“Influence of different storage conditions on the performance of spray-dried yogurt used as inoculum for milk fermentation”

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Short title: Spray-dried yogurt and milk fermentation

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Summary

In present study a commercial drinkable yogurt with and without 4% of added trehalose (as cell protectant) was spray-dried obtaining a powder with low water activity ($a_w$). Total bacterial count in the powder was between 8.48-8.90 log cfu/g. The dried yogurt was stored: i) at 38°C and $a_w = 0.33$; ii) at 38°C in hermetically sealed flasks ($a_w = 0.21/0.22$); iii) in a cyclic temperature chamber (10-20°C) in hermetically sealed flasks ($a_w = 0.21/0.22$). Whole milk was then fermented by adding an inoculum of spray-dried yogurt after storage under the abovementioned conditions. The kinetics of acidification evidenced the presence of a lag time which was strongly dependent on storage conditions. The data was fitted with a logistic type equation from which the lag time was calculated. To evaluate structural differences among samples, Fourier Transform Infrared spectra (FTIR) were recorded. Partial Least Squares (PLS) models enabled a good correlation between lag time of fermentation and FTIR spectra. The lag time for yogurt powder stored at $a_w$ about 0.21/0.22 and cyclic temperature 10-20 °C remained approximately constant over the 12 weeks of storage, while all the other conditions resulted in a dramatic increase. The addition of trehalose had a small influence on lag time and, therefore, as a protectant of lactobacilli.

Keywords: Spray-drying; Yogurt; FTIR; acidification; lag time
Introduction

In response to consumer demand for foods with health benefits, the food industry began to diversify its products. Although most foods containing probiotic bacteria are dairy products, there is also a growing demand for incorporating probiotics in other food products. Spray-drying is widely used in the food, pharmaceutical and other industries because of its ability to efficiently transform a liquid feed into a dry powder (Huang, 2011). Recent references highlight that spray drying technique is a suitable long-term preservation method for liquid and semi-liquid food also used for the stabilization of matrices containing probiotic bacteria, namely yogurt and other dairy products (Barbosa et al., 2017; Reale et al., 2019).

The shelf-life of yogurt can be considerably changed by spray-drying resulting in a stable powder of high quality, without the need for refrigeration (Kumar and Mishra, 2004, Guergoletto et al., 2012). This yogurt powder can also be used as an ingredient for the manufacturing of several food products such as confectioneries, yogurt drinks, fruit mixes, etc, as well as for direct consumption after reconstitution (Kumar and Mishra, 2004). It is to be noted according to the Food and Agricultural Organization (FAO), the microorganisms in yogurt must be viable and in sufficient amount to promote health benefits (Guergoletto et al., 2012); this is usually greater than 6-7 log of colony forming units (cfu) of viable microorganisms per gram of product at the time of being consumed (Aquilina et al., 2013).

Survival of microorganisms in dried matrices or products depends on many factors including species and strain, drying conditions, the use of cell protective agents and storage conditions (i.e., water activity ($a_w$), temperature and time). The decay of viable cell count is the main drawback when dehydrating probiotic formulations, and is associated to the severity of processing and storage conditions (Succi et al., 2014; Fiore et al., 2010). Cell protectants have been used to increase the survival of lactic acid bacteria after drying and storage. Among them, milk powder, whey, trehalose, sucrose, lactose and some oligo and polysaccharides
can be mentioned (Schoug 2009, Meng et al, 2007). Very recently, Stefanello et al. (2018) reported on the role of trehalose as bioprotectant in freeze-drying.

The dried state limits the number of techniques that can be applied to study conformation and stability of intracellular biomolecules (Wolkers and Oldenhof, 2005). One of the few suitable techniques for in situ analysis of biomolecules in the dried state is Fourier Transform Infrared Spectroscopy (FTIR). On account of characteristic molecular vibrations that absorb in the infrared region, information can be derived on molecular conformation and intermolecular interactions of biomolecules in their native environment (Wolkers and Oldenhof, 2005).

