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OBTAINING KEFIR MICROCAPSULES IN POWDER FORM BY SPRAY DRYING

Sousa LJ[1], Santos TC[2], Pedroso LV[2], Vellido JI[2], Silva BB[2] and IRO Pereira[2]*

Luana Jorge De Sousa

*Corresponding author email: isabela.pereira@mackenzie.br

[1]University of São Paulo (USP), Graduate Program in Public Health Nutrition, Department of Nutrition, São Paulo, Brazil

[2]Presbyterian University Mackenzie, Center for Biological and Health Sciences (CCBS), Pharmacy Program, São Paulo, SP, Brazil

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ABSTRACT

Kefir is presented as gelatinous grains which, when inoculated in milk, produces a fermented milk acidified with probiotic activities. The reduction of water activity is a strategy to increase the shelf-life of food products and has been used to increase stability and viability of microorganisms. Obtaining Kefir powder can be a strategy to increase shelf life, reduce storage costs, and increase practicality in the marketing and the use of the product. Spray drying is a thermal process, which must be controlled because the viability of the microorganisms can be affected by numerous factors. This study aimed to obtain a fermented powder product from kefir grains that preserve characteristics and survival of microorganisms after dehydration by Spray Drying. The kefir was fermented in 12 and 25% (w/w) reconstituted skim milk powder and then dried. Grain growth, yield, moisture, nutritional composition, kefiran content and microbiological survival of the obtained powders were evaluated. The product was characterized by differential exploratory calorimetry (DSC) and Scanning Electronic Microscopy (SEM). After drying, the yield and kefiran content of the samples with initial 12% solids were higher than 25% and the moisture was lower. A high survival rate for acidic lactic acid bacteria in the powdered product (10^7 CFU/g) produced at 12% (w/w) was found. However, yeast did not survive the process. Microcapsules observed by SEM exhibited a rounded shape with a preserved surface, while DSC indicated a probable disruption above 50°C. The powder produced at 12% showed better fermentation characteristics, process yield, moisture of the final product and survival of microorganisms. The product contains equivalent to 50mg of kefiran/20g, indicating that in this fermentation and drying conditions, exopolisacarides have been preserved. The microcapsules produced by the Spray Dryer technique have a rounded shape with a preserved surface, without pores, however rough and varied in size. The technique used was efficient by generating intact microcapsules containing potentially probiotic live microorganisms.

Key words: probiotic, *Lactobacillus*, exopolisacarids, kefiran, spray dryer, milk kefir, microcapsules

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INTRODUCTION

Kefir is a potentially probiotic food, which has historical origins in the Caucasus Mountains that contains several strains of Lactobacillus, Leuconostoc, Lactococcus, Acetobacter and Saccharomyces [1]. Its most common form of presentation is as gelatinous grains which, when inoculated in a culture medium such as milk, produces an acidified fermented milk that is slightly carbonated and contains small amounts of alcohol called kefir [2]. Kefir grains have variable size, irregular shape and gelatinous consistency, resembling pieces of cauliflower or popcorn. Its matrix is composed of a web of proteins and polysaccharides, in which bacteria and yeasts involved in the fermentation process are firmly adhered and encapsulated. This polysaccharide matrix is called kefiran [3]. During fermentation, the kefir grains increase in weight and, after being sieved from the fermented milk, they can be reused for a new fermentation process [2]. Several benefits are attributed to kefir and kefiran, such as improved digestion and tolerance to lactose, inhibition of the growth of tumors and pathogenic microorganisms, regularization of intestinal transit, reduction of cholesterol, inhibition of angiotensin converting enzyme (ACE) and modulation of immune system, including allergy and asthma relief [4]. The industrial production of kefir is known in several countries, such as Russia, Canada, Sweden, but there is a growing demand for kefir-based products in several countries [2].

