coffee exhibited the lowest antioxidant capacities by both methods. In instant coffee, antioxidant capacity can be attributed to compounds such as caffeine, chlorogenic acids and melanoidins (Vignoli, Bassoli, & Benassi, 2011). In their study, Najgebauer-Lejko and Sady (2015) evaluated the antioxidant capacity of commercial fermented milks and also found that the addition of other ingredients such as coffee provided high antioxidant capacity. The formulations with lower concentration of instant coffee (F1, F3 and F5) presented low antioxidant capacity and did not differ from each other ($p \ge 0.05$).

3.3. Rheological behavior

The power law model presented a good fit to the rheological data, with coefficient of determination (R^2) higher than 0.98 (Table 2). An increase in shear stress as a function of shear rate was observed (Fig. 3A), demonstrating non-Newtonian rheological behavior of the formulations, which is characteristic of fermented dairy beverages (Ertekin & Guzel-Seydim, 2010). Parameter n of the formulations was less than one (Table 2). Thus, the formulations exhibited pseudoplasticity. Parameter k represents the consistency index, corresponding to the viscosity of the fluid (Doğan, 2011). Overall, it was observed that the addition of milk powder resulted in formulations with higher shear

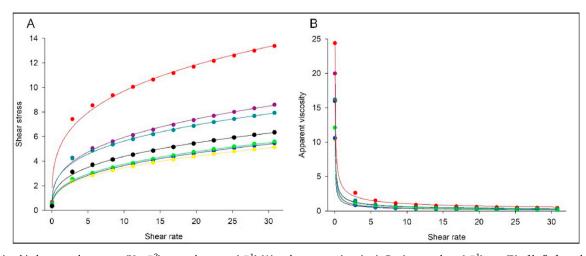


Fig. 3. Relationship between shear stress (N.m⁻²) versus shear rate (s⁻¹) (A) and apparent viscosity (mPa.s) versus shear (s⁻¹) rate (B) of kefir formulations: F1 (\bullet); F2 (\bullet); F3 (\bullet); F4 (\bullet); F5 (\bullet); F6 (\bullet); F7 (\bullet).

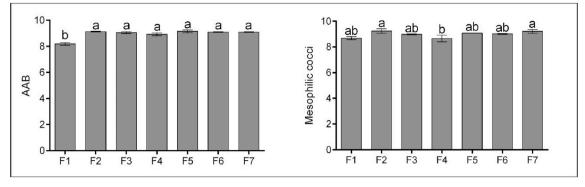


Fig. 4. Counts of acid acetic bacteria (AAB) (log CFU/mL) and mesophilic cocci (log CFU/mL) of kefir formulations (F). Results are expressed as mean \pm standard deviation, three repetitions. Different letters above columns indicate significant difference when compared to each other by Tukey test (p < 0.05).

stress and apparent viscosity (Fig. 3A and Fig. 3B). Therefore, F1 presented the highest responses, while formulations with lower milk powder concentration (F2, F3 and F6) presented the lowest values. This result is corroborated by the value of parameter k (Table 2), being higher for F1. A reduction in apparent viscosity of the formulations was verified as the shear rate increased, exhibiting thixotropic behavior (Fig. 3B).

3.4. Microbiological analysis

The kefir formulations showed no significant difference ($p \ge 0.05$) for *Lactobacillus* spp. and yeast, presenting values between 7.30 and 8.13, 6.34 and 6.78 log CFU/mL, respectively. Counts of acid acetic bacteria (AAB) and mesophilic cocci (Fig. 4) showed significant differences between the formulations (p < 0.05), ranging from 8.19 to 9.16, 8.66 and 9.24 log CFU/mL. F1 presented the lowest AAB value and the other formulations did not present significant difference ($p \ge 0.05$). The present study corroborates the findings by Kim et al. (2015), who reported high AAB viability, above 9 log CFU/mL in kefirfermented milk and yeast count above 7 log CFU/mL.