The objective of this work was to obtain basic information for the development of a powdered drinkable "yoghurt". For this reason, we utilized as raw material a commercial drinkable formulation (see Materials and Methods) containing not only L. bulgaricus and S. thermophilus, but also, L. casei and Bifidobacterium spp. In studies of the stability of yogurt powder it is customary to determine the survival of lactic acid bacteria during storage (Kumar and Mishra, 2004). In the present study a different approach was selected. Instead of enumerating lactic bacteria during storage, whole milk was fermented using an inoculum of spray-dried yogurt previously stored for different times and under different conditions. The measured acidification kinetics by the lactobacilli present in dried yogurt was used as an indicator of the injuries caused by the different conditions of a_w, time and temperature during storage. A FTIR analysis of the stored powder samples was also performed to evaluate the spectral differences among samples, arising from the different storage conditions assayed. The influence of the addition of trehalose, a well-known cell protectant, was also studied.

**Materials & Methods**

**Drinkable Yogurt**

A commercial, drinkable yogurt without added sugar ("SER", Danone, Argentina) was used. The yogurt had the following composition: 8.15% (± 0.02) dried extract, 4.45 % carbohydrates, 3.15 % protein, 0% fat and pH 4.40 (± 0.02).
The list of ingredients included: defatted milk, lactic ferments (Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophilus, Bifidobacterium spp., Lactobacillus casei), modified starch, stabilizers (gelatin, gellan gum), intensive sweeteners and artificial strawberry flavor.

Water activity, moisture content and pH

Water activity ($a_w$) was measured using a dew point hygrometer “Aqualab” Series 3 (Decagon Devices, USA) previously calibrated in the range of interest with standard saturated salt solutions. Moisture content was determined gravimetrically (2g sample) in a forced convection constant temperature oven at 90°C during 6 hours. The pH of fresh yogurt and inoculated milk were measured with a pH meter (Hanna Instruments, Italy) previously calibrated with appropriate buffers. All measurements were carried out in triplicate.

Reagents

Salts for relative humidity control (reagent grade) were obtained from Biopack (Buenos Aires, Argentina). Food grade trehalose (dehydrate) was obtained from Hayashibara Co. Ltd. (Japan).

Spray-drying

The drinkable yogurt, as such or after the addition of 4 % trehalose, was spray-dried in a mini spray-dryer Büchi model B-290 (Büchi Laboratoriums Technik, Switzerland). The main operating conditions were selected so that the air outlet temperature did not exceed 70°C, being this an important condition to obtain an adequate survival of lactobacilli (Zhang et al., 2016). A combination of an inlet air temperature of 140°C and a feed (yogurt) flow rate of 500 g/h allowed to obtain an outlet temperature of 68-70°C as well as a low $a_w$ (about 0.20). The spray-drying runs were performed in duplicate and the yield was around 50%.

Plate counting
Bacterial viability was determined before and after spray-drying and in the presence (4%) or absence (0%) of trehalose. For each determination, samples were rehydrated in 1ml 0.85% w/w NaCl. Bacterial suspensions were serially diluted, plated on MRS agar (de Man et al., 1960) and incubated at 37°C for 48 hours in aerobic conditions. Results were expressed in log cfu/ml and are the average of three independent determinations.

**Storage conditions**
Spray-dried yogurt powders (0% and 4% trehalose) were stored in the following conditions:
(a) hermetically sealed flasks to preserve its initial a_w. Some were kept in a constant temperature oven at 38°C and others in a cyclical temperature chamber where the temperature was maintained at 10°C for 12 hours and then 12 hours at 20°C. This cyclical variation of temperature adequately represents the daily variation of temperature in Buenos Aires between the months of August through November. The temperature of 38 °C is often utilized for studies of accelerated storage of foods (Labuza, 1982).

