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AGAVE JUICE AS AN AGENT FOR PROBIOTIC ENCAPSULATION BY SPRAY DRYING

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ABSTRACT Functional properties of natural food sources create alternatives to develop new products. Dietary fiber promotes several health benefits and the agave juice is a natural resource rich in it. Besides, probiotics are beneficial microorganisms which enrich the naturally present bacteria in human gut. The aim of this study is to evaluate the viability of encapsulated probiotic (*Lactobacillus casei*) when agave juice mixed with maltodextrin is used as an encapsulating agent by spray drying. The viability of *L. casei* was evaluated before and after the spray drying process as well as during storage for 20 days at room temperature. Two different air inlet temperatures, 140°C and 150°C and solution flow rates, 10 and 15 mL/min were evaluated for the spray drying process. Growth of *L. casei* in a model system with and without the encapsulation was also evaluated. Moisture content, a_w and bulk density were determined for the encapsulated probiotic powder. The outlet air temperature for the different spray drying trials was between 65 and 90°C. The final physical properties of the powders were moisture 0,79-3,21 %, bulk density 283-366 kg/m^3 and a_w 0,27-0,33. The spray drying process conditions which produced the highest survival population of microorganisms ($7x10^7$ cfu/g) were 140°C and 10 mL/min. The microorganisms survival of this encapsulated probiotic after 20 days of storage was $9.71x10^5$ cfu/g. The obtained encapsulated probiotic with agave juice-maltodextrin can be a suitable ingredient to develop new functional food products.

Keywords: agave-juice, probiotics, prebiotics, encapsulation

INTRODUCTION Food industry is actually concerned in providing novel products with nutritional characteristics, since consumer attention goes toward functional food. Among food products and ingredient improvements, several studies have been done with probiotics and prebiotics (Brassart and Schiffrin, 2000; Simmering and Blaut, 2001; Chen and Chen, 2007; Anal and Singh, 2007).

Probiotics are defined as “live microbial feed supplements which beneficially affect the host animal by improving its intestinal balance” (Fuller, 1989). However most of the probiotic products are dairy products, fermented or not. Probiotic incorporation to these products can be done as initial starter for fermented products or encapsulated (Simmering and Blaut, 2001). Encapsulation has been an useful tool to improve the delivery of

bioactive compounds, particularly probiotics (Madene et al., 2006; Champagne and Fustier, 2007). Different microencapsulating techniques have been proven to increase the survival of probiotics by up to 90%; these can be classified in two main groups: physical/mechanical and chemical (Kebary et al., 1996; Madene et al., 2006). Among the physical or mechanical techniques are: spray drying, spray chilling, extrusion, and fluidised bed; while among the chemical techniques are simple and complex coacervation, as well as molecular inclusion. Spray drying is most widely used in the pharmaceutical and food industry for probiotic encapsulation due to its inherent attributes such as high production rates and relatively low operational cost (Gibbs et al., 1999).

Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or limited number of bacterial species present in the colon (Gibson and Roberfroid, 1995; Simmering and Blaut, 2001). This concept arose from the observation that inulin and fructo-oligosaccharides (FOS) selectively stimulate the growth of *Bifidobacterium* and *Lactobacillus* which are considered to be beneficial for human health. Many charbohydrates have been reported to exert a prebiotic effect such as FOS, galacto-oligosacchraides and lactulose (Ziemer and Gibson, 1998; Sanz et al., 2006). FOS are commonly found in vegetables, spices and fruits (artichoke, chicory, asparagus, garlic, onion, cereals, banana, citric peels) which are part of the daily diet in humans since many years ago (AAOCC, 2001). Fructans can be present in plants up to 24% of their mass (Chacón-Villalobos, 2006). Several studies have demonstrate that Agave species are rich in fructans (Mancilla-Margalli and Lopez, 2006; Ortiz-Basurto et al., 2008; Urías-Silvas et al., 2008). Even if agave-juice also called "aguamiel" is primarly used for *tequila* production (mexican destilled alcoholic beverage), due to its functional properties, this may be used to add nutritional value to other kind of products. Actually, food manufacturers are promoting the combination of both type of ingredients, pro- and prebiotics in a single product (Simmering and Blaut, 2001).

