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Development of Dried Probiotic Apple Cubes Incorporated with *Lactobacillus casei* NRRL B-442

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Abstract

This work presents the development of a probiotic dried apple snack consisting of dried apple cubes impregnated with *Lactobacillus casei* NRRL B-442. Apple cubes were impregnated with probiotic microorganisms and dried under different temperatures (10 to 60 ºC), with or without application of ultrasound. The viability of *Lactobacillus casei* in the dried apple snack was evaluated studying the effects of drying conditions and ultrasound application (as a drying enhancing technology). A mathematical model was developed to predict the drying kinetics and the inactivation of *Lactobacillus casei*. Drying and microorganism inactivation rates increased with increasing process temperature and with ultrasound application. The concentration of probiotics in the apple snacks was similar to the concentration of microorganisms in commercial probiotic dairy products when the apples were dried at 60 ºC or when ultrasound-assisted air-drying was applied, thus proving that the production of dried probiotic apple snacks is possible and technically viable.

Keywords: Probiotics; *Lactobacillus casei*; drying kinetics; ultrasound; apple.

1. INTRODUCTION

Probiotics were defined by the World Health Organization as "live microorganisms, which when administered in adequate amounts confer a health benefit on the host" (FAO & WHO, 2002). The fortification of foods with probiotic strains is interesting because of the health benefits associated to it, such as: reduction in the risk of cardiovascular disease and type 2 diabetes, anti-inflammatory potential, protection against colitis and epithelial cell damage, reduction of insulin resistance and steatosis (Bron et al., 2017; Lourens-Hattingh & Viljoen, 2001; Marco et al., 2017; Sánchez et al., 2016; Shah, Ding, Fallourd, & Leyer, 2010).

The functional foods market has grown reflecting the concern of consumers in eating foods that are healthier and that brings extra benefits to the human body. Foods containing probiotic microorganisms are among several functional foods that are currently available in the market. In 2016, the global probiotics market was worth 40 billion euros, with yoghurts and other dairy products corresponding to 90% of the probiotic sales. An 38% growth in this market has been projected between 2016 and 2021 (Euromonitor International, 2016).

Several strains of *Lactobacillus* are used as functional ingredient in food production. Most probiotic foods consist of refrigerated dairy products because they provide an environment in which most *Lactobacillus* strains survive for long periods (Havenaar, Brink, & Veld, 1992). The drawbacks to the consumption of probiotic dairy products are that these products usually needs to be kept under refrigeration and they are not indicated for persons with lactose intolerance or that need to control their cholesterol levels. Furthermore, some consumers simply do not like dairy products and would like to eat other kinds of probiotic products.

Lactobacillus casei is used in the food industry and is very popular in fermented milks. Its use has been linked to an increased production of free radicals scavenging enzymes and of pleasant fruity aroma compounds and to the reduction of undesired aldehydes, ketones and secondary esters (Zotta, Parente, & Ricciardi, 2017).

The increasing concern regarding healthy foods has increased the consumption of functional foods and has also increased the consumption of fruits and vegetables. Thus, there is a potential market for probiotic fruit products. Apples are one of the most consumed fruits in the world. They are consumed fresh, in juices and as several dried

products including snack preparations, integral breakfast and other foodstuffs (Biedrzycka & Amarowicz, 2008).

Drying brings several advantages to foods: reduction of storage cost, reduction of packaging cost, reduction of transport cost, increase of shelf life and no requirement of refrigeration (Boudhrioua, Michon, Cuvelier, & Bonazzi, 2002). Probiotics can be added to fresh foods or to dried foods. While fresh foods have shelf-life of a few weeks (e.g. yogurts), dried foods may have shelf-life of several months, e.g. milk powders (Weinbreck, Bodnár, & Marco, 2010). Thus, the development of dried probiotic fruit products is theoretically possible.

The use of ultrasound technology, especially at temperatures below 60 ºC, has been considered unfeasible for microorganism destruction because it is unable to lower the microorganism count at the 5 log reduction required for decontamination (Piyasena, Mohareb, & McKellar, 2003). Thus, ultrasound may reduce the time required to dry apple samples without inactivating the probiotic microorganisms.

Considering the importance of functional foods on human health, the benefits caused by the ingestion of probiotic microorganisms, the increase in consumption of fruits and the interest in novel functional foods, this study was carried out to evaluate the technical viability in producing a probiotic dried apple snack. To accomplish this goal, the influence of drying conditions on the viability of *Lactobacillus casei* NRRL B-442 impregnated in cubes of Granny Smith apples (*Malus sp.*), and the use of ultrasound as a drying enhancing technology were evaluated.