Total storage time was 12 weeks; one sample from each condition was removed each week and kept in a freezer at -72°C until the different analyses were carried out. **Table 1** shows abbreviated codes used for stored samples of dried yogurt.

- Insert Table 1 about here -

**Kinetics of milk fermentation using yogurt powder as inocula**

Pasteurized whole milk was fermented by adding an inoculum of the spray-dried yogurt (previously stored at different conditions as explained in section 2.6) and the kinetics of fermentation were evaluated by determining their rate of acidification (pH decrease). The following procedure was used: 1) sterilization of
glass jars, 2) preheating of milk at 43 °C, 3) inoculation of 20 ml of milk in the jars with 1.0 g of yogurt powder without trehalose, or 1.45 g of yogurt powder with 4% trehalose, and 4) glass jars were kept in a constant temperature bath at 43°C and pH was measured every 30 minutes during a 6 hours period; pH measurements were made in triplicate. An excess of inoculum (1.45 g instead or 1.0 g) was used in the yogurt powder runs with trehalose to compensate for the dilution of yogurt caused by the addition of trehalose.

Fourier Transform Infrared Spectra (FTIR)

About 5 mg of dried yogurt powder stored under the different conditions (as explained in section 2.6) were used to register the FTIR spectra. Samples were placed on the sample holder of an ATR-FTIR Thermo Nicolet iS10 spectrometer (Thermo Scientific, MA, USA). Spectra were registered in the 4000-500 cm\(^{-1}\) range by co-adding 100 scans with 4 cm\(^{-1}\) spectral resolution, using OMNIC software (version 8.3, Thermo Scientific, MA, USA). At least three spectra were recorded.

Data Analysis

Multivariate analysis and data pre-processing as mean centering and extended multiplicative scatter correction were performed on the FTIR spectra, using The Unscrambler software (version 10.2, CAMO, Norway). To evaluate the spectral differences among samples, arising from the different storage conditions assayed, a principal component analysis (PCA) was performed on the FTIR spectra. Taking into account the spectral differences in the PCA scores plot, PLS models were calibrated to determine the lag time (Esbensen, 2005). FTIR spectra covering the whole range of values were used to define the models. To set-up the PLS models, carried out on the raw spectra, 54 spectra were registered for calibration. Leave-one-out-cross validation method (LOOCV) was used for validation, and 7 PLS-factors were used for prediction. Lag times as results of experiments described in Section 2.7 were used as reference values. The reliability and robustness of the calibrated models were determined as a function of their correlation values, R-square, BIAS and their calibration errors (RMSEC).
Results & Discussion

Moisture content, $a_w$ and microbiological analysis of spray-dried yogurt

Fresh drinkable yogurt with (4%) and without added trehalose was spray-dried using the aforementioned operating conditions. The outlet air temperature not only influences the survival of lactic bacteria but also the $a_w$ of the powder, so a compromise value was taken since a decreased outlet temperature improves survival but also increases final $a_w$. The yogurt powder spray-dried with 140°C air inlet temperature and 68/70°C outlet air temperature had the following $a_w$ and moisture content (dry basis): 0.206 ± 0.005 and 5.32 ± 0.15, respectively for yogurt without trehalose, and 0.225 ± 0.001 and 5.16 ± 0.15, respectively for yogurt with 4% trehalose. Kumar and Mishra (2004) suggested that for a better survival of *Streptococcus*, *Lactobacillus* and *Bifidobacterium* spp. during storage the $a_w$ of yogurt powder should be about 0.20, which is in good agreement with the present values. Microbiological analysis indicated that fresh yogurt had a total bacterial count of 8.51 log cfu/ml, plain dried yogurt had 8.48 log cfu/g and dried yogurt added with trehalose (4%) had 8.89 log cfu/g. Reconstituted (to their initial solids content) spray-dried yogurt was also analyzed and total bacterial counts were of 7.81 log cfu/g for 0TR, and 7.96 log cfu/g for 4TR. These values indicate satisfactory survival of yogurt cultures (Zhang et al. 2016, Bielecka and Majkowska, 2000) and are very similar to those reported by Koc et al (2010) for spray-dried yogurt.