One way of improving new products, is using natural resources as encapsulating agents for probiotic encapsulation. Among studies with different carriers demonstrating functional properties, it has been found that some of them are high hygroscopic, due to its molecular structure; therefore it is suggested to be used in combination with other common encapsulating agents which may help to their stability during storage (Langrish and Chiou, 2008; Chiou and Langrish, 2007). Besides, due to the high carbohydrates (including FOS) content of some natural resources, some problems during spray drying may be presented due to its Tg, causing sticking problems in the spray chamber (Ananta et al., 2005); an equilibrium between inlet air temperature for spray drying and high probiotic viability after this process must be found.

The aim of this study is to evaluate the viability of encapsulated probiotic (*Lactobacillus casei*) when agave juice mixed with maltodextrin is used as an encapsulating agent by spray drying.

MATERIALS AND METHODS

Encapsulating agent preparation of agave-juice and maltrodextrin Agave-juice was a mixture of three different agave species juice, *Agave atrovierens*, *A. salmiana* and *A. mapisaga*, recovered by a local agriculture group "Tlachiqueros de la Mañana" from San

Mateo Ozolco, Puebla, Mexico. To prepare the encapsulating solution, 25g of maltodextrin 10DE powder (Globe 19100, CPIngredientes, Mexico) was added to the agave-juice and mixed with a magnetic stirrer at room temperature (25°C). The prepared solution of agave-juice and maltodextrin was characterized measuring the relative density (ρ_L = [kg/m^3]) by the method 962.37 of the AOAC (1995), with Fisher picnometers, and the cinematic viscosity (μ_L = [cP]) using a Cannon-Fenske viscosimeter (Mod. 350-152-I, Cannon Instrument Co., U.S.A.). Moisture content was determined by gravimetric difference, with two-stage water evaporation, submitting the prepared encapsulation solution to a steam bath before drying in a vacuum oven (15 mm Hg, 70°C, 3h).

Probiotic bacteria cultivation and preparation prior encapsulation The strain of *L. casei* NRRL B-1922 used in this study was donated by the National Center for Agricultural Utilization Research of the USDA. This strain was cultivated in MRS broth (Becton-Dickinson Co., U.S.A) at 37°C for 36h, and then it was centrifuged at 10,000 rpm for 10 min. The pellet (microbial mass) recovered was re-suspended in 2mL of destilled and sterile water and added to the encapsulating agent solution (agave-juice-maltodextrin) prepared previously. Before and after spray drying this solution and during storage, the microbial count was done, preparing selected serial dilutions to inoculate them on MRS agar plates using the Spriral Counter Autoplate 4000 (Spiral Biotech, Exothec, U.S.A). Plates were placed in desiccators to generate anaerobic conditions, applying vacuum, and incubated at 37°C 48h or 72 h prior to scan count (QCount 3.0, Spiral Biotech, Exothec, U.S.A.).

Spray drying microencapsulation of probiotics Encapsulation was carried out in a mini-spray dryer (B290, BÜCHI Labortechnik, Switzerland), fixing the nozzle air flux rate at 414-473 L/h, and settling the solution flux rate to 10 or 15 mL/min, with inlet air temperature of 140 or 150°C. The pipe used to spray the encapsulation agent solution wtih the probiotic was cleaned with a chloride aqueous solution at 5% (p/v) and sterile water, previous to each trial. Once the inlet air temperature was reached to the settled condition, it was maintained during 10 min, then distilled sterile water was sprayed (at the settled flux rate condition) during 30 min, to diminish the temperature of the spray dryer chamber (Rodríguez-Martínez et al., 2009). Finally, the encapsulating agent solution inoculated with *L. casei* pellet, was sprayed.

Powder encapsulated probiotic characterization and storage As soon as the encapsulated probiotic powder was obtained, moisture content was determined by gravimetric difference after dried at 70°C in a vacuum oven after 3 h; a_w was measured with an hygrometer (Aqualab, Decagon Devices, U.S.A.), and the bulk density (ρ_p = [kg/m^3]) was determined, weighting the powder occupying 10 mL in a probe. Then, the encapsulated powder was poured in glass bottles, each containing 1 g of powder, and sealed hermetically and stored at 25°C, microbial count was determined for 20 days, taking samples every two days.

