# Evaluation of fermentative activity of lactic cultures for dehydrated yogurt with the use of different additives

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**ABSTRACT**

**Objective**: To evaluate the fermentative activity of dehydrated lactic cultures with the use of different additives and vacuum desiccation, using yogurt as model system.

**Design/methodology/approach**: The yogurt was elaborated with commercial lactic cultures (YF-L705 Yo-Flex CHR HANSEN) and whole milk incubated at 42 °C for 4 h. Yogurt was centrifuged at 6,000 rpm/15 min/4 °C. The supernatant was eliminated and with the precipitate, 6 treatments were established by addition of additives: T1, Without additive, T2, Glycerol, T3, Calcium carbonate, T4, Yeast extract, T5, Glycerol, and T6, Glycerol, Calcium carbonate and Yeast extract; non-dehydrated and freeze-dried yogurt was used as control: T7 and T8, respectively. The precipitate of the treatments with additives was dehydrated in a silica gel in a desiccator and under vacuum conditions. The weight loss was recorded at 0, 24, 48, 72 and 96 h. The precipitate with dehydrated additives was used as milk inoculates for yogurt elaboration. The change of pH was recorded at 0, 1, 2, 3, 4, 5, 6, 7, 8 and 24 h. With the pH and the fermentation time, a model was established to present the change curve in fermentation pH and the Fourier transform infrared spectroscopy (FTIR).

**Results**: The drying time to constant weight was 3 days. The fermentation pH change curve was a Boltzman sigmoidal function and analysis of variance was conducted with its parameters to assess the different fermentation speeds of the different treatments. The dehydrated cultures with Yeast Extract and Calcium Carbonate are associated with a higher fermentation activity of the milk ($p \leq 0.05$). The yogurts manufactured with fresh cultures take 4 to 5 h to ferment and the dehydrated ones take more than 10 h. The infrared spectra showed that the quality of the yogurts produced with fresh or dry cultures are similar, which agrees with other studies.

**Limitations on study/implications**: The dehydrated inoculated with the additives can be used to make yogurt with similar quality as to when inoculate with fresh culture is used, with the disadvantage of the fermentation time being longer. It is possible that this methodology can be used to dehydrate other inoculates based on lactic bacteria, but their effectiveness would have to be assessed experimentally.

**Findings/conclusions**: This study shows an alternative method to dehydrate lactic bacteria in the laboratory with equipment of relatively easy access for any laboratory.

**Keywords**: yogurt, drying, additives, lactic bacteria.

**Citation**: Colorado-Campos, J. A., Bucio-Galindo, A., García-Alamilla, P. & Ramos-Juárez, J. A. (2023). Evaluation of fermentative activity of lactic cultures for dehydrated yogurt with the use of different additive *Agro Productividad*. https://doi.org/10.32854/agrop.v16i8.2436

**Academic Editors**: Jorge Cadena Iñiguez and Lucero del Mar Ruiz Posadas

**Received**: November 17, 2022.
**Accepted**: July 25, 2023.
**Published on-line**: September 25, 2023.

*Agro Productividad, 16*(8). August. 2023. pp: 67-75.



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## INTRODUCTION

Acid-lactic bacteria are Gram-positive, non-sporulated, negative catalases, microaerophilic which produce lactic acid based on the fermentation of glucose or other carbohydrates (Mora-Villalobos *et al.*, 2020). They are broadly distributed in nature. One of the main uses is to elaborate fermented lactic products, such as cheese, jocoque, yogurt (Walstra *et al.*, 2005; Ramírez *et al.*, 2011), or products used in animal feeding such as vitafert (Lazo-Pérez *et al.*, 2017, Citalan Cifuentes *et al.*, 2016; Castillo Mercado *et al.*, 2020), which is an inoculate of lactobacillus from yogurt that is useful for the production of lactic acid and the reduction of pH in fermented foods.