Kinetics of milk fermentation using inoculum of yogurt powder

Whole milk was fermented using inocula of spray-dried yogurt previously stored for different times and conditions. Figure 1 shows the measured kinetics of acidification using yogurt powders inocula; for the purposes of clarity only eight curves were displayed. The standard deviation for pH values ranged between 0.04 to 0.06. The eight conditions plotted in Figure 1 adequately illustrate the general shape of pH curves obtained after mild or drastic storage conditions. However, as
will be shown later, a much larger number of conditions (several combinations of 
\(a_w\), temperature, presence of trehalose and time) will be utilized for further 
calculations. The measured kinetics of fermentation was used as an indication of 
the metabolic activity. Golowczyc et al. (2010) also measured the acidification 
kinetics in milk by fresh and dried lactobacilli to evaluate the injuries caused by 
spray-drying on lactobacilli isolated from kefir grains.

- Insert Figure 1 about here -

In all acidification curves, a period during which the pH remains 
approximately constant (lag time) was observed; in most of them this was followed 
by a rapid fall in the pH values. The lag time is associated with the time for repair 
sublethal injuries experienced by lactobacilli stored under various stress conditions. 
In cases of extreme storage conditions (38°C and \(a_w=0.33\)), the lag time was very 
long and even 400 minutes was not enough for obtaining the final pH of around 
4.5. In order to better understand the relationship between storage condition, time 
and addition of trehalose, all acidification curves were fitted using a logistic type 
equation (eqn.1) (Romano et al. (2016).

\[
pH(t) = \frac{pH_0 - pH_f}{1 + \frac{t}{t'}} + pH_f \\
\]

(Eqn. 1)

where,

\(pH(t)\): is the pH at different fermentations times (every 30 minutes)

\(pH_0\): is pH at the beginning of milk fermentation

\(t\): time (minutes)
The obtained variables were,

- \( c \): time at which the inflection point occurs in the fitted function
- \( p \): exponential adjustment factor
- \( pH_f \): pH at the end of the fermentation

The lag time was calculated as the intersection between the tangent line at \( t = c \) and \( pH_0 \). Figure 2 shows the effect of storage time of yogurt powder (inocula) on the lag time during milk acidification.

It can be seen that the lag time for yogurt powder of both samples stored at the most gentle condition, which was 10/20°C and \( a_w = 0.22 \), remains approximately constant over the 12 weeks of storage (samples codes 0TR-10/20-Ai-Wn and 4TR-10/20-Ai-Wn). For these samples, trehalose showed a modest decrease in the lag period, suggesting a small effect as cell protector; this was only perceived under this gentle storage condition. At all other conditions of \( a_w \) and temperature, the lag time increased rapidly with storage time up to a point (8 weeks) at which all samples either leveled off or tended to vary in a more or less erratic way (data not shown). It is clear that both the \( a_w \) and the temperature of storage are key factors determining bacterial viability during storage; even small variations in \( a_w \) (0.21/0.22→0.33) and temperature (10/20°C→38°C) were highly detrimental to cell functioning.

**FTIR**

FTIR spectroscopy was used to monitor the changes in lag period experimented by spray-dried yogurt stored under different conditions. In order to discriminate spectra according to the physical conditions used for storage, and to avoid the interference of trehalose, strongly absorbing in the infrared region of the
electromagnetic spectrum, two different PCA was carried out on spray-dried yogurt without (Figure 3A) and with the protective compound trehalose (Figure 3B). Samples of yogurt powders spray-dried without trehalose, were mainly distributed along PC2, which explained 25% of the variance. In turn, yogurt powders containing trehalose were mainly grouped along PC1, which explained 58% of the variance. However, regardless of the presence of trehalose, sample grouping was associated to differences in the lag times. FTIR spectroscopy was used by Kher et al. (2007) to examine the conformation of proteins in spray-dried milk protein concentrate prepared from a range of processing conditions and after storing for 4 weeks at 21 °C. They reported that FTIR spectroscopy could be used to predict protein behavior since the obtained data correlated well with changes in solubility of the dry powders on storage.