Fermentation test of the non-encapsulated and encapsulated probiotic Two model broths were prepared based in the MRS broth composition without dextrose, one of them inoculated with the encapsulated probiotic powder (spray dried at 140°C and 15 mL/min) and the other one was kept as a control which was inoculated with non-encapsulated *L. casei*, inoculating 1x10^7 ufc/g aproximately. Microbial count was periodically determined in both broths, as well as acidity (% lactic acid) during 36 h. The growth kinetic of *L.*

casei encapsulated or not, were modeled with the modified Gompertz equation (1), were N is the microbial population at time t, No is the initial microbial population, A parameter is the maximum growth [cfu/g], μ parameter is the maximum growth rate [cfu/g.h], λ parameter is the lag time [h] and e = exp(1).

$$\ln(N/No) = A\exp\left\{-\exp\left(\frac{\mu \cdot e}{A}(\lambda - t)+1\right)\right\} \quad (1)$$

Statistical Analysis The outlet air temperature (T_{out}) of the spray drying trials, as well as the microbial survival were evaluated by ANOVA, and paired comparison test of Fisher with 95% of confidence, using Minitab (v.14, LEAD Technologies Inc., U.S.A.). Furthermore, a paired t-test was also applied to evaluate *L. casei* growth in the model broths when added encapsulated or not.

RESULTS AND DISCUSSION The encapsulating agent solution prepared with agave juice-maltodextrin resulted in a non-clear solution, slightly green, with a moisture content of 69.6 % wt.b and with good fluid properties for spray drying, with low viscosity, μ_L of 1,34 cP and ρ_L of 1130 kg/m^3. The different trials for spray drying were chosen in aim to obtain low outlet air temperatures to enhance microbial survival, and final moisture content < 4% wt.b in the encapsulated powder to assure good physical properties avoiding agglomeration or crystallisation during storage (Gardiner et al., 2000; Boza et al., 2004; Ananta et al., 2005).

Characterisation of the encapsulated probiotic powder The encapsulated probiotic powder resulted in a white fine powder with an average bulk density ρ_p of 326.5 kg/m^3 ± 36.6 which is characteristic when using maltodextrin for spray drying encapsulation (Boza et al., 2004; Fuchs et al., 2006). The average moisture content of 1.92% ± 1.02 (wt.b) and average a_w 0.28 ± 0.08, for the encapsulated probiotic were also suitable values, to assure good physical properties during storage (Ananta et al., 2005) (Table I). Chiou and Langrish (2007) suggest that for spray drying encapsulated powder using natural citric fibers, powders must be kept at an ambient relative humidity under 50% to prevent microorganisms growth; they even conclude that natural fibers, due to its hygroscopic properties, must be used in combination with other powders to achieve moisture stability. In fact, in our study, no powder agglomeration occurred during storage at 25°C for the different encapsulated probiotic powders using agave-juice-maltodextrin matrix.

Probiotic survival after spray drying and during storage The final outlet air temperature (T_{out}) for the different trials was between 66 to 90°C in which the trial carried out with 140°C of inlet air temperature (T_{in}) and flux solution rate (Q_L) of 15 mL/min showed the lowest T_{out}, and significantly different ($p<0.05$) from the rest of the trial (Table II). The pellet added to the encapsulating solution before spray drying

Table I. Physicochemical properties of powders with *L. casei* encapsulated in an agave-juice-maltodextrin matrix by spray drying

Spray drying conditions		Moisture content (% wt.b)	a_w	Bulk density (kg/m^3)
Q_L (mL/min)	T_{in} (°C)			
15	150	2.06 ± 0.94	0.27 ± 0.13	320.7 ± 0.7
15	140	3.32 ± 0.16	0.33 ± 0.01	282.9 ± 0.6
10	140	1.23 ± 0.23	0.29 ± 0.13	355.9 ± 0.6
10	150	1.08 ± 0.41	0.23 ± 0.07	366.1 ± 0.8