Yogurt inhibits the pathogens transmitted by foods due to the action of organic acids (primarily lactic acid) and other compounds such as hydrogen peroxide, acetaldehyde, and bacteriocins that can act as bactericide/bacteriostatic agents (Papadopoulou *et al.*, 2021).

Historically, fermented lactic foods are prepared by inoculating fresh milk with a portion of a previously manufactured product that contains lactic bacteria and other fermented microorganisms. The inoculates used in the industrial production of many fermented products are dehydrated lactic cultures, which have functional advantages compared to fresh cultures, since in addition to being concentrated, they present low volume which makes their frozen storage and commercialization easy, and in addition, they can have several years of useful life.

To process 200 L of milk to yogurt, the inoculate must consist in 5 to 10 L of recently elaborated yogurt, but if a freeze-dried inoculate from some prestigious commercial brand is used, with 20 g of concentrated freeze-dried culture, it is enough to process the 200 L of milk. However, dehydrated inoculates are expensive for their application in animal production, particularly if they are freeze-dried; thus, for example, the cost of 20 g of the freeze-dried inoculate to process 200 L of milk for yogurt is approximately US$10.00.

To obtain inoculates, there are several drying processes (freeze drying and convection drying). Convection drying is a less costly method than freeze drying, and it is used to preserve lactic bacteria (Kets, 1997; Linders, 1996) with the advantage that it can be done at a small scale and with equipment that is relatively easy to get or fabricate. However, convection drying is a somewhat unknown method.

Water is the main constituent of bacteria, and their viability and fermentative capacity is decreased with dehydration; however, their viability can be improved with the addition of agents that protect from osmotic stress (Broeckx *et al.*, 2016). Those protective agents are compatible solutes such as betaine, carnitine, ectoines, sucrose and trehalose that make bacteria more resistant to dehydration (Kets, 1997; Prasad *et al.* 2003); these solutes can accumulate at high levels without interfering with cellular processes. Many compatible solutes have shown effectiveness in enzyme stabilization, under conditions of high concentrations of salt, high temperatures, freezing or thawing and with dehydration processes (Poolman *et al.*, 1998). The design of convection drying protocols can be evaluated using lactic yogurt bacteria as a model system. The fermentative activity of dehydrated inoculates in the substrate to ferment is an indirect, fast and practical measure to understand the functionality of the bacteria (Linders, 1996). FTIR spectroscopy can be used to evaluate the spectral signatures of many products, such as dairy products (Rodríguez-Saona and

Allendorf, 2011), and also to perform exploratory studies of changes in quality during the useful life of the products. In the case of yogurt, FTIR spectroscopy has been used to evaluate the changes during its shelf life; however, it has not been used in the assessment of the fermentative activity of dehydrated bacteria. Based on this, the use of different additives to preserve the fermentative activity of dehydrated lactic cultures was assessed in vacuum desiccation using yogurt as model system.

## MATERIALS AND METHODS

The yogurt was formulated pasteurized cow milk of the brand Lala® (fat 3.3% g; protein 3.1% g; lactose 4.80%) inoculated with freeze-dried culture (0.1 g bacteria L milk) of the brand YF-L705 Yo-Flex® CHR HANSEN and incubated at a temperature of 44 to 45 °C in a FELISA FE-377 thermal bath for a period of 4 to 5 h. Then, it was stored in refrigeration for conservation before using it. The yogurt was centrifuged at 6000 rpm at 4 °C during 15 min; then the lactic sediment was collected, which contained lactic bacteria that were used for the experiments, and the supernatant was eliminated.