- Insert Figure 3 about here -

According to the loading plots in PC1 (Figure 4), the main differences among samples were observed in the 1200-900 cm⁻¹ region, associated to the C-O-C glycosidic linkage, the δCOH and the νC-C vibrational modes (Santos et al., 2014a). For example, 0TR-38-33 samples stored for 12 weeks showed the largest lag times and were observed at the highest PC1 values (Figure 3A, open circles). On the contrary, sample 0TR-10/20-Ai stored for shorter times (squares-open, gray or black in Figure 3A) were observed in the other extreme of the plot. Samples with lag times within these two extremes were observed at intermediate positions. A similar behavior was observed along PC1 for yogurt powders containing trehalose (Figure 3B).

- Insert Figure 4 about here –
A PLS model was defined to determine the lag time directly from the FTIR spectra. In the definition of such model, results obtained in section 3.2 were used as references. The PLS model was calibrated using 54 spectra (correlation of 0.989; $R^2$, 0.977; Root Mean Square Error of Calibration (RMSEC), 15.95; Standard Error of Calibration (SEC), 16.101; BIAS, $-1.907 \times 10^{-6}$), and cross-validated (Figure 5). Using spectra covering a broad range of lag times strengthened the predictive capacity of the model. The mean of the predicted values fitted nicely to those shown in Figure 2 and were not dependent on the presence (or absence) of trehalose in the samples. This broadens the application of the obtained model, supporting its use to investigate unknown samples. Considering that the lag time is closely related to the functionality of yogurt powders, using the defined PLS model, would be valuable to determine this parameter just by registering FTIR spectra.

- Insert Figure 5 about here –

Conclusion

Whole milk was fermented using inocula of spray-dried yogurt (with and without added trehalose) previously stored for up to 12 weeks at different $a_w$ and temperature. The lag time in the kinetics of milk acidification was associated with the impact of the storage condition and the presence of trehalose on the repair sublethal injuries experienced by lactobacilli. Lag times for yogurt powder of samples stored at the most gentle condition, which was 10/20ºC and $a_w=0.22$, remained approximately constant over the 12 weeks of storage. For these samples, trehalose showed only a modest decrease in the lag time, suggesting a small effect as cell protector which was only perceived under this gentle storage condition. At the other conditions of $a_w$ and temperature, the lag time increased rapidly with storage time and the presence of trehalose could not counterbalance the negative effect of temperature and $a_w$. Both, $a_w$ and temperature of storage
were key factors determining sublethal injuries during storage; even small variations in $a_w$ (0.21/0.22→0.33) and temperature (10/20°C→38°C) were highly detrimental of cell functioning.

Partial Least Squares (PLS) models enabled a fairly good correlation between lag time of fermentation and FTIR spectra. Using the defined PLS model, would be valuable to predict lag time just by registering FTIR spectra.

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References

Aureli P, Fiore A, Scalfaro C, Casale M & Franciosa G 2010 National survey outcomes on commercial probiotic food supplements in Italy. *International Journal of Food Microbiology* **137** 265-273

Aquilina G, Bach A, Bampidis V, Bastos ML, Flachowsky G, Gralak, MA, Hogstrand Ch, Leng L, López-Puente S, Martelli G, Mayo B, Renshaw D, Riychen G, Saarela M, Sejrsen K, van Beelen P, Wallace RJ & Westendorf J 2013 Scientific Opinion on the safety and efficacy of Probiotic LACTINA® (*lactobacillus acidophilus, Lactobacillus helveticus, Lactobacillus bulgaricus, Lactobacillus lactis, Streptococcus thermopiles and Enterococcus faecium*) for chickens for fattening and piglets. *EFSA Journal* **11** 317-3183
Barbosa J, Brandao TRS & Teixeira P 2017 Spray drying conditions for orange juice incorporated with lactic acid bacteria. *International Journal of Food Science & Technology* **52** 1951-1958