presented an initial probiotic population average (N_o) of $5.94x10^9$ cfu/g, and the average of *L. casei* survival (log N/N_o x 100) was 73.5%, resulting in a final microbial population above $1x10^7$ cfu/g which is the recommended for probiotic products. The highest probiotic survival of 78 % was obtained with T_{in} of 140°C and Q_L of 10 mL/min, however, the statistical analysis of the probiotic survival after spray drying showed that there was no significance difference ($p>0.05$) among the different spray drying operating conditions (Table II). Gardiner et al (2002) studied the temperature effect when encapsulating *L. paracasei* and *L. slivarius* with skim milk and found out that probiotic survival decreases when increasing the outlet air temperature (T_{out}) for spray drying, ranging from 97% at T_{out} of 70-75°C to 0% at 120°C. Besides, the termorresistance for spray drying process also varied (11%) for the different *Lactobacillus* species studied. Annata et al. (2005) obtained survival of *L. rahnmnosus* of 60% when spray drying with T_{out} of 70°C, when using skim milk added with prebiotic substances as Raftilose®P95 (oligofructose from chicory inulin) and polydextrose.

Table II. Microbial reductions, survival and outlet air temperature (T_{out}) when encapsulating *L. casei* in an agave-juice-maltodextrin matrix by spray drying

Spray drying conditions			Log reductions	Survival
Q_L (mL/min)	T_{in} (°C)	T_{out} (°C)	of viable cells	(Log N/No x 100)
15	150	76^a ± 1.4	2.49^a ± 0.40	74.5^a ± 2.1
15	140	66^b ± 0.1	2.28^a ± 0.27	77.3^a ± 1.6
10	140	85^c ± 2.8	1.95^a ± 0.48	78.0^a ± 2.9
10	150	90^c ± 0.7	3.50^a ± 1.54	64.3^a ± 6.6

[a,b,c] Different letters in columns are significantly different ($p<0.05$)

Microbial reduction of the different encapsulated powders of *L. casei* varied from 0.82 to 1.96 log reductions, when powders were kept during 20 days at 25°C (Figure 1). This results are pretty acceptable (fairly good) when comparing with Boza et al. (2004) study, of encapsulated *Beijerinckia spp*. using maltodextrin 10DE, obtaining 1.89-2.12 log reductions after 30 days of storage at 4°C, which suggests that a higher probiotic survival may be achieved at lower storage temperature.

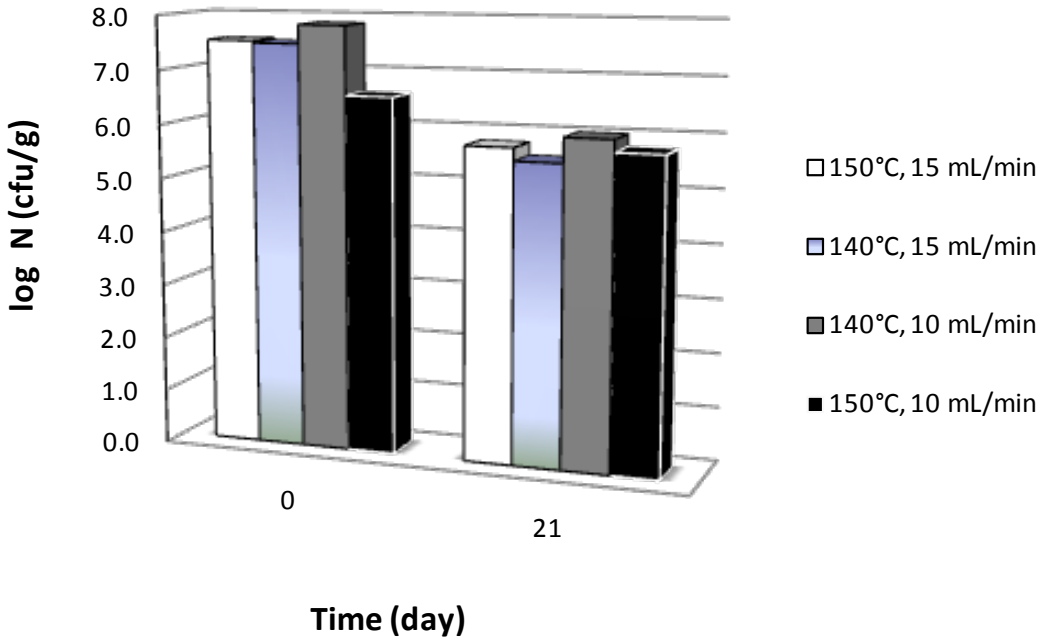

Figure 1. Survival of encapsulated *L. casei* in agave-juice-maltodextrin matrix at different spray drying conditions (inlet air temperature T_{in}, flow rate Q_L), stored at 25°C.