The reduction of water activity is a strategy to increase the shelf-life of food products and has been used to increase stability and viability of microorganisms [5]. Obtaining Kefir powder can be a strategy to increase shelf life, reduce storage costs, and increase practicality in the marketing and the use of the product. However, the development of dehydrated functional probiotic products is a challenge in terms of viability and functionality during the manufacture and storage of the product [6].

Spray Drying has been used as a convenient method for production of bacteria and yeast powder. Spray drying is a technology that has gained a lot of space due to the reduced cost and low drying time when compared, for example, with lyophilized, in addition, it has an efficient drying with a high rate of moisture removal [1]. However, spray drying is a thermal process, which must be controlled because the viability of the microorganisms can be affected by numerous factors, such as inlet and outlet temperatures, type of atomization, air flow direction and counting of initial microorganisms [7]. The initial concentration of solids presents in the liquid feed has a significant impact on the efficiency of the drying operation [8]. A common practice in drying processes is to increase the concentration of solids of the initial sample to optimize the procedure as well as generate considerable energy savings.

Kefir powder was obtained with whole milk [1], but skim milk [5] has been shown to be efficient for drying probiotic cultures by spray-drying. Besides that, the fat reduces

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powder stability by oxidation. Thus, to improve cell viability, in this study, we opted to evaluate the fermentation and drying of kefir in skim milk. In addition, to obtain a higher content of kefiran, it was proposed to dry the contents of the kefir grains together with the fermented milk. The kefiran is a polysaccharide that can have microencapsulation effect and protection of the cells during the drying process [3].

Therefore, the objective of this work was to obtain a fermented powder product from kefir grains and skimmed milk that preserve characteristics and survival of microorganisms after dehydration by Spray Drying. For this, the growth of kefir grains was evaluated in two concentrations of skim milk powder were evaluated.

Experimental

Growth assessment in skimmed milk with different solid concentrations

Kefir fermentation and sample preparation for drying were performed according to popular culture with modifications [2]. To evaluate the effect of solids content on fermentation and drying efficiency, growth of kefir grains in skim milk was evaluated at two different concentrations of solids obtained from the reconstitution of 12% and 25% skim milk powder (w/w), in triplicate, totalling 6 samples. Samples containing a total weight of 500g of reconstituted and sterilized skimmed milk were added with 2% (w/w) kefir grains. The product was stored in a sealed and sterilized glass bottle and kept at room temperature (18-24°C) for 24 hours for fermentation. Milk and bottle were sterilized in autoclave at 115°C by 10min.

The fermentation control was performed by pH measurements at 0 and 24 hours. Immediately after 24 hours of fermentation, the Kefir grains were sieved in plastic sieve treated with ethanol 70% and weighed in analytical scales to verify the weight gain. The kefir grains were then ground and homogenized to the fermented dairy product with a mixer at 12,000 rpm for 3 minutes. After this process, the contents were sieved to remove any non-homogenized material. The contents were placed under refrigeration until reaching the minimum temperature of 10ºC to start the processing by Spray Drying.

Drying and Process performance

The drying was performed in a mini-spray dryer (Lab Maq do Brasil® MSD 1.0) at flow rate of 0.5L/h, inlet and outlet temperature at 130°C and 90-100°C, respectively. Immediately after drying, the kefir powder was weighted. The yield was calculated, discounting the residual moisture content, in relation to initial solids content. Then, the moisture content of the liquid feed and of the powder was determined using a moisture balance (Shimadzu® MOC 120H). The obtained powders were packed in plastic bottles, sealed and kept in a desiccator. The exopolisacarides extraction was realized in kefir powder samples according to Einikev with same modifications [9]. The kefir powder was boiled (1:10, w/v) for 30 min under stirring, centrifuged

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(10,000g, 20 min, 20°C) and the supernatant was mixed with cold 96% ethanol (2:1). After overnight incubation at -20 °C and centrifugation the pellets were redissolved in hot water. The precipitation was repeated three times. The pellets of polysaccharide were reconstituted in water (400 µg/mL) and after hydrolysing, the sugar content was determined by spectrophotometry like described in Einikev [9]. The results were expressed like kefiran concentration.