Bielecka M & Majkowska A 2000 Effect of spray-drying temperature of yogurt on the survival of starter cultures, moisture content and sensoric properties of yogurt powder. *Nahrung* **44** 257-260

de Man JO, Rogosa M & Sharpe ME 1960 A medium for the cultivation of lactobacilli. *Journal of Applied Bacteriology* **23** 130-135

Esbensen KH 2005 Multivariate data analysis. In practice (5th ed.). Esbjerg, Denmark: CAMO process AS

Golowczyc MA, Silva J, Abraham AG, De Antoni GL & Teixeira P 2010 Preservation of probiotic strains isolated from kefir by spray-drying. *Letters in Applied Microbiology* **50** 7-12

Guergoletto KB, Sivieri K, Tsuruda AY, Martins EP, Bertuol de Souza JC, Roig SM, Hiroooka EY & Garcia S 2012 Dried probiotics for use in functional food applications. *Food Industrial Processes- Methods and Equipment*, Dr. Benjamin Valdez (Ed), ISBN: 978-953-307-905-9, In Tech (London, UK). Available from: http://www.intechopen.com/books/food-industrialprocesses-methods-and-equipment/dried-probiotics-for-use-in-functional-food-applications

Kher A, Udabage P, Mc Kinnon I, Mc Naughton D & Agustin MA 2007 FTIR investigation of spray-dried milk protein concentrate powders. *Vibrational Spectroscopy* **44** 375-381

Koç B, Yılmazer MS, Balkır P & Ertekin FK 2010 Moisture sorption isotherms and storage stability of spray-dried yogurt powder. *Drying Technology* **28** 816-822

Kumar P & Mishra HN (2004) Yoghurt powder — A review of process technology, storage and utilization. *Food and Bioproducts Processing* **82** 133-142

Meng XC, Stanton GF, Fitzgerald CD & Roos RP 2007 Anhydrobiotics. *Food Chemistry* **106** 1406-1416
Reale A, di Renzo T, Preziuso M, Panfili G, Cipriano L, Messia MC 2019
Stabilization of sourdough starter by spray drying technique: new
breadmaking perspective. *LWT - Food Science and Technology* **99** 468-475

Romano N, Schebor C, Mobili P & Gómez-Zavaglia A 2016 Role of mono and
oligosaccharides from FOS as stabilizing agents during freeze drying and
storage of *Lactobacillus delbrueckii* subs. *bulgaricus*. *Food Research
International* **90** 251-258

Santos M, Araujo-Andrade C, Tymczyszyn E & Gomez-Zavaglia, A 2014a
Determination of amorphous/rubbery states in freeze-dried prebiotic sugars
using a combined approach of near-infrared spectroscopy and multivariate
analysis. *Food Research International* **64** 514-519

Santos MI, Araujo-Andrade C, Esparza-Ibarra E, Tymczyszyn E, Gómez-Zavaglia
A 2014b Galacto-oligosaccharides and lactulose as protectants against
desiccation of *Lactobacillus delbrueckii* subsp. *bulgaricus*. *Biotechnology
Progress* **30** 1231-1238

Schoug A 2009 A dry phase of life freeze drying and storage stability of
*Lactobacillus coryniformis* Si3 in sucrose-based formulations. Doctoral
Thesis, Swedish University of Agricultural Sciences, Uppsala

Succi M, Sorrentino E, di Renzo T, Tremonte P, Reale A, Tipaldi L, Pannella G, Russo A & Coppola R 2014 Lactic acid bacteria in pharmaceutical
formulations: presence and viability of “healthy microorganisms”. *Journal of
Pharmacy and Nutrition Sciences* **4** 66-75

