**Section: Bioprocessing and Biological Engineering**

# Survival of spray-dried *Lactobacillus kefir* is affected by different protectants and storage conditions

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**Abstract**

Survival of two *Lactobacillus kefir* strains after spray drying in reconstituted skim milk with or without the addition of 12.5 g monosodium glutamate/l, 20 g sucrose/l, or 20 g fructo-oligosaccharides (FOS)/l and during subsequent storage under different conditions of temperature (20 and 30°C) and relative humidity (0, 11 and 23%) was evaluated. After being dried, *Lactobacillus kefir* 8321 and *L. kefir* 8348 had a decrease in viability of 0.29 and 0.70 log c.f.u./ml respectively, while the addition of different protectants improved the survival of both strains significantly. During storage, bacterial survival was significantly higher under lower conditions of relative humidity (0-11%), and monosodium glutamate and FOS proved to be the best protectants.

**Keywords:** *Lactobacillus kefir*, protectants, spray drying, survival

## Introduction

A growing interest has arisen in the inclusion in dried foods of viable probiotics of long-term shelf life at ambient temperatures and of survival in sufficient numbers to provide a health benefit to consumers. To that end, drying techniques to obtain dehydrated probiotic organisms in a viable state have proven useful; and although freeze-drying (lyophilization) has been the most widely used, spray-drying is 4 to 7 times less expensive and is more energy-efficient as well (Ananta et al. 2004).

A successful conservation of a given microorganism by spray drying therefore requires an evaluation of many variables - strain type, the carrier or vehicle of resuspension,

the drying temperature, the time of exposure to heat, the water activity, and the storage conditions- and an optimization of the conditions for survival (Chávez and Ledeboer 2007).

Reconstituted-skim-milk (RSM) powder would appear to be a suitable carrier medium for an efficient spray-drying of probiotic cultures; but a wide variety of protectants - including whey protein, trehalose, monosodium glutamate, gum acacia, glycerol, betaine, adonitol, sucrose, glucose, inulin, lactose, and oligosaccharides - have been added to improve the survival of bacteria during this process (Desmond et al. 2002; Corcoran et al. 2004; Ananta et al. 2005; Morgan et al. 2006; Sunny-Roberts and Knorr 2009). Nowadays, so-called prebiotics are being extensively investigated because many studies have shown that these compounds have beneficial effects on consumer health and in addition enhance probiotic survival during drying (Ananta et al. 2005; Schwab et al. 2007).

Previously we reported that spray-drying was suitable for preserving microorganisms isolated from kefir grains (Golowczyc et al. 2010). In this investigation we studied the effects of monosodium glutamate, sucrose, and fructo-oligosaccharides (FOS) in combination with RSM on the viability of two probiotic *L. kefir* strains during spray drying and the survival of both afterwards during storage under different conditions of temperature and relative humidity.

## Materials and methods

### Bacterial strains and growth conditions

*Lactobacillus kefir* CIDCA 8321 and *L. kefir* CIDCA 8348 were grown in MRS broth (Difco) at 30ºC for 48 h under static conditions to achieve stationary-phase growth. The cells were harvested by centrifugation for 10 min at 7000 x *g* and 4ºC and washed twice by centrifuging with sterile Ringer's solution.

**Preparation of bacterial suspensions**

The cell pellets obtained as described above were resuspended in 11% (w/v) reconstituted skim milk (RSM) and RSM containing 20 g sucrose /l 12.5 g monosodium glutamate/l and 20 g fructo-oligosaccharides (FOS)/l (Beneo P95, Orafti S.A.).

**Spray-drying and storage**

Each sample to be tested was spray-dried in a pilot-scale apparatus (Niro Atomizer, Copenhagen, Denmark) in a vertical cocurrent drying chamber, 0.8 m diam. and 0.6 m ht. Spray drying conditions were: outlet-air temperature at 70°C, inlet-air temperature at 160ºC, and atomizing air pressure at 3 bar. The powder was collected in a single cyclone separator. Samples of the spray-dried powder were placed in sealed glass bottles placed in hermetic jars containing saturated aqueous solutions of lithium chloride and potassium acetate (both of Merck) so as to give relative humidities of 11% and 23%, respectively. A relative humidity of 0% was maintained by equilibrium with silica gel. Jars were held at either 20 or 30°C for 14 weeks and samples were removed periodically for counting.

**Enumeration of survivors**

The enumeration of survivors after spray drying and during subsequent storage in the dried state was performed as previously reported (Golowczyc et al. 2010). The storage-inactivation data were expressed as the logarithm of the fractional survival (logN/No, where N is the bacterial count at a particular storage period and No the value at the beginning of storage - *i. e.,* immediately after spray drying). From these graphs, at each relative-humidity level and for each protective agent, a best-fit straight line was obtained by least-squares

regression analysis and the decimal reduction time ($D$ value = 1/k) - the time required to reduce the viable count by one log at a given temperature—was calculated (see Fig. 1). Thus, $D_{20}$ and $D_{30}$ represent the time (in weeks) required for one log reduction in viability at 20 and 30°C, respectively.

**Water-activity measurements**

The water activity in the spray-dried powders was measured at room temperature in duplicate with an Aqualab Model Series 3 TE instrument (Decagon Devices, Inc.).

**Statistical analysis**

The results were expressed as the mean ± the standard deviation from triplicate samples in each of at least two independent experiments. For statistical comparisons, the analysis of variance (ANOVA) of viable counts before and after spray drying and at regular time intervals during storage was performed. The Student t test was used to determine the significance of differences between the $D$ values. All differences were considered statistically significant at a $p < 0.05$.

**Results and discussion**

Table 1 shows that the survival of *L. kefir* 8321 was better than that of *L. kefir* 8348, and the values for water activity were either equal or lower in the powders when reconstituted skim milk (RSM) alone was used as carrier. *L. kefir* 8321 evinced a reduction of 0.29 log c.f.u./ml when RSM alone was used as a carrier, while the reduction in log cycles was significantly lower ($p<0.05$) when RSM was supplemented with monosodium glutamate or

with FOS (Table 1). Similarly, the addition of monosodium glutamate, sucrose, or FOS markedly increased the number of viable bacteria in *L. kefir* 8348 (Table 1). Water activity is a critical parameter affecting the processability, the handling properties, and the stability of dairy powders (Ross 2002). The water-activity values of samples dried in RSM containing sucrose, monosodium glutamate, or FOS were not significantly different ($p>0.05$) from the samples dried in the presence of RSM alone.

To achieve long-term storage of the spray-dried powder, the environmental conditions, such as the temperature and the relative humidity (RH), are especially influential (Teixeira et al. 1995; Castro et al. 1995; Morgan et al. 2006). Sucrose and monosodium glutamate were originally used effectively as protecting agents in both freeze drying and spray drying (Teixeira et al. 1995; Carvalho et al. 2003; Ferreira et al. 2005; Sunny-Roberts and Knorr 2009); but in recent years the use of prebiotics for this purpose has been further studied, though with variable results (Corcoran et al. 2004; Ananta et al. 2005; Schwab et al. 2007). The FOS are considered prebiotics because those saccharides were demonstrated to increase the number of bifidobacteria as well as inhibit the growth of several pathogens (Fooks and Gibson 2002).

In order to represent the difference in lactobacilli survival upon storage in the presence of the various carrier combinations and at different relative humidities, we plotted death curves. For example, Fig. 1 shows the decrease in the number of viable bacteria during storage at 30°C and 0% RH. From the lethality rate constant (k) of the linear-regression slope, we calculated the *D* values. During storage at 20°C (Fig. 2) a significant increase in the $D_{20}$ values were found in the samples dried with RSM supplemented with FOS and stored at 11% RH and with those dried with RSM supplemented with monosodium glutamate and stored at

0% or 11% RH compared to the data obtained in the presence the unsupplemented carrier (RSM) at the same percent RH (Figs. 2a and 2b). Both strains studied showed a similar survival during storage under these different conditions.

The addition of sucrose gave different results depending on the strain used, and significant differences ($p<0.05$) were observed relative to the presence RSM alone only when *L. kefir* 8321 was stored at 11% RH.

Castro et al. (1995) and Teixeira et al. (1995) found that after freeze drying and spray drying, *L. delbrueckii* subsp. *bulgaricus* showed a similar behaviour during storage at 0 and 11% RH. In contrast, only when RSM supplemented with sucrose was the carrier did *L. kefir* 8321 and *L. kefir* 8348 exhibit no significant differences ($p>0.05$) in their viability when stored at 0% or at 11% RH and 20°C.

In general, upon storage at 23% RH, all samples had the lowest $D_{20}$ value (*i. e.*, the most rapid loss of viability at 20ºC), and no protection was conferred by the different carrier combinations.

Temperature is a critical parameter affecting the survival of microorganisms during storage, with the survival rates being higher at lower temperatures (Teixeira et al. 1995; Desmond et al. 2001; Golowczyc et al. 2010). During storage at 30°C and 23% RH (Fig. 3) there were no significant differences between the samples of strain dried in the presence of RSM with or without the protective agents, whereas a significant increase in the $D_{30}$ values were found upon storage at 11% RH in the samples of either *L. kefir* 8321 dried with RSM containing FOS or *L. kefir* 8348 dried with RSM plus monosodium glutamate (Figs. 3a and 3b). Nevertheless, the highest $D_{30}$ values were observed for strain upon storage at 0% RH after dehydration in the presence of RSM supplemented with any one of the protective agents

(Figs. 3a and 3b). These results are consistent with the observation of Castro et al. (1995) that the autoxidation of fatty acids within bacterial cell membranes is accelerated by increases in the RH, thus affecting the overall lipid profiles. Moreover, that protective effects were conferred in these experiments by the additives to RSM at RH of 0% even at 30ºC—when autoxidation would normally be favored by an elevated temperature—would indicate that at this low RH the protectants were acting to preserve the lipid composition of the bacterial membranes.

Thus, the markedly improved survival values obtained under these conditions would argue for the use of a storage atmosphere with very low water activity in combination with protectants as an effective strategy for preserving dried probiotic microorganisms. Accordingly, Santivarangkna et al. (2008) concluded that in order to maintain the viability of dried cultures, along with a consideration of the storage temperature, the water activity should be kept as low as possible and the packaging used for conservation should be hermetically sealed against the incursion of oxygen and moisture.

**Conclusions**

The proper approach to the use of spray drying for obtaining dehydrated probiotic powders is a complex issue because of the multiple conditions that need to be optimized during drying and storage. This work investigated the influence of three protective agents in addition to storage temperature and different relative humidity on the survival of *Lactobacillus kefir* strains 8321 and 8348 in the dried state. The viability of both strains was significantly higher under 0 and 11% of RH and when the bacteria were dried in the presence of monosodium glutamate and FOS. In contrast, when the carrier was supplemented with sucrose, variable results were obtained depending upon the storage temperature. We

concluded that optimum results with a given microorganism can only be obtained after taking into account that not only dehydration conditions and protectants but also storage has to be considered to maintain viability.

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**Table 1** Influence of different carriers on the survival of *Lactobacillus kefir* strains 8321 and 8348 during spray drying and water activity ($a_w$) values obtained in the spray-dried powders

| | Carriers[a] | Log reduction[b] | $a_w$ |
|---|---|---|---|
| *L. kefir* 8321 | **RSM** | **0.29 ± 0.05** | **0.26 ± 0.03** |
| | RSM + Glut | 0.10 ± 0.03 | 0.26 ± 0.01 |
| | RSM + Suc | 0.25 ± 0.07 | 0.30 ± 0.01 |
| | RSM + FOS | 0.18 ± 0.02 | 0.32 ± 0.01 |
| *L. kefir* 8348 | **RSM** | **0.70 ± 0.11** | **0.29 ± 0.02** |
| | RSM + Glut | 0.16 ± 0.05 | 0.25 ± 0.01 |
| | RSM + Suc | 0.08 ± 0.09 | 0.29 ± 0.01 |
| | RSM + FOS | 0.15 ± 0.05 | 0.33 ± 0.01 |

[a] RSM = reconstituted skim milk (11% w/v) ; glut = 12.5 g monosodium glutamate/l; suc = 20 g sucrose/l; FOS= 20 g fructo-oligosaccharides/l

[b] Represent the difference between the log (c.f.u./ml) of viable microorganisms before and after spray drying. The values are the mean of three independent assays ± standard deviation

**LEGENS TO THE FIGURES**

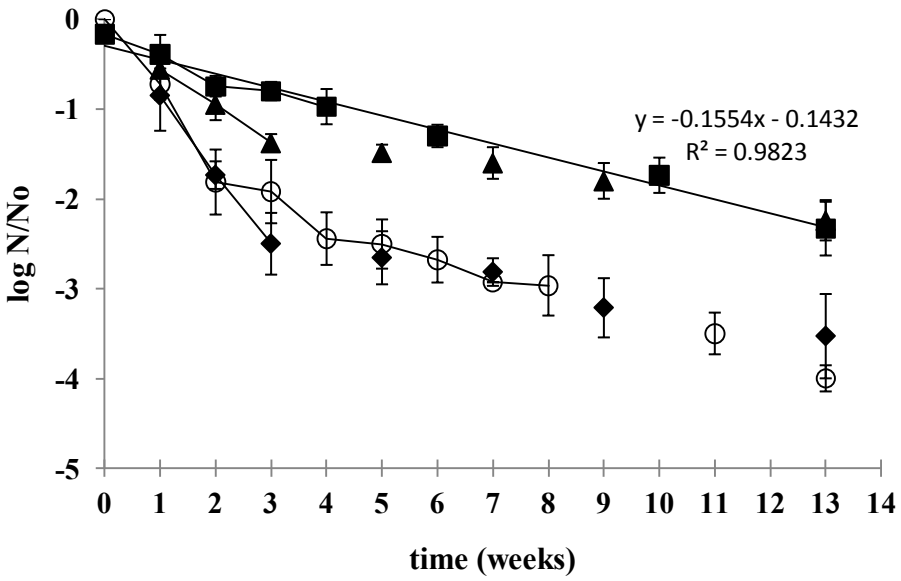
**Fig. 1** Survival of spray-dried *Lactobacillus kefir* CIDCA 8321 during storage at 30ºC in relative humidity of 0% as a function of carrier used during spray drying process: (■) RSM+ monosodium glutamate, (◆) RSM+ sucrose, (▲) RSM+FOS and (○) RSM (control). The straight line was fitted to the data by linear regression analysis and the correlation coefficient is shown. From the lethality rate constant (k) of the linear-regression slope, the $D_{30}$ values were calculated as 1/k. Each point represents the mean of duplicate spray drying experiments and the error bars indicate the standard deviations for the data points.

**Fig. 2** Decimal reduction time (*D*) of *Lactobacillus kefir* CIDCA 8321 (a) and *Lactobacillus kefir* CIDCA 8348 (b) stored at 20°C during 14 weeks of storage, at different relative humidities: 0% ( □ ), 11% ( ■ ) and 23% ( □ ). *D* values were obtained from the slope of survival rate of microorganisms plot dried in RSM (control) or RSM added with 20 g fructo-oligosaccharides/l (RSM+FOS), 12.5 g monosodium glutamate/l (RSM+glut) and 20 g sucrose/l (RSM+suc) by linear regression.

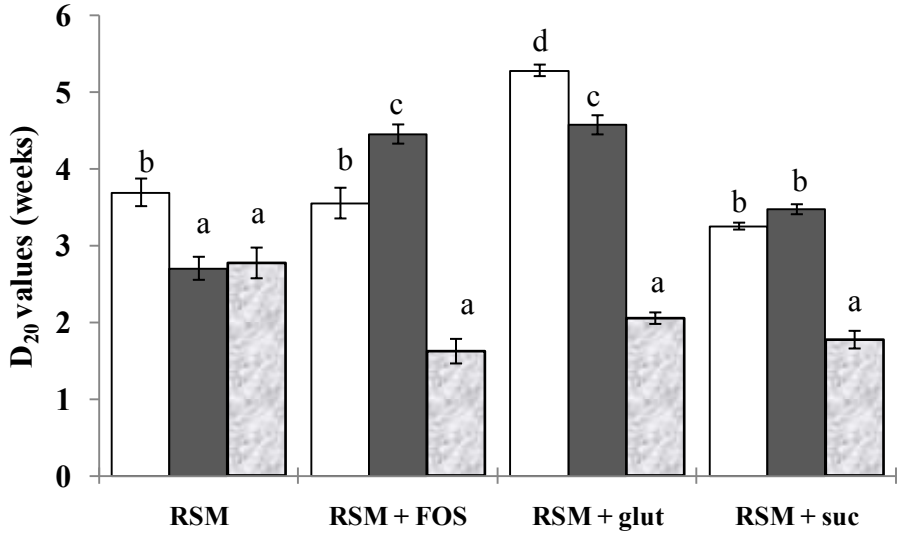
Different letters indicate significant differences at $p < 0.05$ by Student t test.

**Fig. 3** Decimal reduction time (*D*) of *Lactobacillus kefir* CIDCA 8321 (a) and *Lactobacillus kefir* CIDCA 8348 (b) stored at 30°C during 14 weeks of storage, at different relative humidities: 0% ( □ ), 11% ( ■ ) and 23% ( □ ). *D* values were obtained from the slope of survival rate of microorganisms plot dried in RSM (control) or RSM added with 20 g fructo-oligosaccharides/l (RSM+FOS), 12.5 g monosodium glutamate/l (RSM+glut) and 20 g sucrose/l (RSM+suc) by linear regression.

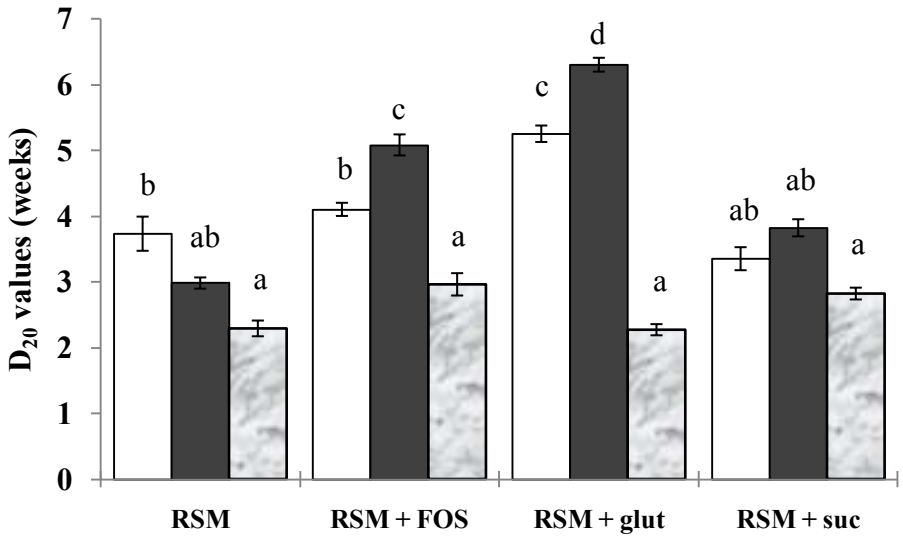
Different letters indicate significant differences at $p < 0.05$ by Student t test.




Golowczyc et al., Fig. 1

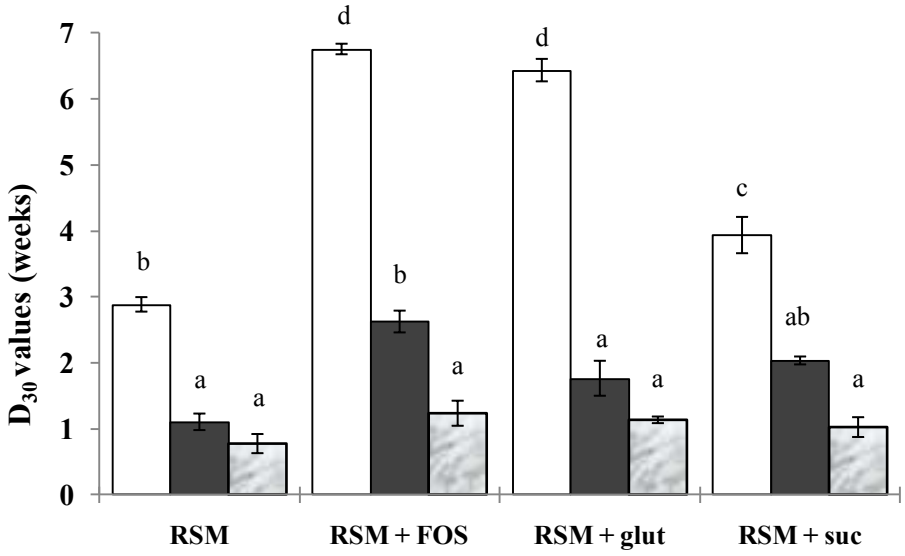



a




b

Golowczyc et al., Fig. 2

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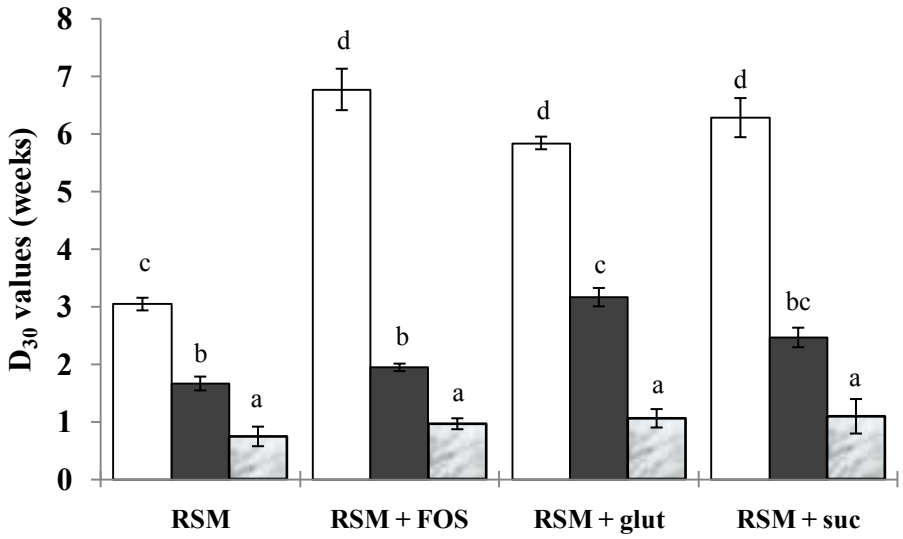


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Golowczyc et al., Fig. 3