2. MATERIALS AND METHODS

2.1. Sample preparation

Apples (*Malus sp.* var Granny Smith) were purchased in a local market (Valencia, Spain). The fruits were washed with water, peeled, and cut into cubes of 8.7 x 8.7 x 8.7 mm using a cutting device. The samples were immediately immersed in 1% (w/w) citric acid and 2% (w/w) ascorbic acid solution, for 10 min, to prevent oxidation.

The moisture content was determined by heating in a vacuum drying oven at 70 ºC and 800 mbar for 48h, according to the AOAC method 934.06 (AOAC, 1997). This analysis was carried out in triplicate.

2.2. Microorganism and Inoculum Preparation

The *Lactobacillus casei* strain NRRL B-442, obtained from the NRRL Culture Collection (Peoria, Illinois, USA), was used in this study. The lyophilized cells were cultivated in the Man, Rogosa and Sharpe (MRS) broth (Himedia, India) at 37 °C for 8 h (Pereira, Maciel, & Rodrigues, 2011). The initial pH of the culture medium was adjusted to 6.5 with H_3PO_4. This culture was mixed to a 50% v/v sterile glycerol solution. The glycerol stock culture was frozen (-20 °C) and stored in sterile screw cap tubes containing 8 mL of the culture suspension.

The inoculum was prepared from the stock culture of *Lactobacillus casei*. The culture was propagated in 100 mL of MRS broth at 37 °C for 6 h to obtain an initial cell concentration of approximately 9.0 log counting forming units per milliliter (log CFU/mL).

The MRS broth has a very strong taste, which could interfere with the taste of the dried probiotic apple cubes. Thus, the *Lactobacillus* cells were removed from the MRS broth and suspended in a neutral solution. To accomplish this transfer, the *Lactobacillus* culture was centrifuged at 4 °C and 10000 rpm for 5 min. The supernatant containing the MRS broth was removed and the centrifuged cells were suspended in 90 mL of a sterile saline solution (0.9% w/v of NaCl). This culture was used as inoculum for the apple cubes, and contained approximately 9.0 log counting forming units per milliliter (log CFU/mL).

The inoculation was carried out immersing the fresh apple samples (25 g) for 5 min at room temperature in a Becker containing 100 mL of the probiotic suspension. Gentle agitation (50 rpm) was applied during immersion to ensure a more uniform incorporation of microorganisms in all apple cubes. The inoculation was carried out prior to drying because it may allow the diffusion of microorganisms into the samples and can enhance the adhesion of the microorganisms to the surface of the samples.

2.3. Drying experiments

The inoculated apple samples (25 g) were dried at 10, 40 and 60 ºC, with or without application of ultrasound. Air velocity was set at 1 m/s. These conditions were chosen considering that 10 ºC is the temperature used to store *Lactobacillus*, that 60 ºC

is a traditional temperature for food drying, and that 1 m/s is also a traditional velocity for food drying (Jangam, Law, & Mujumdar, 2010).

The drying experiments carried out at 40 ºC and 60 ºC were done in an ultrasound-assisted convective drier comprised of an aluminum-vibrating cylinder (internal diameter 10 cm, height 31 cm, and thickness 1 cm) driven by a piezoelectric composite transducer (21.7 kHz) used to apply ultrasound (García-Pérez, Cárcel, Fuente-Blanco & Riera, 2006). The drying experiments carried out at 10 ºC were done in a low temperature ultrasound-assisted convective drier, like the hot air ultrasound-assisted convective drier, but inserted in a controlled temperature chamber. Construction details of the drier can be found in García-Pérez et al. (2012a).

In both cases, the apple cubes (25 g) were put inside the aluminum-vibrating cylinder, in which hot or cold air passed through. Air velocity (± 0.1 m/s) was measured using a digital vane anemometer (Wilh Lambrech model 1468). Air relative humidity (± 2.5%) and air temperature (± 0.5 ºC) were measured using a digital thermohygrometer (KDK Galtech sensor).

The ultrasound-assisted experiments were carried out using an acoustic power of 20.5 kW/m^3, which is defined as the electric power supplied to the ultrasonic transducer divided by the volume of the drying chamber. Ultrasound was applied continuously during drying. The experiments carried out without application of ultrasound were done in the same equipment, but without turning on the ultrasonic generator.

The weight of the samples was measured every 5 min, allowing to calculate the moisture content of the samples. Drying was carried out until the sample weight reduced by 80%. Prior experiments showed that the water activity of the samples, at this weight reduction level, is below 0.6 (condition that prevents microorganism growth). The experiments at each drying condition were done in triplicate.