Stefanello RF, Machado A, Cavalheiroa C, Santos M, Nabeshima E, Copetti M & Fries L 2018 Trehalose as a cryoprotectant in freeze-dried wheat sourdough
production. *LWT - Food Science and Technology* **89** 510-517

Reale A, di Renzo T, Preziuso M, Panfili G, Cipriano L, Messia MC 2019
Stabilization of sourdough starter by spray drying technique: new
breadmaking perspective. *LWT - Food Science and Technology* **99** 468-475

Wolkers WF & Oldenhof H 2005 *In situ* FTIR assessment of dried *Lactobacillus
bulgaricus*: KBr disk formation affects physical properties. *Spectroscopy* **19**
88-99
Zhang Y, Lin J & Zhong Q 2016 Effects of media, heat adaptation and outlet temperature on the survival of *Lactobacillus salivaris* NRRL B-30514 after spray drying and subsequent storage. *LWT-Food Science and Technology* 74 441-447
Figure legends:

Figure 1:
Kinetics of milk fermentation using yogurt powder inocula for some of the storage conditions for samples with (black reference) and without (grey reference) trehalose. Data shown are the average of three independent determinations. Error bars for pH are not observed because they overlap with data symbols.

Figure 2:
Effect of storage time of yogurt powder on the lag time during milk acidification. Data shown are the average of three independent determinations.

Figure 3:
PCA performed on the spray-dried yogurt samples, stored at different $a_w$ and temperatures. A: without trehalose; B: with trehalose. Squares: 0TR-10/20-Ai or 4TR-10/20-Ai; circles: 0TR-38-33 or 4TR-38-33; triangles: 0TR-38-Ai or 4TR-38-Ai. Full black symbols (squares, circles and triangles): samples stored for one week; full gray symbols: samples stored for 6 weeks; empty symbols: samples stored for 12 weeks.

Figure 4:
1-D Loading plots in PC1 and PC2, performed on the FTIR spectra of powder yogurts without (A) and with (B) trehalose.

Figure 5:
Predicted vs reference values for lag time.
Table 1- Description of abbreviated codes used for stored samples of dried yogurt

| Code          | Trehalose (%) | Storage temperature (ºC) | a_w of dried yogurt during storage |
|---------------|---------------|--------------------------|-----------------------------------|
| 0TR*          | 0             | -                        | -                                 |
| 4TR(**)       | 4             | -                        | -                                 |
| 0TR-10/20-Ai-Wn | 0            | 10 ↔ 20 (***)            | 0.21                              |
| 4TR-10/20-Ai-Wn | 4            | 10 ↔ 20                  | 0.22                              |
| 0TR-38-Ai-Wn  | 0             | 38                       | 0.21                              |
| 4TR-38-Ai-Wn  | 4             | 38                       | 0.22                              |
| 0TR-38-33-Wn  | 0             | 38                       | 0.33                              |
| 4TR-38-33-Wn  | 4             | 38                       | 0.33                              |

(*) 0TR = 0% of trehalose addition
(**) 4TR = 4% of trehalose addition
(***) Cyclic temperatures: 12 hours cycles (20ºC and 10ºC) resembling the daily variation of temperature in a temperate climate region.
Ai = As is; in relation to the water activity of the powder
Wn: weeks stored, n = 1 to 12
Figure 1:
Figure 2:

![Graph showing lag time (minutes) vs. storage time (weeks) for different conditions. The graph includes multiple lines representing different storage conditions, such as 0TR-38-33, 4TR-38-33, 0TR-10/20-Ai, 4TR-10/20-Ai, 0TR-38-Ai, and 4TR-38-Ai. The x-axis represents storage time in weeks, ranging from 0 to 12, and the y-axis represents lag time in minutes, ranging from 0 to 350. The graph shows variations in lag time across different storage conditions and times.]
Figure 3:
Figure 4:
Figure 5: