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Suitability of kefir powder production using spray drying

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Abstract

Spray drying was applied for the production of kefir powder. The survival of microorganisms after drying, storage and simulated gastrointestinal (GI) conditions was investigated. Kefir was obtained by fermentation of milk and whey permeate, and was dehydrated directly (traditional kefir) or using different carriers (skim milk, whey permeate and maltodextrin). Low survival (5.5 log and less than 2 log CFU/g for lactic acid bacteria and yeast respectively) of microorganisms was achieved when kefir was dehydrated without thermoprotectants (carriers). In contrast, survival of the microorganisms was significantly improved in the presence of different carriers. When skim milk (SM) was used as the carrier medium, lactic acid bacteria (LAB) survival was above 9 log CFU/g. In contrast, viability of yeast was dramatically reduced after spray drying in these conditions. When whey permeate was used as the carrier medium, LAB survival was 8 log CFU/g and yeast survival was above 4 log CFU/g. LAB in the kefir powder survived better the simulated GI conditions when spray drying was conducted in SM. LAB in kefir powder sample dehydrated in SM and SM plus maltodextrin remained stable for at least 60 days at 4 °C. Our results demonstrated that spray drying of kefir is a suitable approach to obtain a concentrated kefir-derived product.

Keywords: kefir; spray drying; lactic acid bacteria; yeast

1. Introduction

Kefir is a traditional fermented milk originated many centuries ago in the Caucasus Mountains. It is obtained by fermentative activity of 'kefir grains', which have a complex microbial composition consisting of a mixture of lactic acid bacteria (10^8 CFU/g), yeast (10^6–10^7 CFU/g), and acetic acid bacteria (10^5 CFU/g) coexisting in a complex symbiotic association in a protein–polysaccharide matrix (Garrote, Abraham & De Antoni 2001, 2010; Bourrie, Willing, & Cotter, 2016).

Kefir is considered as a probiotic-fermented product and it is also a source of probiotic strains (Gul, Mortas, Atalar, Dervisoglu, & Kahyaoglu, 2015; Bengoa et al., 2018). Many health benefits have been related to its regular consumption, such as anti-infectious activity, anticarcinogenic activity, antitumoral, hypocholesterolaemic and immunomodulating effects (Ahmed et al., 2013; Prado et al., 2015).

Kefir is mainly produced from cow, ewe, goat, or buffalo milk by mean of traditional procedures. In addition, soy milk and whey permeate were also used as fermentable substrates for kefir production (Londero, Hamet, De Antoni, Garrote & Abraham, 2012; Gamba, De Antoni & Peláez, 2016).

Due to the complexity of the microbiota and variability in the manufacturing procedures, some difficulties have been encountered in producing kefir of reproducible quality. For this reason, industrial-scale production rarely utilizes kefir grains for fermentation. Instead, to reduce batch-to-batch variability, starter cultures containing microbes that have been isolated from kefir are used (Assadi, Pourahmad & Moazami, 2000).

Even though freeze-drying is the most commonly used drying method in the microbiological industry, it is expensive and time-consuming. For this reason, several authors have explored alternative drying processes for the preservation of microorganisms. In this context, spray drying is a rapid and cost-efficient method that can produce dry powder with suitable properties, such as specific residual moisture content, good flowability, and uniform shape and size distribution (Pentewar, Somwanshi & Sugave, 2014; Sosnik & Seremeta, 2015). This technique is a promising process for mass production of dry probiotic preparations (Desmond, Stanton,

Fitzgerald, Collins & Ross, 2002; Corcoran, Ross, Fitzgerald & Stanton, 2004; Sunny-Roberts & Knorr, 2009, Lavari, Páez, Cuatrin, Reinheimer & Vinderola, 2014). Although spray drying can lead to an acceptable stability of products containing microorganisms, high temperatures involved in this process require a certain thermotolerance and its main limitation is the loss of viability that occurs during drying and subsequent storage of dehydrated products. In order to minimize this injury and to obtain more stable products, several thermoprotectants are commonly used. Skim-milk (SM) powder could be a suitable carrier medium for an efficient spray drying of probiotic cultures; but a wide variety of thermoprotectants, e. g..whey proteins, trehalose, monosodium glutamate, sucrose, glucose, inulin, lactose, and oligosaccharides have been also tested to increase survival of bacteria during this process (Desmond, Stanton, Fitzgerald, Collins & Ross, 2002; Corcoran, Ross, Fitzgerald & Stanton, 2004; Sunny-Roberts & Knorr, 2009).

Dehydrated kefir has extended shelf life even without refrigeration but it is necessary to optimize the preservation and storage of large quantities of product. The objective of this work was to determine the effect of different carriers on the viability and resistance to simulated gastric conditions of kefir microorganisms after spray drying.

2. Materials and methods

2.1. Kefir culture

AGK1 kefir grains from the CIDCA (Centro de Investigación y Desarrollo en Criotecnología de Alimentos) collection were used as fermentation starters. Microbiological analysis of these grains has demonstrated the presence of microorganisms belonging to the species *Lactobacillus plantarum*, *L. kefiri*, *Lactococcus lactis* subsp. *lactis*, *Leuconostoc mesenteroides*, *Saccharomyces spp*. and *Acetobacter spp* (Garrote, Abraham & De Antoni 2001). In addition, these grains lead to products with antifungal and immunomodulating activities (Gamba, De Antoni & Peláez, 2016; Iraporda et al., 2017).

Kefir was made using two types of fermentation substrates: A - commercial ultra-high temperature (UHT) low fat milk (Sancor, Santa Fe, Argentina) and B - whey permeate 20% w/v (Arla Foods Ingredients S.A., BsAs, Argentina). Grains were inoculated in both substrates in a

proportion of 10% w/v and incubated for 24 h at 20 °C. Kefir grains were separated from the fermented product by filtration with a previously disinfected plastic sieve.

2.2. Samples for spray drying

2.2.1. Kefir dehydrated directly. Kefir obtained using UHT milk (solid content 11% w/v) and whey permeate (solid content 20% w/v) as fermentation substrates was spray dried immediately after fermentation without addition of thermoprotectants (carriers). The pH of kefir was between 4.0 and 4.4 on both fermentation substrates.

2.2.2. Kefir dehydrated with different carriers. Kefir obtained in UHT milk was dehydrated in the presence of different carriers with potential thermoprotectant ability. To this end, 200 ml of kefir were centrifuged at 10000 rpm and 4°C for 15 min (Sorvall Centrifuge). The supernatant was discarded, and the pellet was suspended in 200 ml of a carrier suspension (20% of dissolved solids) before spray drying. The carriers used (Table 1) were skim milk (Svelty, Nestlé, Argentina), whey permeate (Arla Foods Ingredients S.A., Buenos Aires, Argentina) and maltodextrin (Maltodextrin DE 12, Ingredion, Buenos Aires, Argentina). When indicated, samples were neutralized to pH 7 by addition of small volumes of 5M NaOH (Anedra, Buenos Aires, Argentina).

2.3. Spray drying procedure

The samples were dehydrated using a laboratory-scale spray-dryer (BUCHI Mini Spray Dryer B-290) at a constant air inlet temperature of 135°C and the feeding flow at 10 mL/min. The air flow at the nozzle was 473 L/h and the drying air flow was 30 m^3/h. In these conditions, the outlet temperature was between 66°C and 69°C. Cell suspensions were atomized and sprayed into the drying chamber by using a two-fluid nozzle. All samples were stabilized at room temperature and thoroughly mixed prior to spray drying process. The obtained powder was collected in sterile polypropylene recipients and stored at 4°C tighten sealed in the dark. Three independent replicates were performed for each sample.

2.4. Spray drying yield

The spray drying yield was defined as the ratio (percentage) between the collected solids at the end of the drying process and the dissolved solids in the feeding solution. It was calculated as follows:

$Y\% = (wf/wi)x100$,

where Y% is the yield, wf is the weight of powder collected after spray drying and wi is total dissolved solids in the solution before the process. Powder present on the inside wall of the cyclone was not considered as part of the yield.

2.5. Microbiological analysis

Yeast and lactic acid bacteria in kefir were assessed by plate counts at each stage (fermented milk, carrier suspension and reconstituted dried kefir samples). Before spray drying, 10 mL of kefir obtained as above indicated and 90 mL saline (0.85% NaCl), were vortexed for 1 min, serially diluted and plated . After spray drying, dried kefir samples were rehydrated to the initial solid content before dilution and plate counts. Appropriate dilutions were plated in triplicate on YGC (Biokar, France) or MRS (Difco, Beauvais, France) to assess yeast and lactobacilli counts respectively. Incubations were performed for 24 h at 30°C (YGC) or 48 h at 37°C (MRS).

Survival rates (N/N_0) were expressed as the ratio of colony forming units per milliliter (CFU) of kefir before drying (N_0) and that of kefir powder after drying (N). Both N and N_O were expressed per gram of dry matter.

2.6. Water activity and moisture content measurements

The water activity of the samples was measured after drying by using the dew point method with an Aqualab 4TEV (Decagon Devices, USA) at 25ºC after stabilization at this temperature for 1 hour. Standard salt solutions (Decagon) of known water activity were used for calibration.

The residual moisture content of spray-dried samples was determined by oven-drying the powders at 102 ºC, determining the difference in weight, and expressing the weight loss as a percentage of the initial powder weight.