Viability of non-encapsulated and encapsulated probiotic Growth kinetics of the encapsulated *L. casei* was evaluated in comparison to non-encapsulated cells in a model system. It was observed that probiotic encapsulation with agave-juice-maltodextrin enhanced *L. casei* growth (Figure 2). Results from the paired t-test ($4.84 > t_{\alpha/2,8} = 2.306$) applied to the growth data, showed a significance difference ($p<0.05$). Moreover, the model of the modified Gompertz equation (1) described in a suitable way ($R^2 \sim 0.97$) the experimental data of *L. casei* growth (Table III). From the parameters of the modified Gompertz equation, the maximum growth (A) and the growth rate (μ) are 64% and 52% higher, respectively, for encapsulated *L. casei* than when it is not, demonstrating the prebiotic effect of agave-juice on the probiotic growth. However other studies are suggested to evaluate if the encapsulated probiotic powder accomplishes its functional properties in humans, as in-vitro acid and bile salt tolerance.

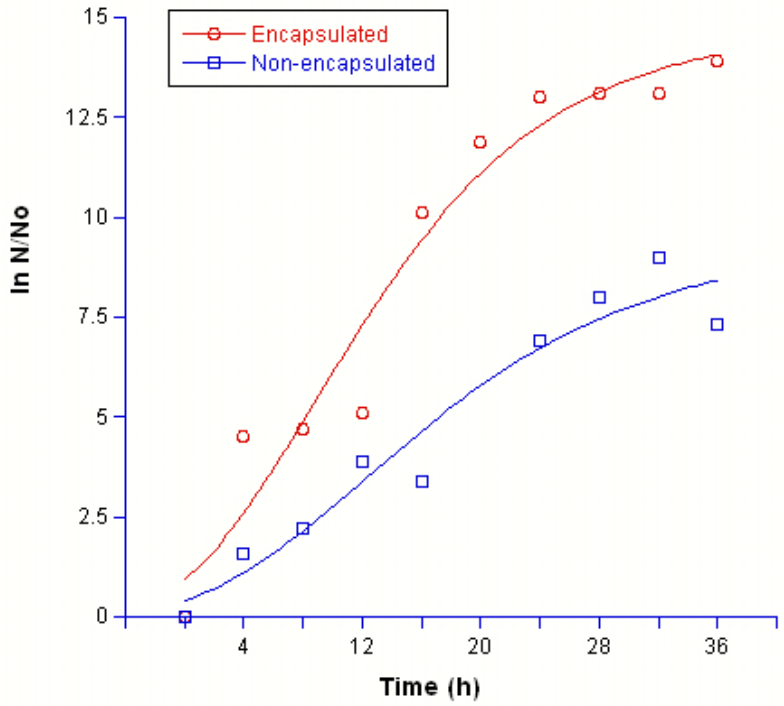

Figure 2. Growth kinetics of *L. casei* in a model system when added encapsulated with agave-juice-maltodextrin (140°C, 15 mL/min) or not, modelled with the modified Gompertz equation (1).

Table III. Parameters of the modified Gompertz model (ec. 1) for *L. casei* growth in a model system, encapsulated or not

L. casei	A ln[cfu/g]	μ [cfu/g.h]	λ [h]	R^2
Encapsulated	14.731	2.456	1.013	0.9735
Non-encapsulated	9.414	1.282	1.355	0.9700

CONCLUSION Agave-juice-maltodextrin as an encapsulating agent for *L. casei* by spray drying demonstrated to be a good protective matrix, resulting in a high microbial survival after the process and obtaining acceptable survival during storage at 25°C after 20 days. Furthermore, the growing rate of *L. casei* was enhanced when it was encapsulated with agave-juice-maltodextrin rather than non-encapsulated, which suggests

that this matrix works as prebiotic. In aim of diversifying food products with functional properties, agave-juice is a good alternative to be used as a carrier in combination with maltodextrin for probiotic encapsulation by spray drying.

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