Kefir is considered a probiotic product with numerous therapeutic benefits (Otles & Cagindi, 2003). However, to achieve the desired functionality from the intrinsic microbiota, the product must have minimal cell viability which, according to the Food and Agriculture Organization of the United Nations/World Health Organization - FAO/ WHO (2003), recommendations, is at least 4 and 7 log CFU/g of yeast and bacteria respectively. Therefore, the kefir formulations developed in the present study were in accordance with these specifications. The concentration, type and characteristics of the ingredients added in the preparation of fermented milks play a fundamental role in the endogenous bacteria activity of the product (Baú, Garcia, & Ida, 2013).

3.5. Sensorial analysis

Formulation 2 was defined as optimal based on the highest overall desirability value and was used for the Temporal Dominance of Sensations test (TDS) and acceptance and purchase intention tests. TDS evaluates the sequence of dominant product sensations over a pre-defined time, identifying and classifying the intensity of perceived sensations as dominant from the beginning to the end of perception. The dominant sensation is defined as the one that catches the consumer's attention, not necessarily the most intense sensation. One of the advantages of this method is that it does not require prolonged consumer training, as well as evaluating several attributes simultaneously (Di Monaco, Su, Masi, & Cavella, 2014).

TDS curves (Fig. 5) show the attributes over time for the evaluated formulation. The coffee flavor sensation was significantly dominant throughout most of the analysis time. The maximum dominance rate

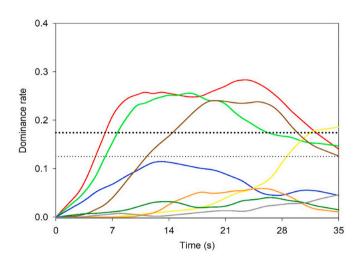


Fig. 5. Temporal dominance of sensations (TDS) curves for Coffee flavor (—); Bitter taste (—); Acid taste (—); Residual flavor (—); Sweet taste (—); Cappuccino flavor (—); Aerated texture (—); Viscous texture (—); Chance (·····); Sig. level (······).

(DRmax) was 0.28 and the maximum dominance time (TDRmax) was 23.50s. The time when the dominance rate was 90% or greater of DRmax was 16.40s. Bitter taste and cappuccino flavor were significantly dominant with DRmax and TDRmax time of 0.26 and 16.50 and 0.24 and 19.50s, respectively. It was observed that the residual flavor sensation was significantly dominant, with a dominance rate of 0.19 from 31s. The other sensations (acid taste, sweet taste, aerated texture and viscous texture) did not present significant dominance, being below the significance level.

The results of the acceptance test are shown in Table 3. The attributes appearance, aroma, texture and overall appearance were higher than 6, being between liked slightly and liked very much. The taste was the only attribute that had the lowest acceptance score. However, this attribute was not rejected, with a score between 5 and 6, which

Table 3Sensory characteristics of kefir formulation (F2).

Sensory attributes	Score
Appearance	7.67 ± 1.25
Aroma	7.33 ± 1.41
Taste	5.82 ± 1.90
Texture	6.91 ± 1.66
Overall acceptability	$6.60 ~\pm~ 1.58$

Values expressed as means \pm standard deviation, 122 consumers.

corresponds to neither liked nor disliked and like slightly, which may be justified by the dominance of bitter and residual taste sensations as observed in TDS (Fig. 5). Regarding the purchase intention test, it was found that the product had a grade of 3.36 ± 1.08 , being between 3 and 4, which corresponds to not knowing if it would buy and probably would buy. Of the total participants, 55.74% said they knew kefir. Of this group that knows kefir, only 67.65% have consumed this product. Thus, it can be observed that kefir, despite its many beneficial effects on the health, still has low popularity among consumers.

4. Conclusions

The addition of different amounts of skim milk powder, instant coffee and refined sugar into the fermented milk modified the physicochemical, rheological and microbiological properties of the formulations. In general, the higher addition of milk powder contributed the highest values of dry extract, ash, protein, energy value, pH, luminosity and viscosity, as well as lower acetic acid bacteria count. Instant coffee has provided extra nutritional benefits, resulting in beverages with better antioxidant properties.

The formulations exhibited non-Newtonian behavior, being the power law model adequate to describe this behavior. Moreover, it was verified that the formulations presented microbiological characteristics necessary for the attendance of probiotic property.

The formulation with the highest coffee addition was determined as optimal by the desirability function. In TDS, this formulation had a predominant coffee flavor, followed by the bitter taste and cappuccino flavor. At the end of the analysis, the residual flavor was perceived. Moreover, this formulation showed good acceptance and purchase intent. The present study showed that skim milk powder, instant coffee and refined sugar can be added to produce new kefir based products.

Author contributions section

Vimercati, W. C., Araújo, C. da S., and Macedo, L. L. were responsible for the study conception, for the study methodology, for the formal analysis and for writing an original manuscript preparation. Fonseca, H. C. and Guimarães, J. S. were responsible for the study methodology and for the formal analysis. Abreu, L. R. and Pinto, S. M. were responsible for supervision for study and for the project administration.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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References

- AOAC (2005). Official methods of analysis (18th ed.). Maryland, USA: Association of Official Analytical, Chemists International.
- Baú, T. R., Garcia, S., & Ida, E. I. (2013). Optimization of a fermented soy product formulation with a kefir culture and fiber using a simplex-centroid mixture design. *International Journal of Food Sciences & Nutrition*, 64(8), 929–935.
- Bensmira, M., Nsabimana, C., & Jiang, B. (2010). Effects of fermentation conditions and homogenization pressure on the rheological properties of Kefir. *LWT - Food Science and Technology*, 43, 1180–1184.
 Cais-Sokolińska, D., Wójtowski, J., & Pikul, J. (2016). Rheological, texture and sensory
- Cais-Sokolińska, D., Wójtowski, J., & Pikul, J. (2016). Rheological, texture and sensory properties of kefir from mare's milk and its mixtures with goat and sheep milk. *Mljekarstvo*, 66(4), 272–281.
- Derringer, G., & Suich, R. (1980). Simultaneous optimization of several response variables. Journal of Quality Technology, 12(4), 214–219.
- Dertli, E., & Çon, A. H. (2017). Microbial diversity of traditional kefir grains and their role on kefir aroma. LWT - Food Science and Technology, 85, 151–157.

Di Monaco, R., Su, C., Masi, P., & Cavella, S. (2014). Temporal dominance of sensations: A

review. Trends in Food Science & Technology, 38(2), 104-112.

- Doğan, M. (2011). Rheological behaviour and physicochemical properties of kefir with honey. Journal Für Verbraucherschutz Und Lebensmittelsicherheit, 6(3), 327–332.
- Ertekin, B., & Guzel-Seydim, Z. B. (2010). Effect of fat replacers on kefir quality. Journal of the Science of Food and Agriculture, 90, 543–548.
- FAO/WHO (2003). Joint FAO/WHO food standards programme. Codex standard for fermented milks (Codex Stan 243-2003). Codex Alimentarius Commission.
- Fiorda, F. A., Pereira, G. V. M., Thomaz-Soccol, V., Medeiros, A. P., Rakshit, S. K., & Soccol, C. R. (2016). Development of kefir-based probiotic beverages with DNA protection and antioxidant activities using soybean hydrolyzed extract, colostrum and honey. LWT - Food Science and Technology, 68, 690–697.
- Garofalo, C., Osimani, A., Milanović, V., Aquilanti, L., De Filippis, F., Stellato, G., et al. (2015). Bacteria and yeast microbiota in milk kefir grains from different Italian regions. *Food Microbiology*, 49, 123–133.
- Giarola, T. M. O., Pereira, C. G., & Resende, J. V. (2015). Fortification with iron chelate and substitution of sucrose by sucralose in light uvaia sherbet (*Eugenia pyrtformis* Cambess): Physical, chemical and sensory characteristics. *Journal of Food Science & Technology*, 52(9), 5522–5533.
- Guimarães, Í. C. O., Leão, M. H. M. R., Pimenta, C. J., Ferreira, L. O., & Ferreira, E. B. (2012). Development and description of light functional dulce de leche with coffee. *Ciência e Agrotecnologia*, 36(2), 195–203.
- Gul, O., Atalar, I., Mortas, M., & Dervisoglu, M. (2018). Rheological, textural, colour and sensorial properties of kefir produced with buffalo milk using kefir grains and starter culture: A comparison with cows' milk kefir. *International Journal of Dairy Technology*, 71, 73–80.
- Hall, S., Desbrow, B., Anoopkumar-Dukie, S., Davey, A. K., Arora, D., McDermott, C., et al. (2015). A review of the bioactivity of coffee, caffeine and key coffee constituents on inflammatory responses linked to depression. *Food Research International*, 76, 626–636.
- John, S. M., & Deeseenthum, S. (2015). Properties and benefits of kefir a review. Songklanakarin Journal of Science and Technology, 37(3), 275–282.
- Kaczyński, Ł. K., Cais-Sokolińska, D., & Rudzińska, M. (2018). Cholesterol oxidation products in kefir from goats' milk during storage. *International Dairy Journal*, 85, 35–40.
- Kim, D.-H., Chon, J.-W., Kim, H., Kim, H.-S., Choi, D., Hwang, D.-G., et al. (2015). Detection and enumeration of lactic acid bacteria, acetic acid bacteria and yeast in kefir grain and milk using quantitative real-time PCR. *Journal of Food Safety*, 35(1), 102–107.
- Kim, M., Oh, S., & Imm, J. Y. (2018). Buffering capacity of dairy powders and their effect on yoghurt quality. *Korean Journal for Food Science of Animal Resources*, 38(2), 273–281.
- Koh, W. Y., Utra, U., Rosma, A., Effarizah, M. E., Rosli, W. I. W., & Park, Y.-H. (2018). Development of a novel fermented pumpkin-based beverage inoculated with water kefir grains: A response surface methodology approach. *Food Science and Biotechnology*, 27(2), 525–535.
- Laureys, D., Aerts, M., Vandamme, P., & De Vuyst, L. (2018). Oxygen and diverse nutrients influence the water kefir fermentation process. *Food Microbiology*, 73, 351–361.
- Lee, K.-H., Wu, T.-Y., & Siow, L.-F. (2013). Spray drying of red (*Hylocereus polyrhizus*) and white (*Hylocereus undatus*) dragon fruit juices: Physicochemical and antioxidant properties of the powder. *International Journal of Food Science and Technology*, 48, 2391–2399.
- Li, B., Hayes, J. E., & Ziegler, G. R. (2014). Interpreting consumer preferences: Physicohedonic and psychohedonic models yield different information in a coffeeflavored dairy beverage. *Food Quality and Preference, 36*, 27–32.
- Moraes, P. C. B. T., & Bolini, H. M. A. (2010). Different sweeteners in beverages prepared with instant and roasted ground coffee: Ideal and equivalent sweetness. *Journal of Sensory Studies*, 25, 215–225.
- Najgebauer-Lejko, D., & Sady, M. (2015). Estimation of the antioxidant activity of the commercially available fermented milks. Acta Scientiarum Polonorum Technologia Alimentaria, 14(4), 387–396.
- Nguyen, T., Kuchera, M., Smoot, K., Diako, C., Vixie, B., & Ross, C. F. (2016). Consumer acceptance of a polyphenolic coffee beverage. *Journal of Food Science*, 81(11), S2817–S2823.
- Nunes, C. A., & Pinheiro, A. C. M. (2013). *SensoMaker, version 1.8*. Lavras, Brazil: UFLA. Otles, S., & Cagindi, O. (2003). Kefir: A probiotic dairy-composition, nutritional and
- therapeutic aspects. *Pakistan Journal of Nutrition*, 2(2), 54–59.
 Pineau, N., Schlich, P., Cordelle, S., Mathonnière, C., Issanchou, S., Imbert, A., et al. (2009). Temporal dominance of sensations: Construction of the TDS curves and comparison with time-intensity. *Food Quality and Preference*, 20, 450–455.
- Purutoğlu, K., İspirli, H., Yüzer, M. O., Serencam, H., & Dertli, E. (2019). Diversity and functional characteristics of lactic acid bacteria from traditional kefir grains. *International Journal of Dairy Technology*, 70, 1–10.
- Rufino, M. S. M., Alves, R. E., Brito, E. S., Moraes, S. M., Sampaio, C. G., Pérez-Jiménez, J., Saura-Calixto, F. D., et al. (2006). Metodologia científica: Determinação da atividade antioxidante total em frutas pelo método de redução do ferro (FRAP). *Comunicado Técnico on-line Embrapa*, 1–4. http://www.cnpat.embrapa.br/cd/jss/ acervo/Ct_125.pdf, Accessed date: 23 August 2019.
- Rufino, M. S. M., Alvez, R. E., Brito, E. S., Moraes, S. M., Sampaio, C. G., Pérez-Jiménez, J., Saura-Calixto, F. D., et al. (2007). Metodologia científica: Determinação da atividade antioxidante total em frutas pela captura do radical livre DPPH. *Comunicado Técnico on-line Embrapa*, 1–4. http://www.cnpat.embrapa.br/cd/jss/acervo/Ct_127. pdf, Accessed date: 23 August 2019.
- Satir, G., & Guzel-Seydim, Z. B. (2015). Influence of kefir fermentation on the bioactive substances of different breed goat milks. LWT - Food Science and Technology, 63, 852–858.

- Sharifi, M., Moridnia, A., Mortazavi, D., Salehi, M., Bagheri, M., & Sheikhi, A. (2017). Kefir: A powerful probiotics with anticancer properties. *Medical Oncology*, 34(183), 1–7.
- Costa, M. R. D. A., Alencar, E. R. D. E., Santos Leandro, E. D. O. S., Mendonça, M. A., & Ferreira, W. F. D. E. S. (2018). Characterization of the kefir beverage produced from yam (*Colocasia esculenta* L.), sesame seed (*Sesamum indicum* L.) and bean (*Phaseolus* vulgaris L.) extracts. Journal of Food Science & Technology, 55(12), 4851–4858.
- Silva, C. F. G., Santos, F. L., Santana, L. R. R., Silva, M. V. L., & Conceição, T. A. (2018). Development and characterization of a soymilk kefir-based functional beverage. *Food Science and Technology*, 38(3), 543–550.

Stone, H. S., & Sidel, J. L. (1985). Sensory evalution practices. London: Academic Press311.

- Tan, G., & Korel, F. (2007). Quality of flavored yogurt containing added coffee and sugar. Journal of Food Quality, 30, 342–356.
- Teijeiro, M., Pérez, P. F., Antoni, G. L., & Golowczyc, M. A. (2018). Suitability of kefir powder production using spray drying. Food Research International, 112, 169–174.
- Vignoli, J. A., Bassoli, D. G., & Benassi, M. T. (2011). Antioxidant activity, polyphenols, caffeine and melanoidins in soluble coffee: The influence of processing conditions and raw material. *Food Chemistry*, 124, 863–868.
- Yoon, J.-W., Ahn, S.-I., Kim, H.-N., Park, J.-H., Park, S.-Y., Kim, J.-H., et al. (2017). Qualitative characteristics and determining shelf-life of milk beverage product supplemented with coffee extracts. *Korean Journal for Food Science of Animal Resources*, 37(2), 305–312.