Evaluation of the survival of microorganisms

To verify empirically that the microorganisms present in Kefir remained viable and would be capable of fermentation, a sample of the powder from each obtained batch was added in 50mL of UHT whole milk (4% w/v). Whole milk (UHT) was used as control. Fermentation was performed with pH measurements at 0 and 24 hours. For these evaluations, a sterile plastic container was used, which was kept at room temperature 22°±2 during fermentation. The method was originally developed by our research group.

Process improvement

To increase the stability of the product obtained in the previous tests, it would be necessary to reduce the moisture content. To do this, an increase of the inlet temperature was proposed. Thus, the 12% solids condition was repeated in a new triplicate increasing the inlet temperature to 135°C. The obtained powders were analysed for yield, centesimal composition and survival of microorganisms.

Centesimal composition and microbiological analysis

The results were expressed in g/100g of dry matter. Carbohydrates were calculated by difference, subtracting the contents of ash, protein, lipids, and lactic acid from 100 g, according to the methodology proposed by AOAC [16]. A sample of kefir liquid feed was lyophilized (Liofilizador Interprise I, Terrone®) for each replicate for comparison of nutritional composition before and after spray drying.

For microbiological analysis [5], samples were diluted at 10^{-1} until 10^{-6} in saline-peptone solution. 100 µL from each dilution were inoculated in MRS (Agar to Lactobacillus, according to De Man, Rogosa and Sharpe) media in microaerophilic conditions (after inoculation, a new media layer was added in overlay) to access Lactic Acid Bacteria (LAB) and PDA (Potato-Dextrose agar) media pH 3,5 (adjusted with tartaric acid) to estimate yeasts. The survival rate of lactic bacteria and yeasts (N / No) was expressed as the quotient of the kefir colonies before drying (No) and after drying (N).

Scanning Electronic Microscopy (SEM)

The Kefir powder sample was fixed by a deposition layer of gold ions and placed in a chamber with a pressure around 0.1 mbar using Argon (BAL-TEC, model SCD 050 Sputter Coater) according to Toba [17]. The morphological analysis of the

microcapsules was obtained through JEOL scanning electron microscope images, model JSM 6510.

Differential exploratory colorimetry (DSC)

The thermal analysis of freshly produced samples of kefir powder (12% solids) was carried out in a differential scanning calorimeter, apparatus: SDT Q600 V20.9 Build 20 which were heated at 25°C to 600°C in separate compartments under isothermal conditions and subjected to the same input power variation in the oven 5°C / min in Nitrogen atmosphere, according to Guangiang [18].

Statistical analysis

The results (n=3) were presented as mean ± standard deviation. The statistical analysis was performed by Student's T Test using the program Microcal Origin® 2.0. It was compared 12% vs 25%, 12% vs Control and 25% vs Control when pertinent. Significant difference was considered with * and/or **ϕ** when $p<0.05$ for comparison between each 2 variables.

RESULTS AND DISCUSSION

Growth assessment and process performance using skimmed milk with different solid concentrations

The weight of Kefir grains and the reduction of pH of the milk was evaluated to indicate fermentation with growing of microorganisms and lactic acid production in 2 different concentrations of skimmed milk powder (12 and 25% w/w). The initial pH at 12% and 25% of skimmed milk was similar, however the final pH at 12% was lower ($p = 0.003$), indicating that the fermentation was more efficient in this condition (figure 1). A sample of the product obtained from each solid concentration was added to UHT milk to evaluate the fermentative capacity by pH measurement. The addition of the powders per se caused a decrease in the pH of the milk in relation to the control (figure 2 *$p<0.05$ for comparison between initial (0) control pH and the samples 12% and 25%). However, the samples containing the powder obtained at 12% and 25% did not differ in the initial pH, but they differed in the final pH ($^{\phi}p<0.05$ for comparison between pH from 12% and 25% after 24h). This indicates that the kefir powder obtained with 12% of skimmed milk favoured the fermentation of UHT milk, suggesting a better survival of microorganisms.