### Treatments and experimental design

In a completely random design, six treatments (additives) with three repetitions were distributed. The treatments were: T1: without additive, T2: Glycerol, T3: Calcium carbonate, T4: Yeast extract, T5: Glycerol and Calcium carbonate, T6: Glycerol, Calcium carbonate and Yeast extract. The experimental unit consisted of aluminum trays of 5 cm diameter. Each tray was added with 2 g of the yogurt precipitate and 0.2 mL of the additive according to the treatment (Figure 1). The treatments were dried during 5 days

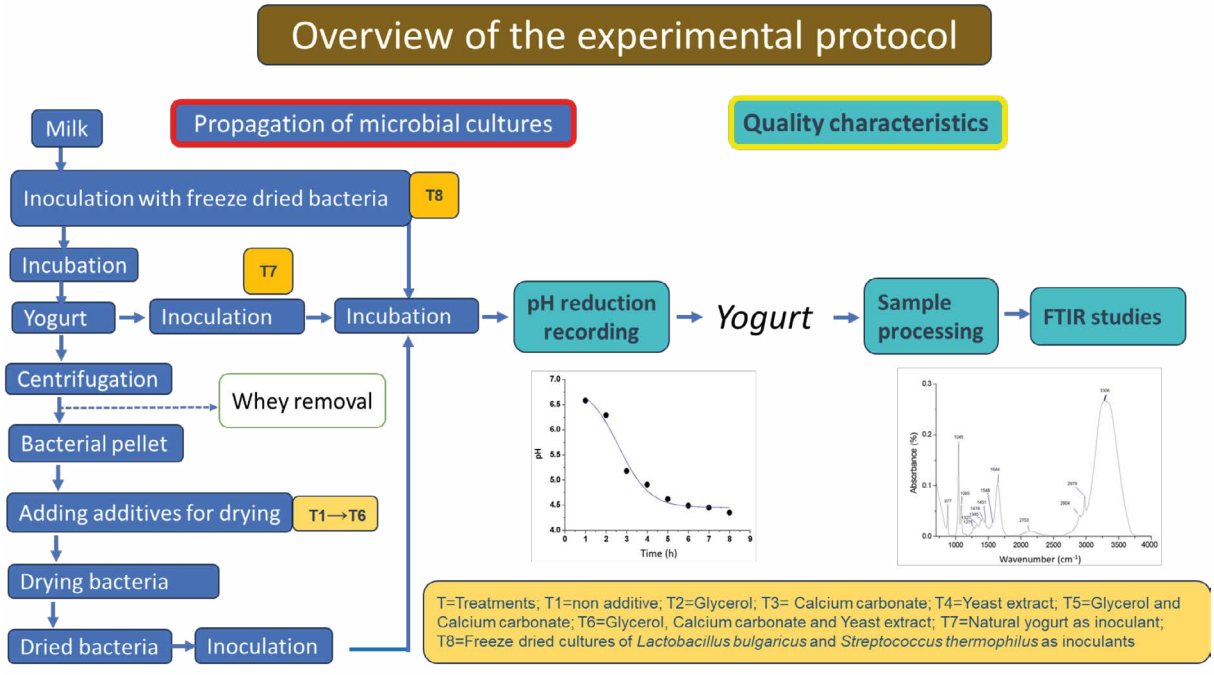


**Figure 1**. Flow diagram of the experiment and additives used in the experiment.

in a desiccator under vacuum conditions (Nalgene NALGE07022). The weight loss of the sample in the desiccator was measured on day 0, 1, 2, 3, 4 and 5. Fermentative activity tests of the treatments studied were conducted at 72 h of drying, since no changes were observed after that time in the weight of the samples. The dehydrated product (0.5 g) was used to inoculate 50 mL of whole milk (composition described earlier) and the pH was measured at 0, 1, 2, 3, 4, 5, 6, 7 and 8 h with a potentiometer (HANNA HI-2211, United States of America). In the fermentation tests, fresh yogurt inoculates (Treatment 7) and a freeze-dried culture (Treatment 8) were used as control groups. Treatment 7 was inoculated with 2 g of natural yogurt elaborated with the freeze-dried culture (without centrifuge and without dehydrating). Treatment 8 was inoculated with 0.005 g of a freeze-dried culture.