2.4. Drying and cell inactivation modeling

A diffusion model applied to cubic geometry was proposed to model the drying kinetics. The model assumed uniform temperature inside the cubes and negligible shrinkage (Equation 1), which could be assumed because of the small size of the samples (less than 1 cm^3) and the small changes in size during the drying process (less than 10% in volume). These assumptions were based on experimental and simulation data presented by Magalhães (2016) who studied the humidity and temperature profiles

in apple cubes drying under the same conditions used in this study; and found that the temperature gradient inside the samples was lower than 2 ºC.

The external resistance to mass transfer was considered and incorporated into the model as one of the boundary conditions required to solve the model (Equations 2 to 4) (Crank, 1975). The external resistance to mass transfer was considered and calculated because the incorporation of microorganisms in the apple samples can impose a higher resistance to moisture diffusion from the surface of the fruit sample to the air. Then, Equations 2 to 4 reflect how the moisture flows by diffusion towards the solid surface and then how it moves by convection to the drying air. The symmetry of the cubic samples was used as the second boundary condition of the model (Equation 5).

$$\frac{\partial W_p(x,y,z,t)}{\partial t} = D_e \left(\frac{\partial^2 W_p(x,y,z,t)}{\partial x^2} + \frac{\partial^2 W_p(x,y,z,t)}{\partial y^2} + \frac{\partial^2 W_p(x,y,z,t)}{\partial z^2} \right) \quad (1)$$

$$-D_e \rho_{ds} \frac{\partial W_p(L,y,z,t)}{\partial x} = k[a_w(L,y,z,t) - \varphi_{air}] \text{ at } t > 0 \text{ and } x = L \quad (2)$$

$$-D_e \rho_{ds} \frac{\partial W_p(x,L,z,t)}{\partial y} = k[a_w(x,L,z,t) - \varphi_{air}] \text{ at } t > 0 \text{ and } y = L \quad (3)$$

$$-D_e \rho_{ds} \frac{\partial W_p(x,y,L,t)}{\partial z} = k[a_w(x,y,L,t) - \varphi_{air}] \text{ at } t > 0 \text{ and } z = L \quad (4)$$

$$\frac{\partial W_p(0,y,z,t)}{\partial x} = 0 \; ; \frac{\partial W_p(x,0,z,t)}{\partial y} = 0 \; ; \frac{\partial W_p(x,y,0,t)}{\partial z} = 0 \quad (5)$$

where, a_w is the water activity (dimensionless) on the surface of the sample, D_e is the water effective diffusivity (m^2/s), k is the external mass transfer coefficient ($kg/m^2.s$), L is the cube size (m), t is the time (s), W_p is the water content in the samples (dry basis - dimensionless), x is the width position (m), y is the length position (m) and z is the height position (m), ρ_{ds} is the sample density (kg/m^3), φ_{air} is the air humidity (kg_{water}/kg_{air}). a_w was estimated from the moisture content and the sorption isotherm data reported in the literature (Veltchev & Menkov, 2000)

A first order kinetic model was used to model the viability of the probiotic microorganism during drying. The deactivation rate of the microorganism (k_d) was modeled as a function of temperature.

$$\frac{dN_d}{dt} = -k_d N_d \quad (7)$$

where k_d is the microorganism death rate constant (min^{-1}), N_d is the number of microorganisms (CFU/g).

It must be stated that during the development of the model, the microorganism availability was tentatively modeled as a function of temperature and moisture content in the samples. All simulations showed that the microorganism was only sensitive to process time and temperature and that the effect of moisture content was not significant in the model.

The model was solved applying an implicit finite difference method (García-Pérez, Ortuño, Puig, Carcel, & Perez-Munuera, 2012b), for which a computational algorithm was written in MATLAB v7.9.0 (MathWorks Inc., USA). The moisture content of the samples (experimental data) was used as initial condition for the simulations. The moisture content was assumed uniform in the sample.

The estimation of the effective water diffusivity (D_e) and the external mass transfer coefficient (k) was done fitting these parameters on the experimental profiles. The Levenberg-Marquardt method was used to estimate the model parameters.

The percentage of explained variance ($\%VAR$) was calculated to evaluate the goodness of fits to the experimental data (Equation 6).

$$\%VAR = \left(1 - \frac{S_{xy}^2}{S_y^2}\right) 100 \quad (6)$$

where, S_{xy} is the estimated standard deviation and S_y is the sample standard deviation.

2.5. Number of Viable Cells

The number of viable cells in the apple cubes was determined placing 3 g of sample in a stomacher bag containing 18 mL of sterile saline solution and mixing it by one cycle. Serial decimal dilutions of each sample were plated in triplicate on MRS Agar (Himedia, India) and incubated at 37 °C for 72 h. The number of viable cells was determined as colony forming units (CFU) and the results were expressed as CFU/g (Pereira et al., 2011; Silveira, Fontes, Guilherme, Fernandes, & Rodrigues, 2010).

3. RESULTS AND DISCUSSION

3.1. Drying experiments

The inoculated apple cubes presented an average initial moisture content of 8.7 ± 0.1 kg $_{water}$/kg $_{dry\ matter}$ (d.m.). Figure 1 shows the drying kinetics of apple cubes carried out at different drying temperatures (10, 40 and 60 °C) with or without ultrasound application. Only the falling rate step of drying was observed when drying the apple cubes.

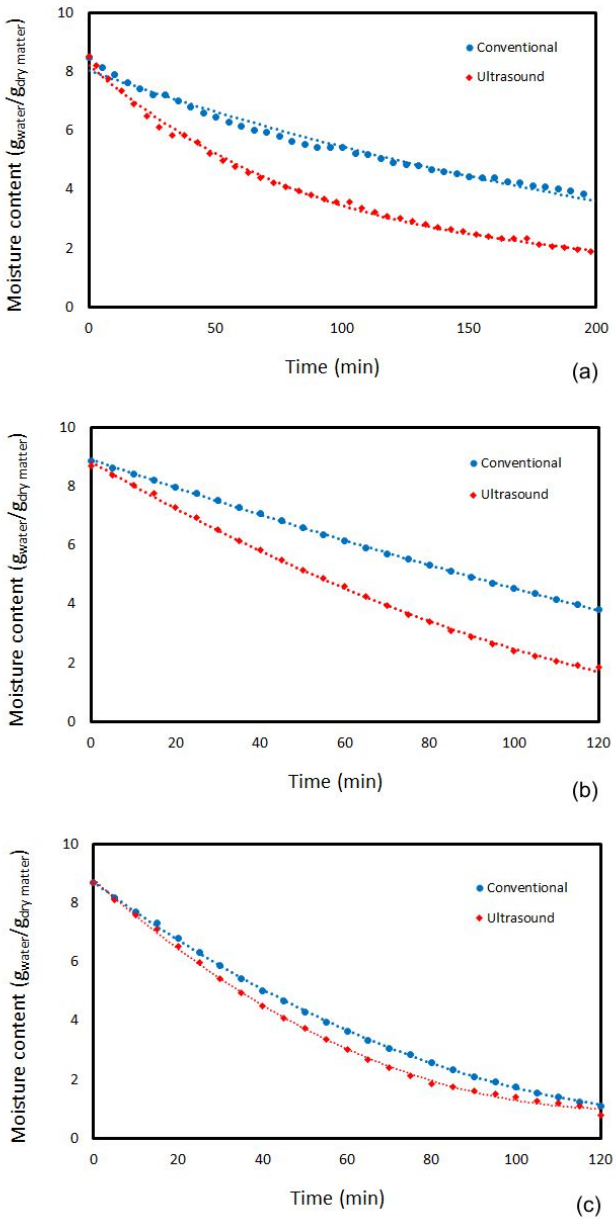

Figure 1. Experimental drying kinetics of inoculated apples samples at 10 (a), 40 (b), and 60 ºC (c) with and without ultrasound application (20.5 kW/m^3).

Drying was faster with increasing drying air temperature, as expected for drying processes. Higher temperatures increased both water mobility inside the product and energy available in the medium for water evaporation facilitating the drying process. After 2 h of convective drying, the moisture content of the samples dried at 10, 40 and 60 ºC was 4.3 ± 0.3, 3.9 ± 0.3 and 1.4 ± 0.3 kg $_{water}$/kg $_{d.m.}$ respectively. The relative humidity of the air was between 6 and 11% during the drying experiments.

The application of ultrasound increased the drying rate in all cases, significantly reducing the drying time. After 2 h of ultrasound-assisted drying, the moisture content of the samples was 2.8 ± 0.2, 1.9 ± 0.2 and 0.9 ± 0.2 kg $_{water}$/kg $_{d.m.}$, respectively for the samples dried at 10, 40 and 60 ºC. The mechanical effects of ultrasound (sponge effect and micro-stirring at interfaces) were the main responsible for increasing the drying rate (Fernandes, Rodrigues, Cárcel, & García-Pérez, 2016; Gallego-Juárez, Rodríguez-Corral, Gálvez-Moraleda, & Yang, 1999).

3.2. Drying kinetic modeling

The drying kinetics of the inoculated apple cubes were modeled applying the diffusion model (Equations 1 and 2). The proposed model fitted the data adequately, providing a percentage of explained variance (%VAR) higher than 99.7% for all operating conditions used in this work (Table 1).

Table 1. Effective water diffusivity and mass transfer coefficient identified during drying of inoculated apple samples.

Temperature (ºC)	D_e (10^9 m^2/s)	k (10^3 kg water/m^2/s)	VAR (%)
Conventional convective drying			
10	0.48 ± 0.02	2.66 ± 0.10	99.7
40	1.24 ± 0.08	2.70 ± 0.05	99.9
60	2.13 ± 0.11	3.56 ± 0.16	99.9
Ultrasound-assisted convective drying			
10	0.92 ± 0.02	5.99 ± 0.22	99.7
40	2.33 ± 0.11	4.21 ± 0.18	99.9
60	2.80 ± 0.13	6.31 ± 0.35	99.9

The influence of temperature in the effective diffusivity was modeled using Arrhenius equation and its influence is presented in Equations 9 and 10.

$$D_e(conv) = 9.77 \times 10^{-6} exp\left(-\frac{2807.8}{RT}\right) \quad (9)$$

$$D_e(US) = 2.08 \times 10^{-6} exp\left(-\frac{2171.9}{RT}\right) \quad (10)$$

The effective diffusivity of the experiments carried out at 60 ºC was 4.4 times greater than the values obtained at 10ºC, for the conventional convective air-drying. The mass transfer coefficient also increased with increasing drying temperature. These trends were expected because it is common knowledge that the energy available for water mobility and for water evaporation increases at higher temperatures resulting in a higher drying rate. Similar effect has been reported previously by several authors (Nascimento, Mulet, Ascheri, Carvalho, & Cárcel, 2016; Srikiatden & Roberts, 2006).

Ultrasound application increased the water effective diffusivity and the mass transfer coefficient (Table 1). The application of ultrasound increased the effective diffusivity by 91.7, 87.9 and 31.5%, respectively for drying temperatures of 10, 40 and 60 ºC. Similar relationship between ultrasound effects and drying temperature was observed with the mass transfer coefficient. The *k* coefficient was 77% higher when ultrasound was applied at 60 ºC and 125% higher at 10 ºC. Thus, the application of ultrasound provided the drying process with additional energy, which was more significant at low drying temperatures (10 ºC) than at higher drying temperatures (60 ºC).

The influence of temperature in the external mass transfer was also modeled using Arrhenius equation and its influence is shown in Equations 11 and 12.

$$k(conv) = 1.45 \times 10^{-2} exp\left(-\frac{491.3}{RT}\right) \quad (11)$$

$$k(US) = 8.47 \times 10^{-3} exp\left(-\frac{98.1}{RT}\right) \quad (12)$$

The changes on the values of effective diffusivity and mass transfer coefficient suggest that ultrasound had a greater effect on external transport than on the internal transport. These results corroborate with other works that have reported that the influence of power ultrasound on the internal resistance to mass transfer is heavily

dependent on the internal structure of the material (Fernandes, Gallão, & Rodrigues, 2009; Fernandes, Rodrigues, Law, & Mujumdar, 2010; Garcia-Noguera, Weller, Oliveira, Rodrigues, & Fernandes, 2010; García-Pérez, Cárcel, Riera, & Mulet, 2009; Rodrigues & Fernandes, 2007; Rodrigues, Oliveira, Gallão, & Fernandes, 2009).

The increase in the effective diffusivity and in the mass transfer coefficient results in a faster drying process, which can be important for the development of the dried probiotic apple snacks. However, the effect of temperature and ultrasound on the degradation of the probiotic needs to be evaluated to determine the optimal operating condition for the process and to determine if there are upper and lower limits for the operating conditions to ensure the production of a probiotic snack.

The water effective diffusivity obtained herein for inoculated apples was higher than the values reported for beetroot (0.3 to 0.9×10^{10} m^2/s) (Vallespir, Cárcel, Marra, Eim, & Simal, 2017), which is expected given the higher porosity of apples; but it was within the same order of the effective diffusivity of pineapples (1.9 to 3.7×10^{10} m^2/s) (Correa, Rasia, Mulet, & Cárcel, 2017), that also has a porosity like apples. The mass transfer coefficient, however, was lower than the value reported for beetroot (21.8 to 32.4×10^4 kg/m^2s) and pineapples (47.0 to 80.0×10^4 kg/m^2s), which may be due to the higher mass transfer resistance that may be imposed by the prebiotic cells that were incorporated into the apple samples.

3.3. Viability of the probiotic microorganism

The initial content of microorganisms in the inoculated apples samples was $3.16\times10^8 \pm 2.20\times10^7$ CFU/g (8.5 ± 0.5 log CFU/g). Drying affected the count of *Lactobacillus casei* in the apple samples and a reduction of the initial value of CFU/g was observed.

Drying carried out at the lowest temperature (10 ºC) presented a better preservation of the microorganisms than drying at higher temperatures (Figure 2). For example, after 100 min of drying at 60 ºC without ultrasound application, the number of microorganisms (CFU/g) was reduced from $3.16\times10^8 \pm 2.20\times10^7$ to $2.92\times10^5 \pm 1.87\times10^4$ CFU/g (8.5 ± 0.1 to 6.3 ± 0.3 log CFU/g). Similar reduction was observed in the experiments carried out at 40 ºC.

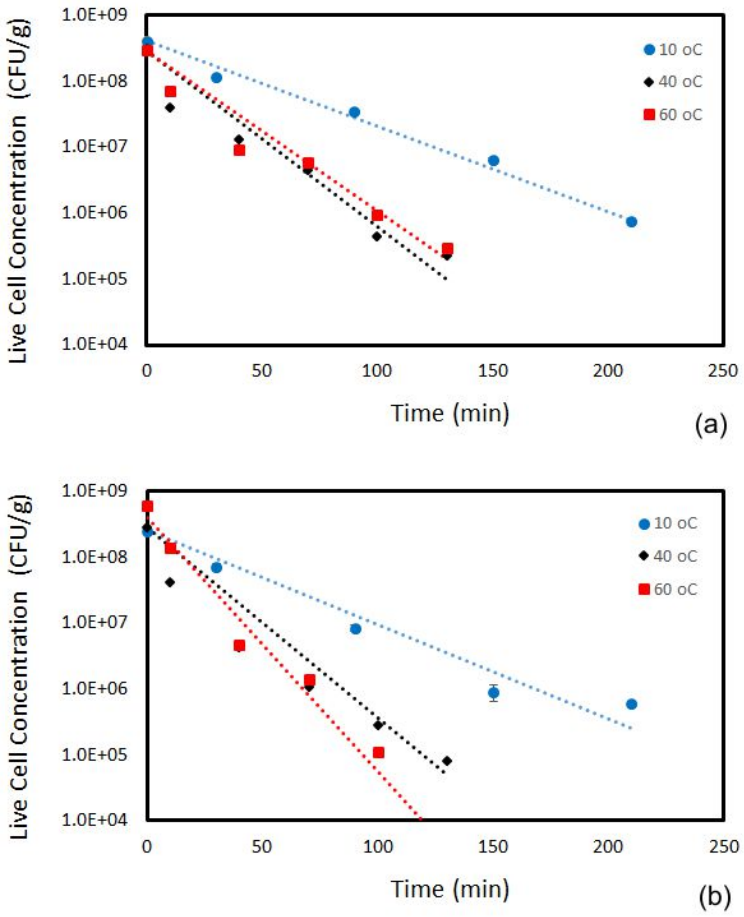

Figure 2. Live *Lactobacillus casei* cell count as a function of time during drying at different temperatures by the conventional (a) and ultrasound-assisted (b) air drying.

The method of inoculation of the microorganisms in the samples (by immersion of the samples in the probiotic suspension) has partially contributed to the reduction of microorganism count. As it was carried out, part of the microorganisms was located at the surface of the samples and was directly subjected to heating. *Lactobacillus casei* is a heat sensitive microorganism, thus any thermal treatment will impact the viability of this microorganism and its viability will be dictated by the drying temperature and by the time spent at this temperature.

Having all the microorganism inside the sample would minimize this problem because the microorganism would be protected by the food matrix against the heated air. We tried this solution, but it showed to be impractical because the suspension of microorganism needed to be injected in each sample of apple. In an industrial application, this injection method in such small samples would be impractical.

The kinetics for the microorganism decay was modeled and the values of the kinetic rate constants for *Lactobacillus casei* inactivation are presented in Table 2. The model proved satisfactory for the process carried out with ultrasound and for the process carried out without ultrasound application. The model fitted adequately the experimental data, resulting in an R^2 higher than 0.98 and the F-test analysis showed that the model was significant at a 95% level of confidence.

Table 2. Inactivation rate of *Lactobacillus casei* as a function of temperature and air-drying technology.

Temperature (°C)	**Inactivation rate - k_d** (10^2 min^{-1})	**VAR** (%)
Conventional convective drying		
10	3.01 ± 0.03	99.7
40	5.82 ± 0.08	99.5
60	5.40 ± 0.11	98.9
Ultrasound-assisted convective drying		
10	3.16 ± 0.02	99.6
40	6.42 ± 0.07	98.9
60	9.98 ± 0.22	98.2

An increase in the process temperature increased the inactivation rate of the microorganism, which is in accordance to the fact that *Lactobacillus casei* is a heat-sensitive microorganism. The mechanical effects of ultrasound caused more damage to the microorganisms and affected the CFU count of the samples. At low temperature (10 °C), the influence of ultrasound could be considered negligible, but the influence was significant at higher temperatures.

Although the temperature plays a major role in the inactivation rate of the microorganism, its effect on the drying time has also to be considered. The microorganism loss of viability will be a function of process temperature and processing time. An increase in process temperature will lead to a higher loss of viability of the microorganism, as will an increase in processing time. The viability of the cells as a function of drying time and temperature is presented in Figure 3.

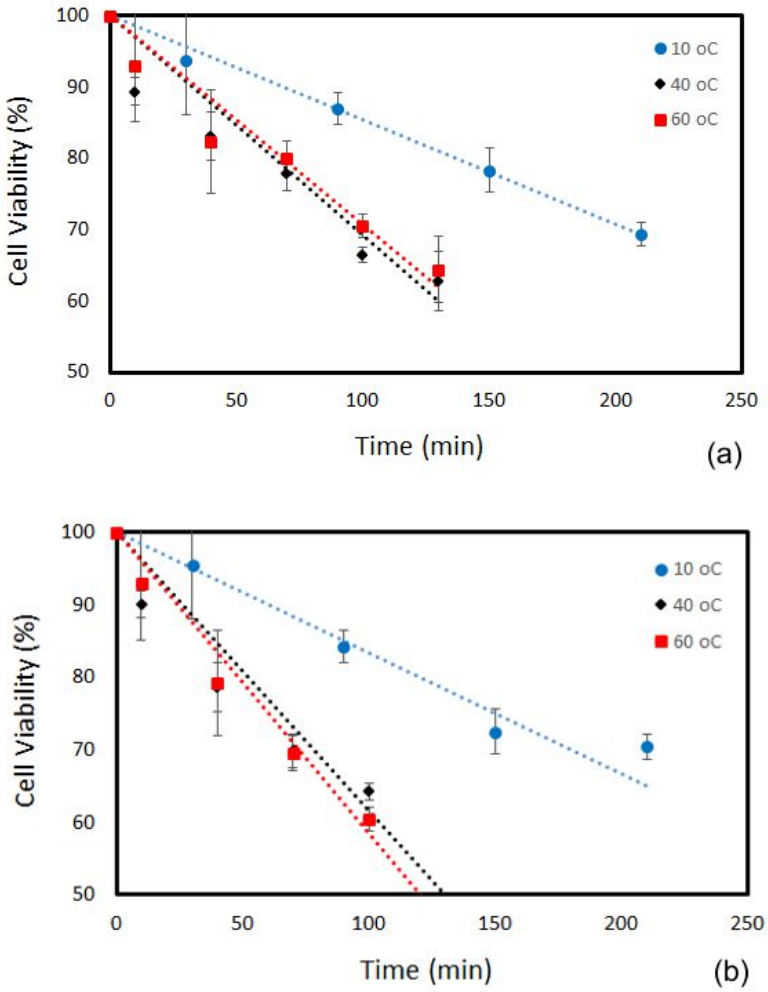

Figure 3. Evolution of cell viability of *Lactobacillus casei* inoculated in fresh apple samples as a function of time during drying at different temperatures by the conventional (a) and ultrasound-assisted (b) air drying.

Figure 4 presents the microorganism viability at the end of the drying process, marked by the reduction on the weight of the sample by 80% and a water activity of 0.35 ± 0.05. At 60 ºC, the high temperature could have a major impact on the microorganism survival, but the thermal damages were limited because of the short drying process. The cell viability under this temperature (86 ± 2%) was the highest among the temperatures tested in this work. The use of ultrasound-assisted air drying improved the microorganism viability. The reduction on the drying time attained by applying ultrasound resulted in a higher probiotic content despite the higher microorganism inactivation rate. The dried probiotic apple ended with 86 ± 2% of cell

viability when ultrasound was applied while 77 ± 2% of cell viability was attained with the conventional drying process.

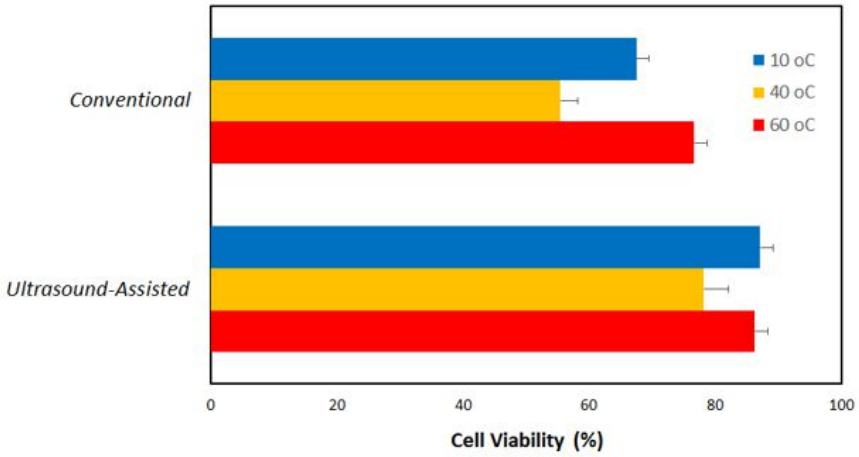

Figure 4. Cell viability of *Lactobacillus casei* inoculated in fresh apple samples at the end of drying at different temperatures by the conventional and ultrasound-assisted air drying.

The cell viability operating at 10 ºC and at 40 ºC without ultrasound application, were lower than 67%. At 10 ºC, the lower process temperature had a small influence over the microorganism survival, but the longer process time reduced the cell viability. At 40 ºC, the drying process was faster than at 10 ºC but the higher microorganism inactivation rate resulted in a higher damage to the microorganisms.

The use of ultrasound-assisted air drying improved significantly the microorganism viability at these two temperatures (10 and 40 ºC). The concentration of live cells in both conditions was 30% higher than the concentration obtained using the conventional drying process. The reduction in drying time achieved applying ultrasound was positive and the decay of probiotic microorganism was lower. The process carried out at 10 ºC retained 87 ± 2% of the initial probiotic count.

The number of viable microorganisms was above 10^6 CFU/g after drying at 60 ºC or when ultrasound was applied. This value is adopted by the food industry to label a product as probiotic (Fonteles, Costa, Jesus, & Rodrigues, 2012; Pereira, Almeida, Lima, Costa, & Rodrigues, 2014; Sheehan, Ross, & Fitzgerald, 2007), thus all apple samples dried under these conditions could be labeled as probiotic.

The samples dried at 10 and 40 ºC by the conventional drying method presented a significant lower number of CFU/g and the final product would not be labelled probiotic, unless a higher initial CFU count is used in the probiotic immersion solution.

The viability of microorganisms improved with the application of ultrasound, thus from a technical point of view, the use of ultrasound has a major advantage towards the final product.

Probiotic products must maintain its functionality and the viability of the microorganisms throughout the storage period (Sheehan et al., 2007). Also, the presence of sufficient number of viable bacterial cells is required to provide health benefits (Reid, Beuerman, Heinemann, & Bruce, 2001). Within the operating conditions studied herein, the apple samples dried at 60 ºC and the samples dried using ultrasound-assisted air drying achieved the conditions to be considered a stable dry product (marked by a moisture content of 0.24 kg water/kg d.m. and water activity of 0.4) and maintained a microorganism count required to be considered probiotic (5.4 ± 0.2 log CFU/g applying conventional drying and 5.3 ± 0.1 log CFU/g applying ultrasound-assisted drying). These results prove that dry apple cubes can be considered as a viable vehicle for probiotic cultures when the adequate drying conditions are applied.

To become a good probiotic, the product should have a high number of viable cells, which must be maintained during the storage of the food and which must survive to the gastrointestinal conditions and reach alive to the intestine to assure a genuine probiotic effect. Many studies have been carried out with *Lactobacillus casei* in these aspects, and the option to use this microorganism was due to their resistance when stored under dried condition ($a_w < 0.6$) and because they survive in great numbers in the gastrointestinal conditions (Marco et al., 2017; Sanders & Marco, 2010).

4. CONCLUSIONS

Drying reduced the number of viable cells of *Lactobacillus casei* inoculated in apple samples, but drying at 60 ºC or applying ultrasound provided a dried product with the required number of viable cells to produce a probiotic product (10^6 CFU/g). The reduction on cell viability depended on temperature and ultrasound application during drying. The application of ultrasound under these conditions helped to reduce the drying time without significantly affecting the viability.

Apple cubes impregnated with *Lactobacillus casei* probiotic presented good cell viability at the end of drying. The probiotic dried fruit could be used as raw material in the food industry or be consumed as a snack. An intake of about 100 g of dried probiotic apple would impart in an intake of about 100 million (CFU) of the probiotic bacteria.

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