2.7. Survival in simulated gastric solution

Survival of dehydrated microorganisms in simulated gastric solution was performed according to Grimoud et al. (2010). Each sample was centrifuged (at 10000 rpm, 4°C for 15 min) and

pellets were suspended in 5 ml of sterile gastric solution (GS) to reach cell concentrations of approximately 8 log CFU/g. The GS consisted of 7.25 g/L NaCl, 0.52 g/L KCl, 3.8 g/L $NaHCO_3$ and 3.0 g/L of porcine pepsin (Sigma-Aldrich). The solution was mixed thoroughly for 10 min and pH was adjusted to 2.5 with HCl solution (36.5 g/L). After one hour of incubation at 37°C with agitation, microbial counts were evaluated. After gastric simulation, samples were washed with sterile PBS and pellets were suspended in 5ml of intestinal solution. Intestinal solution was formulated as follows: 1.3 g/L NaCl, 0.25 g/L KCl, 0.65 g/L $NaHCO_3$, 1.0 g/L, porcine pancreatine (Sigma-Aldrich) and 1.5 g/L bile salts (Sigma-Aldrich). The pH was adjusted to 8 with NaOH solution (200 g/L). After 3 h of incubation at 37°C microbial counts were determined. All centrifugations were performed at 6000 rpm for 10 min. Only non-neutralized samples suspended in carrier were tested in this assay.

2.8. Storage conditions

Samples obtained using carrier solutions were stored for 60 days at 4 °C without fixing the relative humidity. The samples were analysed at different time intervals to determine the microbial viability by plate counts. One gram of spray dried powder was rehydrated in 9 ml of saline solution (0.85% w/v NaCl), vortexed for 1 min and maintained at room temperature for 30 min. Bacterial suspensions were serially diluted, plated on MRS agar and incubated for 48 h at 37 °C.

2.9. Statistical analysis

All experiments were done in triplicate using three independent kefir samples. Analysis of variance (ANOVA) of viable counts corresponding to the different treatments was carried out using the statistical program Statgraphics Centurion XVII (Statistical Graphics Corp, USA). Means were compared by the Tukey test and differences were considered significant at $p < 0.05$.

3. Result and discussion

3.1. Kefir dehydrated directly

Both UHT milk and whey permeate as fermentation substrates allowed to obtain a fermented product with lactic acid bacteria and yeast counts higher than 8 log CFU/g and 6 log CFU/g respectively (Fig. 1a y b). After spray drying, viability of lactic acid bacteria in kefir powders

elaborated in UHT milk was 5.5 log CFU/g while yeast counts were below the detection limits (less than 2 log CFU/g) (Fig. 1a). These findings represent a significant loss in cell viability due to spray drying. In contrast, viability of lactic acid bacteria and yeasts in samples elaborated in whey permeate were of 6 log CFU/g and 4 log CFU/g respectively (Fig. 1b). Taken together, these results indicate lower thermal resistance of yeasts as compared with bacteria and a trend of high survival when spray drying was performed in samples prepared in why permeate. These results are in agreement with those reported by Golowczyc et al. (2010) related to the survival rates of the yeast *Saccharomyces lipolytica* CIDCA 812 isolated from kefir grains during the spray drying in similar conditions to those studied in this work. In addition, Atalar & Dervisoglu (2015) reported that no yeasts were detected in the kefir powder obtained by using similar processing conditions.

According to the Food and Agriculture Organization (FAO) and World Health Organization (WHO) guidelines (2002), a probiotic food supplement should reach at least a population of 10^7 CFU/g to be considered with probiotic potential. Therefore, samples obtained by spray drying of traditionally elaborated kefir did not fulfill this prerequisite.

It is known that the combined effects of heat and mechanical stress result in cellular damage that led to a loss of viability of microorganisms (Chávez & Ledeboer, 2007). These cellular injuries include DNA and RNA denaturation, ribosomal damage, dehydration and destabilization of plasma membrane due to water removal (Teixeira, Castro, Mohacsi-Farkas & Kirby, 1997; Garre, Raginel, Palacios, Julien & Matallana, 2011).

Typical pH of kefir beverages is between 4.0 and 4.4 and the measured values in this study were in this range. Acids generated during kefir fermentation were present in the drying medium and may cause unfavorable conditions for microorganisms' survival. It is worth to note that usually, kefir is made from milk with low solids content (11% w/v) that is not appropriate for spray drying. Higher solids content might increase yield, but when kefir was elaborated with 20% w/v skim milk, it resulted in a high density product that is very difficult to process by spray drying. It is reported that an increase in the amount of total solids in the sample may increase the

performance and the microorganisms viability due to the thermoprotective effect of not-fat milk solids (Corcoran, Ross, Fitzgerald & Stanton, 2004).

3.2. Kefir microorganisms dehydrated using carrier solutions

As a strategy to increase microbial viability and to optimize conditions for dehydration, the effect of whey permeates, skim milk and maltodextrin as spray drying carrier solutions was studied. The use of carriers generates more favorable conditions for dehydration process, increasing the viability of microorganisms in the product (Sunny- Roberts & Knorr, 2009). The yield, water activity and survival of lactic acid bacteria and yeasts in the dehydrated product were analyzed.

In order to produce economically feasible cultures for industrial applications, the culture medium for biomass production must be optimized for high biomass yield and reduction of the production costs. Our results showed that the use of carriers allows for biomass yields between 23 and 35% depending on the carrier solution used (Table 2). The lower values were obtained when WP was used as carrier (23.3%) while the neutralized samples showed the highest biomass yield (35%). It is difficult to make comparisons to other reports due to the diversity of variables that are considered (microorganisms, drying media, concentration of solids, drying parameters).

The biomass yield values obtained during the production of dehydrated product were the result of the higher stickiness of these products on the walls of drying. In these sense, Arslan, Erbas, Tontul & Topuza (2015) obtained similar product yield values (39 -54%) using different carriers. Similarly, Chandralekha et al. (2016) reported that yields after spray drying were 39.5% and 25.8% using skim milk and maltodextrin respectively.

Water activity (a_w) is related to the availability of free water in the sample and is an important parameter that determines the stability and shelf life (Rahman, 2010). According to Schmidt (2004), water activity values between 0.001 and 0.25 are related to high stability. In the present work, all samples showed a_w values around 0.2 - 0.3 (Table 2) which would provide an advantage in storage. According to several reports, optimal moisture content of spray dried powders is between 4 and 7% for storage stability (Ananta, Volkert, & Knorr 2005; Chavez &

Ledeboer, 2007). The moisture content values obtained of the spray dried samples are shown in Table 2. The moisture content of all the spray dried samples with different carrier materials ranged between 5.2 to 5.9% except WP and WP-MD neutralized samples, which has significantly higher moisture content values (ranged between 7.8 to 8.6%). These high values obtained in the neutralized samples could explain the low stability of these samples during storage (Fig. 3b).

3.3. Survival of microorganisms

3.3.1. Lactic acid bacteria survival

Viable lactic acid bacteria in dehydrated kefir samples obtained by using different carriers were assessed (Table 2). All the carriers evaluated lead to an increase in the survival of lactic acid bacteria increased as compared to traditional kefir (Fig 1a). It can be hypothesized that these results are related to the increase in the amount of total solids (and hence non-fat milk solids) with thermoprotectant effect (Corcoran, Ross, Fitzgerald & Stanton, 2004).

The reported total solids contents of the drying media usually range from 20 to 30% (w/v). These values have been considered as optimal to ensure high survival of different LAB strains (Huang et al., 2017). Furthermore, values of total solids content are a relevant technological issue because it impacts on the drying process productivity, energy costs and encapsulation effect. In this context, in this work, higher solids content (20% w/v) in the sample probably generates a more favorable condition for dehydration. When kefir microorganisms were suspended and dehydrated in carriers containing skim milk, lactic acid bacteria survival was greater than 9 log CFU/g.

It has been reported that whey permeate is an appropriate carrier medium for spray drying of lactobacilli (Golowczyc et al., 2013; Hugo, Bruno & Golowczyc, 2016; Eckert et al., 2017). Our results demonstrated that when kefir microorganisms are suspended and dehydrated in whey permeate (WP) and whey permeate plus maltodextrin (WP-MD), lactic acid bacteria survival was above 8 log CFU/g. However when kefir microorganisms are suspended and dehydrated in WP and WP-MD and neutralized to pH 7, lactic acid bacteria survival was above 9 log CFU/g. These findings are in agreement with results reported by Golowczyc et al. (2013) that

demonstrated that acidic conditions during dehydration of kefir-derived lactobacilli is a main factor affecting survival. It could be hypothesized that acid compounds became concentrated during the spray drying process thus enhancing deleterious effects on microorganisms.

3.3.1. Yeast survival

Viable yeast counts of kefir samples suspended in the different carriers after spray drying are shown in Table 2. When kefir microorganisms were suspended and dehydrated in skim milk (SM) and skim milk plus maltodextrin (SM-MD), values of viable yeasts were below the detection limits (100 CFU/g). In contrast, when kefir microorganisms were suspended and dehydrated in neutralized skim milk (SM neut) and skim milk plus neutralized maltodextrin (SM-MD neut) yeast survival was above 3 log CFU/g. These findings demonstrate that appropriate neutralization of the suspension medium is crucial to maintain yeast viability during the drying process. When kefir microorganisms were suspended and dehydrated in whey permeate (WP) and whey permeate plus maltodextrin (WP-MD), yeast survival was above than 4 log CFU/g and no significant differences were found compared to the neutralized samples.

The low thermotolerance of yeasts has been reported (Abidas, Teixido, Usall, Solsona, & Vinas, 2005; Golowczyc, Silva, Abraham, De Antoni & Teixeira, 2010). This susceptibility could be related to the increase of the cell surface-to-volume ratio (s/v) that in turn causes membrane condensation and restructuration during dehydration process (Lemetais, Dupont, Beney & Gervais, 2012). The improvement of viability in WP can be related to the thermoprotective effect of lactose. Indeed, Arao, Suzuki & Tamura (2002) found a protective effect of various saccharides (including lactose) on the resistance of yeast to high temperatures and concluded that this effect is related to the mean number of equatorial OH groups in the sugar molecule.

3.4. Survival in simulated gastrointestinal conditions

Survival to the gastrointestinal transit is a crucial requirement for probiotic microorganisms. In this context , low pH and antimicrobial action of pepsin in the stomach besides with the presence of bile salts in the intestine, constitute barriers that orally administered microorganisms have to cope with (Nagata, Y., Hashiguchi, K., Kamimura, Y., Yoshida, M. & Gomyo, T., 2009).

When kefir microorganisms were suspended and dehydrated in skim milk (SM) and skim milk plus maltodextrin (SM-MD), lactic acid bacteria reduction did not exceed 1 log CFU/mL at the end of the incubation in the simulated gastric solution (Fig. 2). Similarly, Silva et al. (2018) observed that probiotic *L. casei* 01 present in cheese showed a mean survival reduction of about 1 log after simulated gastrointestinal conditions. We demonstrated here that when kefir microorganisms were suspended and dehydrated in whey permeate (WP) and whey permeate plus maltodextrin (WP-MD), lactic acid bacteria decreased 1.5 and 2 log CFU/mL respectively at the end of the simulated gastric solution. These results indicate that skim milk as drying medium allows for the obtention of a dehydrated product with lactic bacteria that can better resist the passage through the gastrointestinal tract. Our findings are in agreement with those of Ilango, Pandey & Antony (2016) that reported high survival rates (98-99%) in simulated gastric and intestinal conditions of encapsulated lactic acid bacteria after spray drying in skim milk as drying medium. Skim milk is commonly used as a drying medium because it prevents cell damage by stabilizing the constituent biomolecules of the cell membrane. In addition, it creates a porous structure in the dehydrated product and contains proteins that provide a protective cover for the cell (Abadias , Teixido, Usall, Benabarre & Vinas, 2001). It can be hypothesized that spray dried bacteria using milk as a carrier have suffered less damage during the drying process thus becoming more resistant to the passage through the GI simulated conditions. Noteworthy, yeasts did not survive the passage through the simulated gastrointestinal conditions (data not shown).

3.5. Shelf life study

Our results demonstrated that lactic acid bacteria in kefir powder samples dehydrated in skim milk (SM) and skim milk plus maltodextrin (SM-MD) remained stable for at least 60 days (Fig. 3a). In a recent study, values of viable *Lactobacillus casei* 01 in cheese were 10^8 CFU/g after storage for 60 days at 4 ºC (Silva et al., 2018). In contrast, in the present study that lactic acid bacteria in kefir powder samples dehydrated in neutralized skim milk (SM neut) or neutralized skim milk plus maltodextrin (SM-MD neut) showed a decrease in viability ranging from 2 to 3 log CFU/g after 20 days of storage (4 ºC). The same result was obtained with samples

dehydrated in whey permeate (Fig. 3b). When analyzing yeast viability, it can be noted that samples dehydrated in whey permeate allowed for higher survival even at 40 d storage. Other conditions lead to the preservation of viability after 20 days but no yeasts were observed after 40 days (data not shown).

Storage at refrigeration temperatures has demonstrated to be a suitable condition for the preservation of dried cultures (Wang, Yu & Chou, 2004; Simpson, Stanton, Fitzgerald & Ross, 2005). Viability during storage is the result of several relevant variables that include initial number of microorganisms, composition of the carrier medium, water activity, storage temperature, oxygen content and relative humidity in packaging (Chávez & Ledeboer, 2007). All these issues must be considered in order to optimize long-term survival of probiotics in spray dried products.

4. Conclusion

Spray drying of kefir lead to many advantages for storage and transportation. Results reported herein, demonstrated that the selection of suitable carriers for the spray drying process is crucial for the viability of kefir microorganisms during both drying process and storage. Even the viability of the more susceptible microorganisms present in kefir, yeasts, was improved when appropriate conditions are used.

Our findings support the use of spray drying as a potential method for obtaining dehydrated products derived from kefir. Further studies are necessary to improve yeast survival and to assess the impact of spray drying and storage on the diversity of kefir microbiota.

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Table 1. The different samples tested

Samples	**Carrier medium description**
SM	Skim milk 20% w/v, pH=5.85
SM neut	Skim milk 20% w/v, pH=7
SM-MD	Skim milk 20% w/v + Maltodextrin 20% w/v, equal parts, pH=5.78
SM-MD neut	Skim milk 20% w/v + Maltodextrin 20% w/v, equal parts, pH=7
WP	Whey permeate 20% w/v, pH=4.88
WP neut	Whey permeate 20% w/v, pH=7
WP-MD	Whey permeate 20% w/v + Maltodextrin 20% w/v, equal parts, pH=4.71
WP-MD neut	Whey permeate 20% w/v + Maltodextrin 20% w/v equal parts, pH=7

Table 2. Biomass yield, water activity, moisture content, viable lactic acid bacteria and yeast after spray drying in dehydrated kefir sample resuspended in different carriers

Sample*	Yield (%)	a_w	% moisture	Lactic Acid Bacteria (log CFU/g)	Yeast (log CFU/g)
SM	33.0 ± 3.1	0.21 ± 0.02	5.4 ± 0.06[a]	8.9 ± 0.4[a]	0 [a]
SM neut	35.9 ± 3.1	0.18 ± 0.01	5.7 ± 0.03[a]	8.8 ± 0.6[a]	0 [a]
SM-MD	29.9 ± 0,6	0.20 ± 0.01	5.5 ± 0.03[a]	9.5 ± 0.4[a]	4.1 ± 0.4[b]
SM-MD neut	37.1 ± 1.6	0.21 ± 0.02	5.2 ± 0.37[a]	8.8 ± 0.3[a]	2.4 ± 0.7[c]
WP	23.3 ± 2.5	0.21 ± 0.02	5.2 ± 0.15[a]	8.1 ± 0.07 [b]	5.2 ± 1.1[b]
WP neut	34.5 ± 3.9	0.29 ± 0.01	8.6 ± 0.09[b]	8.1 ± 0.1[b]	3.9 ± 0.7[b]
WP-MD	24.9 ± 1.2	0.21 ± 0.01	5.9 ± 0.28[a]	9.4 ± 0.6 [a]	4.5 ± 0.06[b]
WP-MD neut	32.8 ± 3.6	0.29 ± 0.01	7.8 ± 0.17[b]	9.1 ± 0.3[a]	3.9 ± 0.6[b]

* skim milk (SM), neutralized skim milk (SM neut), skim milk plus maltodextrin (SM-MD), neutralized skim milk plus maltodextrin (SM-MD neut), whey permeate (WP), neutralized whey permeate (WP neut), whey permeate plus maltodextrin (WP-MD) and neutralized whey permeate plus maltodextrin (WP-MD neut). The different superscript indicates the significant difference ($p < 0.05$).

Fig. 1: Microbial counts of lactic acid bacteria and yeast from kefir elaborated in UHT skim milk **(A)** and from kefir elaborated in 20% whey permeate **(B)** dehydrated directly, before (dark grey) and after (light grey) spray drying process. The *** symbols indicate that the difference is significant ($p<0.001$) when values before and after drying are compared.

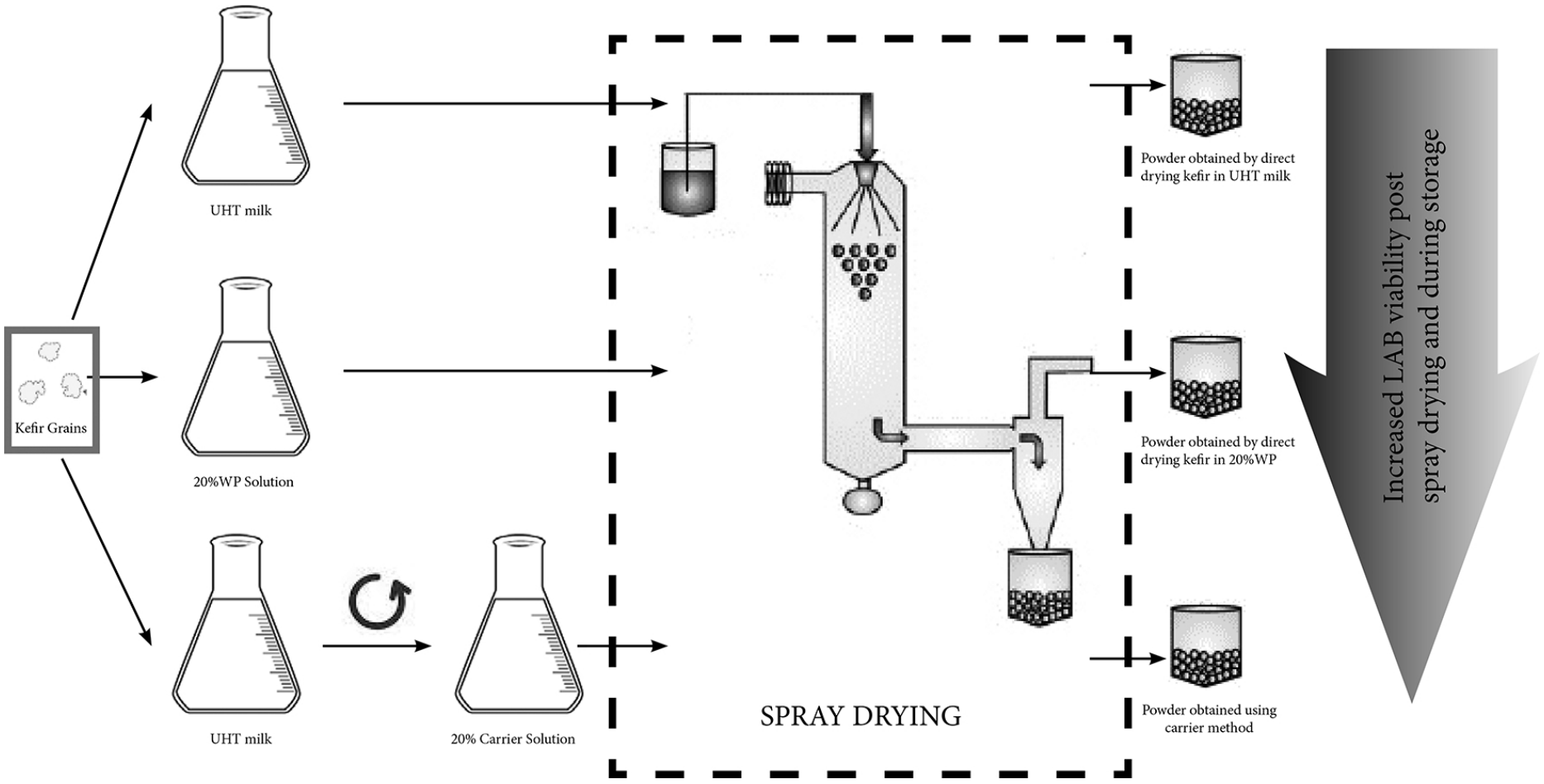
Fig. 2: Survival rate in simulated gastric solution for lactic acid bacteria after spray drying in dehydrated kefir with different carriers: skim milk (SM), skim milk plus maltodextrin (SM-MD), whey permeate (WP), whey permeate plus maltodextrin (WP-MD). Graph shows lactic acid bacteria counts after each step of the process: Dark grey for initial counts, light gray for counts after gastric simulation and black for counts at the end of the process. The ** symbol indicates a significant difference ($p<0.001$), and the *** symbol indicates a very significant difference ($p<0.0001$) after each step compared with the initial counts.

Fig. 3: Shelf life of lactic acid bacteria using different carriers. **(A)** UHT skim milk samples: SM (○), SM neut (●), SM-MD (Δ), SM-MD neut (▼); and **(B)** Whey permeate based samples: WP (○), WP neut (●), WP-MD (Δ), WP-MD neut (▼). Results were expressed as logarithmic values of relative survival fraction ($\log N/N_0$) as a function of storage time at 4 ºC.

Graphical abstract

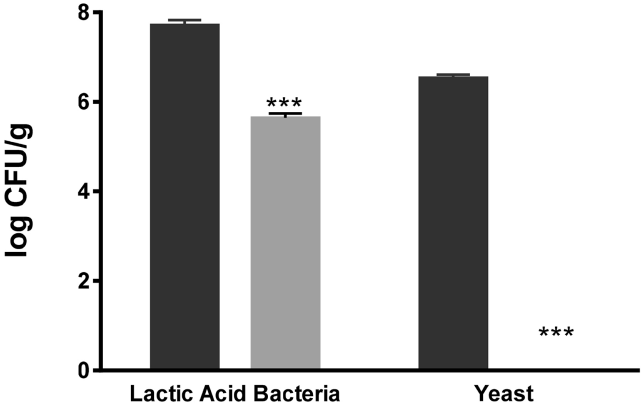
Highlights

- It is possible to use spray drying to produce kefir powder
- Lactic acid bacteria survive better than yeasts in the drying process
- Carrier solutions considerably increased the viability of kefir microorganisms
- Whey permeate is a suitable carrier for dehydration
- Dehydrated lactic acid bacteria survive gastrointestinal conditions

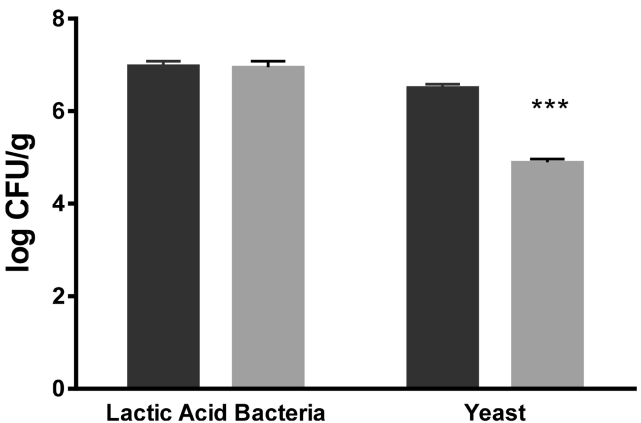


Graphics Abstract

A


8
6
4
2
0
log CFU/g
***
***
Lactic Acid Bacteria
Yeast

B


8
6
4
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log CFU/g
***
Lactic Acid Bacteria
Yeast

Figure 1

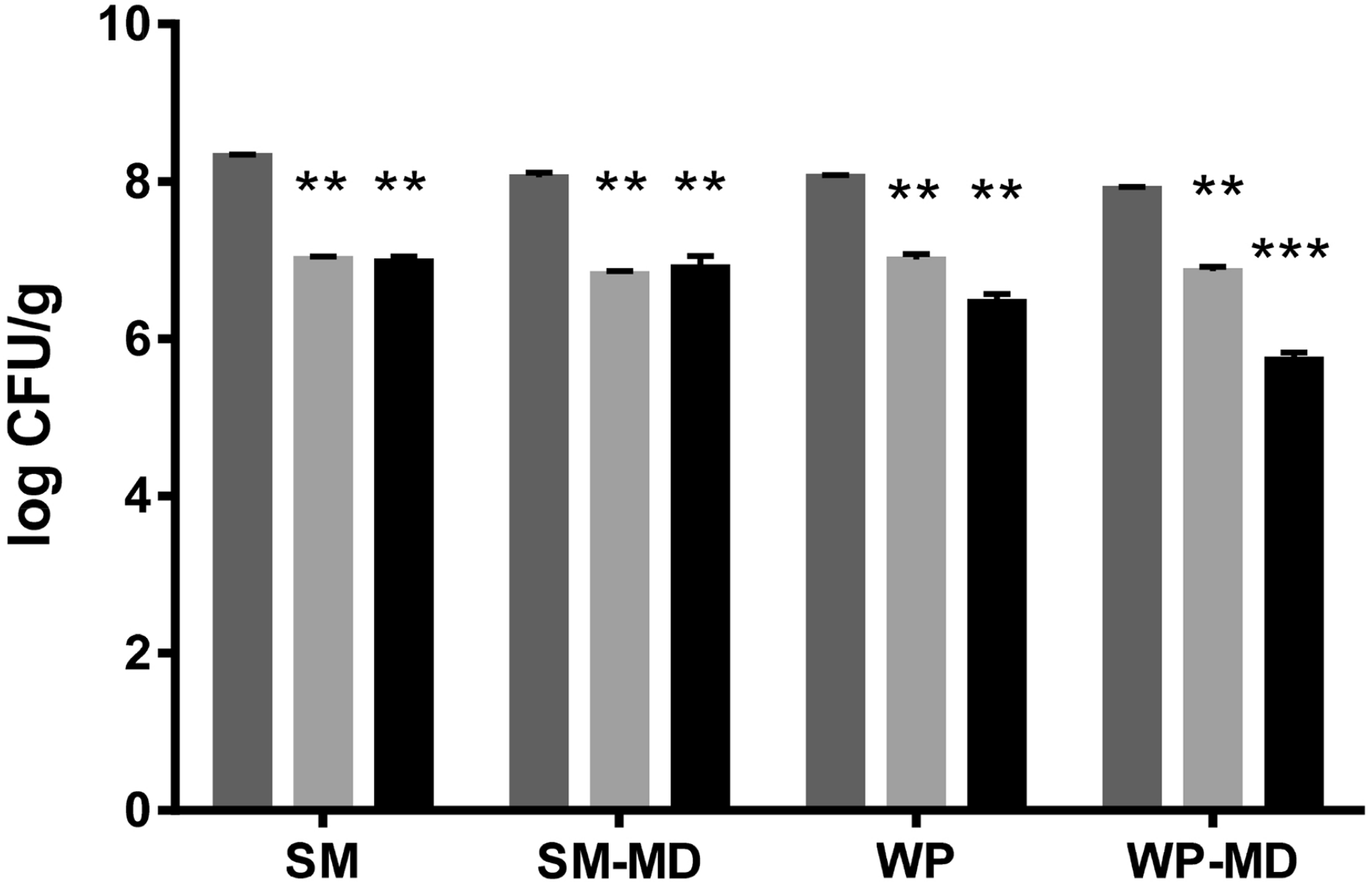
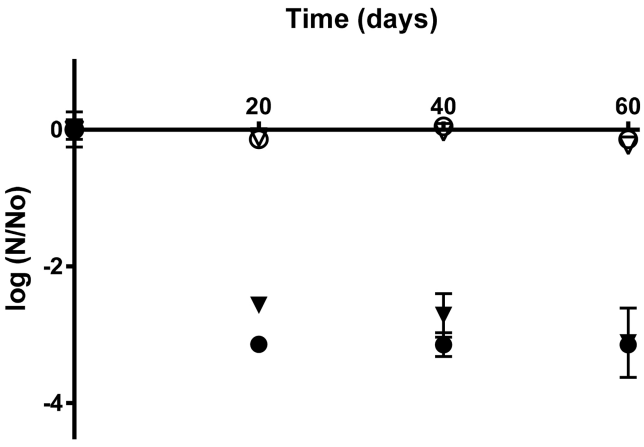


Figure 2

A



B

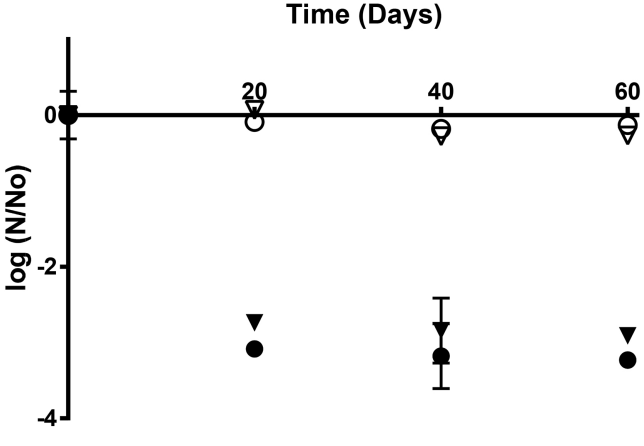


Figure 3