The pH values found at different times were graphed to model and monitor the fermentation in milk using the Boltzman equation with the Microcal Origin software version 6 (Navrátil *et al.*, 2004), adjusted in the following way:

$$pH = \frac{pH_{initial} - pH_{final}}{1 + e^{(x-x_0)/d_x}} + pH_{final}$$

The values of the constants ($pH_{initial}$, $pH_{final}$, $x_0$, $d_x$) were estimated through the non-linear adjustment curves of the Microcal Origin 6.0 software (Northampton, MA, USA). In the Boltzmann sigmoidal function, Y denotes the pH that changes in time t. The parameters $pH_{initial}$ and $pH_{final}$ correspond to the positions of two asymptotes of the curve $Y$(t) (superior and inferior), $x_0$ is half the time that passes between $pH_{initial}$ and the $pH_{final}$ (Figure 2). From this, it is inferred that $2x_0$ is the time at which yogurt reaches the finalization pH.

## FTIR spectral signature

Following the procedures by Subramanian and Rodríguez-Saona (2009), 1 mL of yogurt was placed in a vial, centrifuged at 14,000 rpm for 3 min and the precipitate was discarded. Of the supernatant, 0.5 mL was taken and placed in a new vial, and after that

$$Y = pH = \frac{Initial\ pH - Final\ pH}{1 + e^{(x-x_0)/d_x}} + Final\ pH$$

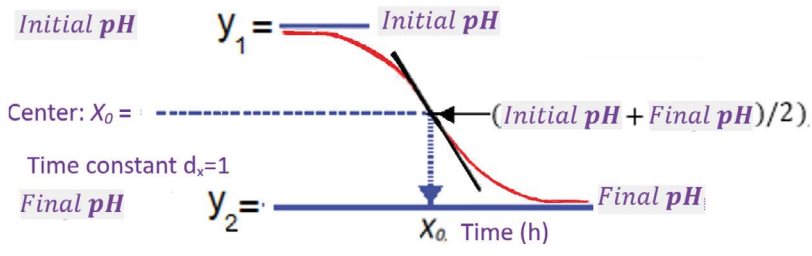


**Figure 2**. Boltzman function, modified from Microcal origin version 6.

0.5 mL of distilled water was added plus 0.5 mL of chloroform to separate the complex fat in the sample from the other portions of the sample; it was centrifuged again under the same conditions mentioned in previous steps, discarding the most viscous part that the chloroform contained with dissolved fats. Finally, 200 $\mu$L of the supernatant was collected and 200 $\mu$L of absolute ethanol from the last mixture was added to precipitate complex proteins of the supernatant; 100 $\mu$L was taken in a new vial to freeze it until its reading, using the trial sample in a FTIR analysis. The vibrational analysis was carried out by using Fourier transform infrared spectrophotometer (FTIR) (Perkin Elmer, Frontier, EUA), using diamond attenuated total reflectance (ATR) controlled with software for Windows©, in the wave number interval of 400 to 4000 $cm^{-1}$ with a resolution of 1 $cm^{-1}$ and 32 scan., A pre-treatment baseline correction and softening in the Spectrum software of the FTIR Perkin-Elmer spectrophotometer (Perkin-Elmer) was conducted with the spectra obtained. The data of the spectra were exported in ASCII format and analyzed using MagicPlot.

## RESULTS AND DISCUSSION

Yogurt was produced with the typical fermentation kinetics with duration of 4 or 5 h incubating at 45 °C (Soukoulis *et al.*, 2007). With the yogurt centrifuge and the removal of the aqueous part, the precipitate was left with approximately 25% of the original weight of the yogurt. Precipitate drying on the trays was done for three days, and after the third day, the samples did not lose weight anymore (Figure 3).

The portions of milk inoculated with the dehydrated yogurt inoculates presented pH reduction profiles where the three phases present in the entire fermentation process are distinguished: 1) latency phase, lag or start of pH decrease, 2) logarithmic phase (quick decrease of the pH), and 3) final decrease of pH until reaching stable values. Two examples of fermentation kinetics are presented in Figure 4. The finalization time of the fermentation differed significantly between freeze-dried and dehydrated inoculates. These data could be modelled in a predictive way with the Boltzman function (Figure 4). The coefficients of determination reached are very high, indicating a good adjustment of the data to the model selected within the whole interval of values of the variables. This model has already been

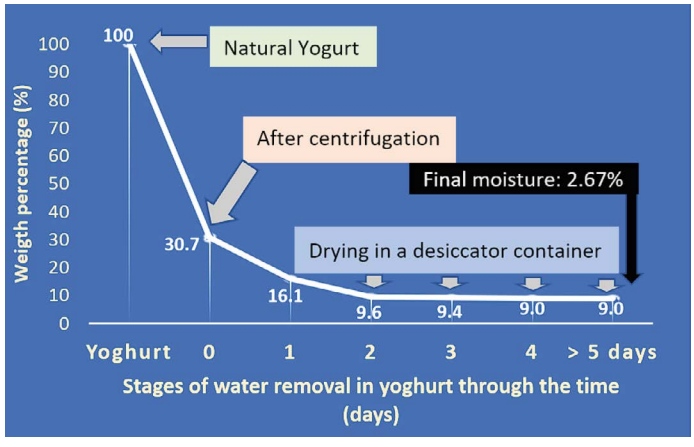


**Figure 3**. Stages of water removal from the yogurt.

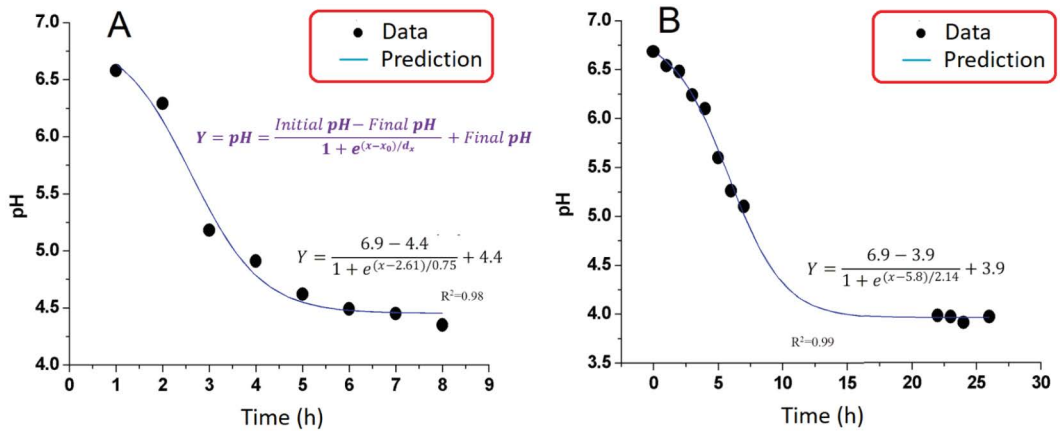


**Figure 4**. pH reduction curve of the yogurt inoculated with a) freeze-dried yogurt and b) dehydrated yogurt using the yeast extract as additive.

used previously when assessing the kinetics of lactose disappearance after fermentation of the Kombucha dairy product (Kanurić *et al.*, 2018), although they have not been used to describe the pH decrease. The yogurts manufactured with fresh cultures take 4 h to ferment, and the dehydrated exceed 10 h (Figure 4).

The parameters of pH reduction kinetics of the milk, fermented with different treatments of dehydrated inoculates and fresh yogurt, based on modelling with the Boltzman function equation, are presented in Table 1. The milks inoculated with fresh and freeze-dried yogurt reduced the pH significantly in less time ($X_0$) than the other treatments (Table 1); the samples from treatment 3 and 4 followed, which contain calcium carbonate and yeast extract, respectively. $X_0$ is the mean reduction time of the pH.

The $pH_{initial}$ of the natural yogurt was significantly higher than the other treatments. It can be assumed that this value was because of interpolation of the parameters of the Boltzmann equation where a slope with great pH reduction speed of this treatment is included; and which compared to the other treatments probably does not have alkalinizing agents that can affect the inferior and superior limits of the kinetics. In the case of the $pH_{final}$, the highest values were for the treatment inoculated with freeze-dried yogurt, probably due to some effect of the inoculate and because of the way in which it is prepared

**Table 1**. Parameters of the pH reduction kinetics based on the Boltzman function.

| Experimental treatments | Initial pH | Final pH | $X_0$ | $d_x$ |
|---|---|---|---|---|
| 1) SN, without additives | 6.6a | 3.9a | 6.3cd | 1.9b |
| 2) Glycerol | 6.7a | 3.9ab | 6.8d | 2.1b |
| 3) Calcium carbonate | 6.8a | 4.0bc | 5.9c | 2.3b |
| 4) Yeast extract | 6.9a | 3.9ab | 5.8c | 2.2b |
| 5) Glycerol and Calcium carbonate | 6.8a | 4.0c | 6.2cd | 2.1b |
| 6) Glycerol, Calcium carbonate and Yeast extract | 6.7a | 4.0c | 6.8d | 2.0b |
| 7) Natural yoghurt (Unflavored and Unsweetened) | 8.1b | 4.0bc | 1.3a | 1.8b |
| 8) Starter cultures for yoghrt (freeze dried). | 7.0a | 4.4d | 2.6b | 0.8a |

for its functionality. It is unknown whether it has slightly neutralizing encapsulated agents. The dehydrated cultures that contain yeast extract and calcium carbonate are associated with a fast fermentation activity of the milk ($p \leq 0.05$).

The FTIR spectral data of the yogurt extract samples elaborated with fresh yogurt inoculates and from one of the dehydrated yogurt samples (Treatment 4) are shown in Figure 5. The characteristic peaks of the yogurt sample manufactured with fresh cultures are similar to those of yogurts with dehydrated inoculates, and no difference is reflected in qualitative quality between the yogurt samples from the two cultures, since the spectra were similar.

Several absorption peaks of the infrared electromagnetic radiation were identified in both spectral graphs, which are characteristic in many fermented dairy products (Rodríguez-Saona *et al.*, 2006, 2017). Two peaks are characteristic of lactose at 1046 and 1086 (Rodríguez-Saona *et al.*, 2017), a spectral absorption peak at 1640 $cm^{-1}$ has been reported with an amide I belonging to the union of carboxyl and amino groups of amino acids, which in the case of this study could be derived from casein, which similar to many other proteins has been reported to have spectral peaks around 1650, 1550 and 1250 $cm^{-1}$ due to amide I, amide II and III (Hewavitharana and van Brakel, 1997; Papadopoulou *et al.*, 2021; Derrick *et al.*, 2000).

Authors such as Derrick *et al.* (2000) observed that the peaks of highest infrared absorption of casein samples are from stretching of C-H groups at 3100-2800 C-H, stretching of C=O groups at 1660-1600, flexion of C-N-H groups at 1565-1500, and flexion of C-H groups at 1480-1300. They are also very common in the samples extracted from cheeses (Subramanian and Rodriguez-Saona, 2009) and from yogurt in this study.

Mixtures of $\alpha$-casein and $\beta$-lactoglobulin have also been reported at 1650 $cm^{-1}$ (Susi, 1972) that are united through SH groups, with the resulting proteins from the milk treatment for yogurt. Other authors also say that it is a wave length where there

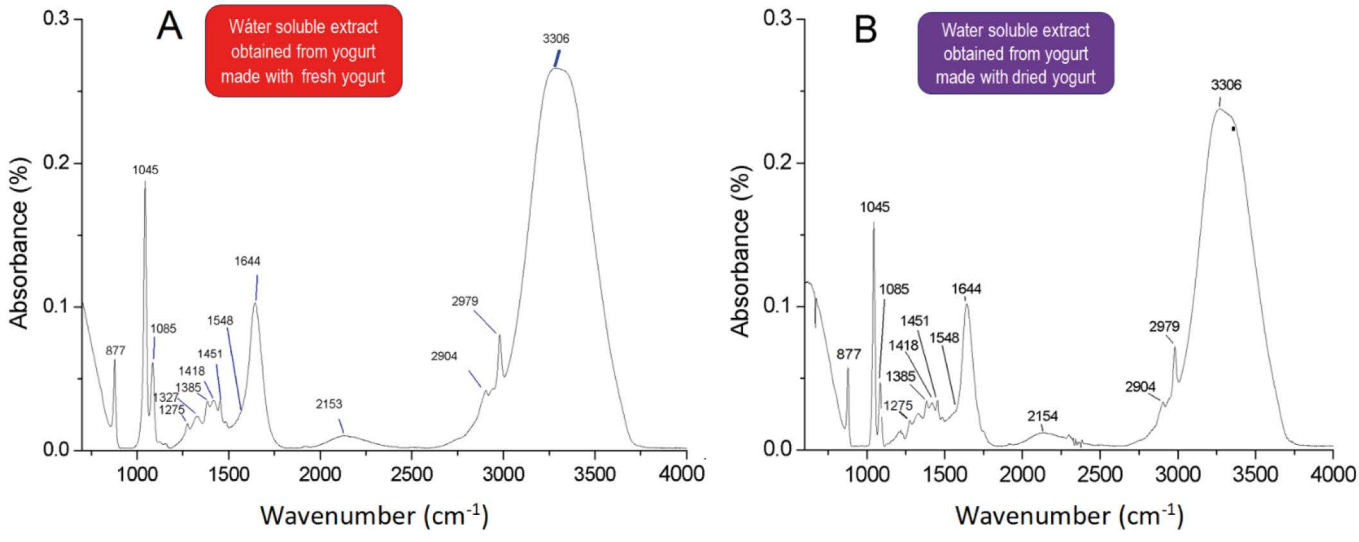


**Figure 5**. FTIR spectra of yogurt samples manufactured with fresh (A) and dehydrated (B) yogurt inoculates.

is water, and carboxyl acids from organic acids (Fagan, 2014) and water (O-H), which could be lactic acid. More recently, Karadeniz *et al.* (2021) analyzed different samples of exopolysaccharides, commonly produced by *Streptococcus thermophilus* by FTIR spectroscopy. The example showed a wide band in around 3287 $cm^{-1}$ attributed to the stretching vibration of hydroxyl groups from carbohydrates and C-H stretching around 2925 $cm^{-1}$. The band of 1638 $cm^{-1}$ is associated with the traction vibration of C=O and corresponds to the characteristic absorption of the polysaccharides. All the spectra showed typical bands of polysaccharides. All the spectra showed typical bands of polysaccharides near 1026,9 $cm^{-1}$ within the region of digital signature (1200-950 $cm^{-1}$). This is novel about convection dehydrated bacteria, their fermentative activity and the assessment of yogurt fermentation products by FTIR. It is possible for this methodology to be used to dehydrate other inoculates based on lactic bacteria, but their effectiveness would have to be evaluated experimentally. The equipment used is of relatively easy access for any laboratory.

## CONCLUSIONS

Dehydration with the additives used and vacuum desiccators allows obtaining dehydrated inoculates that can be used to make yogurt with spectral characteristics similar to when inoculation is done with fresh culture, with the disadvantage of a longer fermentation time.

## ACKNOWLEDGEMENTS

The authors wish to thank CONACyT for granting a scholarship for Master's degree studies to the first author.

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