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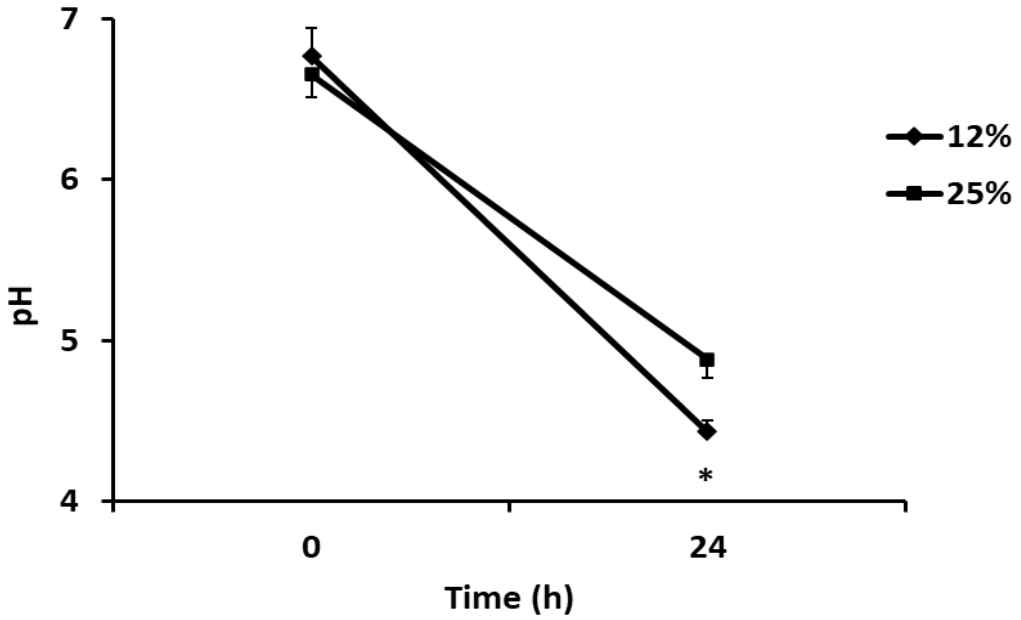

Figure 1: pH variation of kefir during fermentation of skimmed milk at 12% and 25% of solids after 24h (*p=0.003)

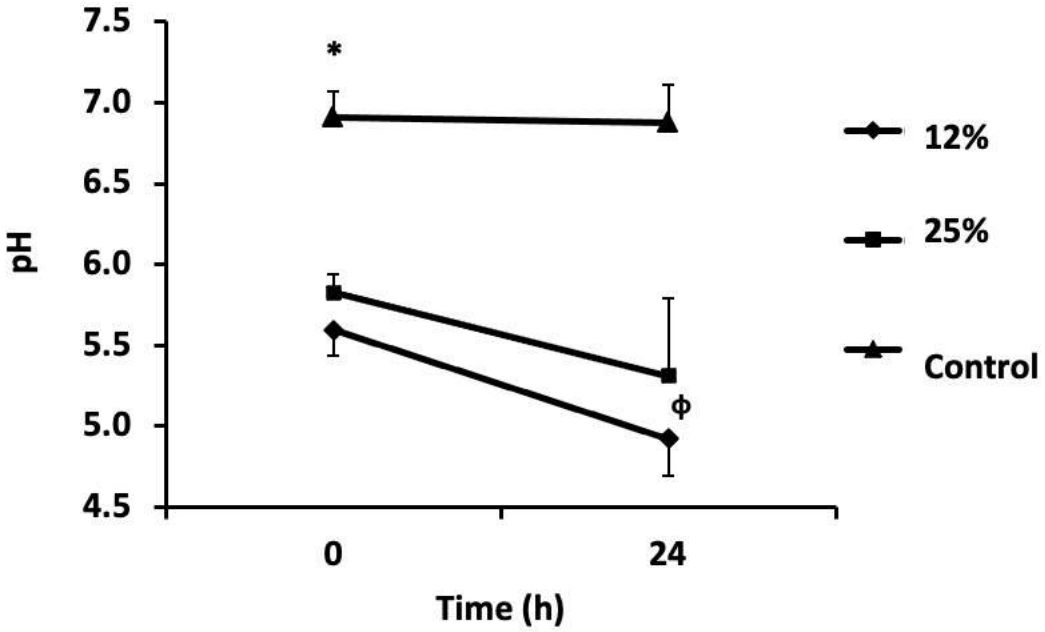

Figure 2: pH changes of fermented milk obtained from the addition of powdered kefir obtained from fermentation of 12 and 25% (w/w) of skimmed milk

(*p<0.05 to comparison between initial (time 0) control pH and 12% and 25%; ᶲp<0.05 to comparison between pH from 12% and 25% at 24h)

The growth of Kefir grains (table 1) at 12% and 25% of skimmed milk were similar, indicating that the increase in total solids to 25% did not increase the growth of kefir

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grains. Each sample at the end of the spray drying process had its yield percent evaluated based on the initial weight of total solids and final weight of the obtained powder. As it can be observed in table 1, the average procedural yield of the 12% solids samples was higher than that of 25% about 10%. After weighting, the moisture of the products obtained was determined. The 12% samples had a lower moisture content than the 25% samples of skim milk.

The sample fermented with 12% of skimmed milk had the best results. Although grain growth was similar to that of the 25% sample (table 1), the sample fermented with 12% skimmed milk had a lower pH (figure 1) indicating greater fermentation efficiency. In addition, the drying process using this sample generated a product with lower moisture and higher yield compared to the 25% sample (table 1). The reconstitution of the powder obtained by the process of spray drying with 12% of skimmed milk favoured the fermentation of the integral UHT milk with reduced pH and organoleptic characteristics like kefir (figure 2). In addition, increasing the spray dryer inlet temperature to 135°C using fermented samples with 12% skimmed milk provides a product with good yield and survival of lactic bacteria (table 2).

Moisture is an important parameter and closely related to the shelf-life and conservation of the product. In the study conducted by Atalar and Dervisoglu [1], the moisture content of the powder obtained by drying the kefir by Spray Drying with initial solids concentration of 11% ranged from 1.75% to 4.9%, with outlet temperatures of 93°C and 69°C, respectively. As the equipment, used in the present study, is different from that of Atalar and Dervisoglu [1], to obtain similar outlet temperature (about 90°C), the inlet temperature should be 130°C. Thus, it was expected that these conditions could produce dust with similarly surviving moisture and microorganisms. In our study, when the inlet temperature was 135°C, the outlet temperature was on average 95°C, a temperature higher than that of the Atalar and Dervisoglu [1] study. Thus, the inlet temperature of 130°C was chosen first to preserve the surviving microorganisms (table 1). But the results found showed a high moisture in that condition that would affect the stability of the product (table 1). In this manner, it was found that the temperature employed significantly interfered with the moisture of the final powdered product.

Process improvement

The previously performed condition at 12% solids resulted in better product characteristics when compared to 25% (table 1, figures 1 and 2). However, the moisture content of 6,7% (table 1) still high for maintenance of product stability [10]. To achieve moisture reduction while preserving microorganisms, a minimum increase in the inlet temperature was applied. Thus, the 12% solids condition was repeated in a new triplicate increasing the inlet temperature to 135°C. The table 2 shows that the increase of the inlet temperature to 135°C improved the average

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process yield and reduced the powder moisture in relation to the results obtained at 130ºC (table 1) for the concentration of 12% of skimmed milk. The initial count (No) of BAL (table 2) ranged from 10^6 to 10^8 and the survival rate ranged from 10^{-1} to 10, but in all replicates, the final count (N) was 10^7.

Table 3 shows the alterations on nutritional composition of kefir powder dehydrated by spray drying (atomized) in relation to kefir composition lyophilized, representing the liquid feed (12% of solids) before drying. The results were presented in relation to dry matter for better comparison.

The yield obtained of 39% relative to the 12% sample (table 2), although apparently low, is considered good since no additives have been used and because the equipment works on a laboratory scale and yields are limited by physical factors. Wang and Langrish [11] demonstrated in their work that laboratory dryers have low yields, and that mini spray dryers rarely provide yields above 50%, even with the application of large amounts of additives. The kefir powder produced at 12% solids show a high concentration of exopolisacarides (table 1), represented by kefiran. Kefiran quantity in kefir of different brands in Russia varied greatly, approximately at 50–200 mg/l [9]. The kefir powder produced at 12% solids contains equivalent to 50 mg of kefiran/20g or 200 mg/80g of product, indicating that in this fermentation and drying conditions, exopolisacarides have been preserved. To reconstitute 1 L of liquid kefir, 120 g of powder is required, providing 300 mg of kefiran—33% more than the maximum concentration reported by Einikeev [9]. This fact shows that kefir grains utilization in drying process contributes to the increase of kefiran content.

Atalar and Dervisoglu [1] did not mention the yield obtained in their study, but they found much lower survival (N/No) (10^{-2}) in their study than ours, but with the use of kefir in whole milk. We believe that the reason for such improvement in the survival found by us, is the use of skimmed milk and the grinding of kefir grains along with fermented milk. Skimmed milk is efficient as a microencapsulant of bacteria [5]. Atalar and Dervisoglu [1] also did not observe survival of kefir yeasts after drying by spray drying.

Characterization: Scanning electron microscopy and Differential scanning calorimetry (DSC)

Scanning electron microscopy (SEM) allowed to observe the external structure of microcapsules produced by the Spray Dryer technique (figure 3). Figure 4 shows the variation of heat flux as a function of the temperature of microencapsulated Kefir powder (DSC).

The microcapsules produced by the Spray Dryer technique have a rounded shape with a preserved surface, without pores, however rough and varied in size. Soukoulis [12] produced microcapsules of pure culture of *Lactobacillus acidophilus* with

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diameter of 10µm, very similar to those obtained in the present study (Figure 3). The variety of diameters of microcapsules observed is explained by the probable diversity of microorganisms found in kefir. Elevating the inlet temperature results in the formation of large microcapsule particles, while dehydration at lower heat transfer rates favours the production of fine powders due to partial collapse of the microparticles [13].

In figure 4 (DSC), we can observe two changes the first suggests a modification in the crystalline structure, probably the evaporation of water. While on the second, denaturation or decomposition of proteins with consequent probable rupture of the microcapsules (glass transition temperature-Tg about 55°C). Tg is directly related to the physical (stickiness and agglomeration) and structural (pore collapse, changes in texture and rehydration capacity) stability and the occurrence of biochemical reactions (lipid oxidation, texture or color changes based on enzymatic reactions) of dehydrated products during storage [12]. Soukoulis obtained a glass transition temperature of 74.3°C for Lactobacillus acidophilus microparticles with skim milk [12]. In our study, the acidic pH of the product resulting from fermentation may have caused a reduction in Tg [14]. A Tg of 55°C suggests that the powder may begin to lose stability if stored at temperatures close to or above this value, but stability is also the result of several other factors, such as water content and water activity [15].

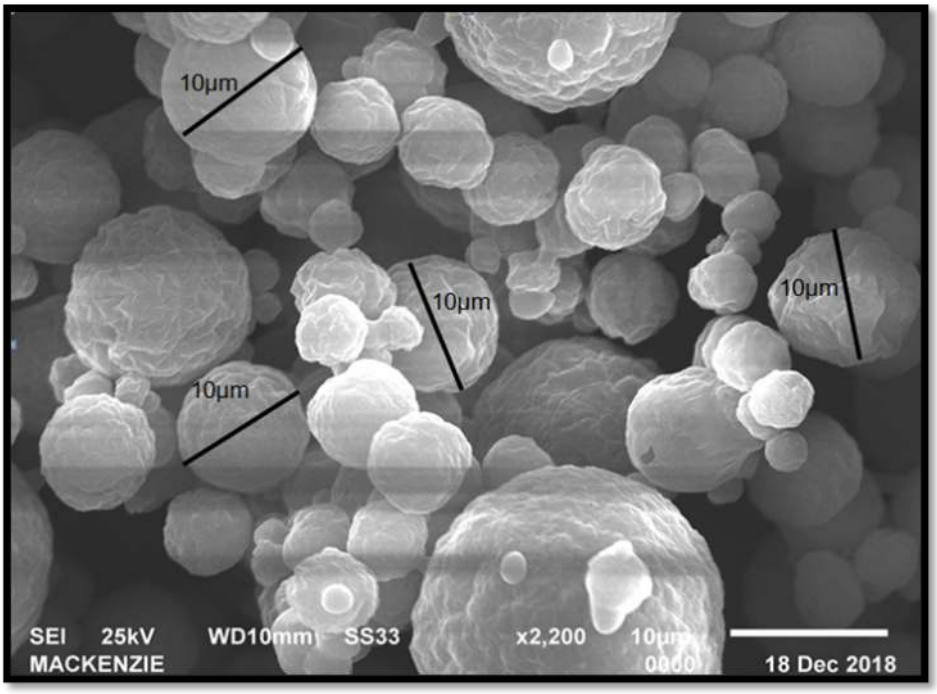

Figure 3: Image obtained by scanning electron microscopy of the casein microcapsules with Kefir after drying by Spray Dryer (2200x)

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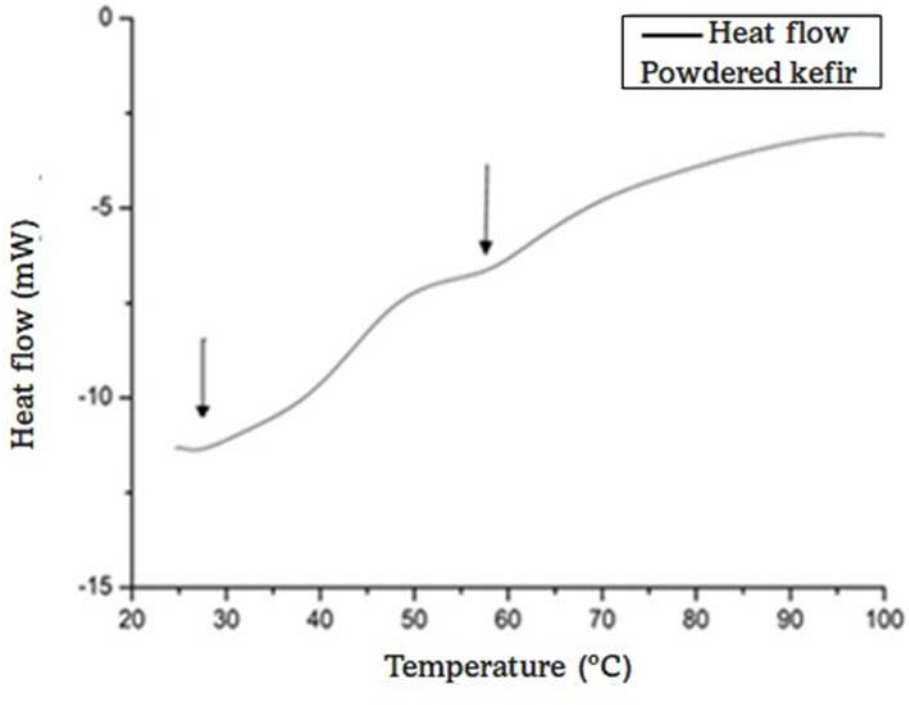

Figure 4: Variation of heat flux as a function of the temperature of microencapsulated kefir powder (DSC)

The increase of the concentration of solids of the skimmed milk did not favour the growth of the kefir grains. Of the two concentrations of skim milk tested (12 and 25%, w/w), the 12% showed the best characteristics of fermentation, process yield, final product moisture and microorganism survival. The inlet temperature of 135°C improves the quality of powder kefir and favoured the survival of lactic acid bacteria, but not yeast.

CONCLUSION AND RECOMMENDATIONS FOR DEVELOPMENT

Powder produced with 12% skimmed milk showed better fermentation characteristics, process yield, moisture and survival of microorganisms. The technique used was efficient by generating intact and spheric microcapsules containing potentially probiotic live microorganisms. The continuity of this study is necessary to evaluate the strains of surviving microorganisms, the stability and the possible biological effects of the product generated. The powder obtained is versatile and can be used as an ingredient in various food products and supplements. However, further studies are necessary to evaluate its safety and reliability for human consumption.

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Conflict of interest

The authors declare no conflict of interest.

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Table 1: Characteristics of fermentation and powdered kefir obtained with skimmed milk at 12% and 25% (w/w) and inlet temperature at 130°C. Results presented as mean ± standard deviation. *p<0,05

Solids (%w/w)	Grains growth (%w/w)	Yield (%w/w)	Moisture (%w/w)	Kefiran (mg/g)
12	54±10.4	*32.7±1.5	*6.7±0.3	*2.5±0.18
25	49±5.9	22.2±2.8	10.0±1.3	0.6±0.14
p value	0.463	**0.005**	**0.046**	**0.00016**

Table 2: Characteristics and survival of microorganisms in powdered kefir obtained in 3 repetitions for fermentation with skimmed milk at 12% (w/w) and inlet temperature at 135°C. No (initial count of microorganisms), N (final count of microorganisms), LAB (lactic acid bacteria). *p<0,05 to means comparison of Yield and Moisture between data from 12% solids at 130°C (table 1) and 135°C

Replicate	Yield (%w/w)	Moisture (%w/w)	Count of Microorganisms	No (CFU/g)	N (CFU/g)	(N/No)
1	40.10	4.38	LAB	$1.2x10^6$	$1.0x10^7$	10
			Yeast	$3.3x10^4$	0	-
2	38.67	3.99	LAB	$2.4x10^6$	$3.5x10^7$	10
			Yeast	$4.0x10^4$	0	-
3	39.07	4.38	LAB	$2.0x10^8$	$1.5x10^7$	10^{-1}
			Yeast	$9.5x10^5$	0	-
Mean ± DP	*39.0±0.7 p=0.0008	*4.3±0.2 p=0.0025	LAB	$6.8x10^7$	$2x10^7$	6.7
			Yeast	$3.4x10^5$	0	-

Table 3: Comparison of centesimal composition of Atomized and Lyophilized Kefir considering dry matter. *p<0.05

SAMPLE (g/100g)	Protein	Lipids	Ashes	Carbohydrates	Latic Acid
Atomized	28.51±1.93	*2.79±1.87	0.81±0.06	*66.88±3.43	0.99±0.15
Lyophilized	31.31±0.39	3.13±1.79	0.83±0.02	57.13±1.56	1.13±0.1
p-value	0.14	**0.03**	0.91	**0.01**	0.16

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