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Research Article

Evaluation of Spray Drying Conditions in the Survival of *Bifidobacterium bifidum* in a Product Based on Whey and Mango Pulp of the Variety Magdalena River (*Mangífera indica L.)*

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Abstract: Food incorporated with probiotics provides essential nutrients to the consumers and at the same time has a positive effect on the human health. This study aimed to determine the optimal conditions for the spray drying process for achieving the maximum survival of *Bifidobacterium bifidum* ATCC 11863 in a beverage based on whey and mango pulp, using maltodextrin as an encapsulating agent. A central composite design with 22 experiments was used, in which different variables. Such as concentrations of maltodextrin (15-35%), inlet temperatures (120-160°C), outlet temperatures (65-77°C) and rotor speed (20000-28000 rpm) were tested to obtain the optimal conditions for the intended new food product. Also, physicochemical variables of the developed food product were determined, such as water activity, humidity, the angle of repose, particle size and yield of the final product. The results showed that *B. bifidum* was affected by the concentration of maltodextrin. The *B. bifidum* survival ranged from of 59.16%; the product was incorporated with 20% maltodextrin, to 76.36% probiotic survival when 25% maltodextrin more used for the food product development. In this regard, the treatment 13 presented a microbiological count of 2.3×10^{11} CFU/g, is this the highest survival percentage of *B. bifidum*.

Keywords: Encapsulation, particle size, probiotics, spray drying, water activity

INTRODUCTION

This study aimed to determine the optimal conditions during the spray drying process to achieve the maximum survival of *Bifidobacterium bifidum* in a beverage based on whey and mango pulp (*Mangíferaindica L.*), with the characteristics of being highly soluble, with low water content (including low water activity) and low hygroscopicity.

The genera Bifidobacterium comprises Gram-positive microorganisms that regularly growth in humans. These microorganisms have been recognized as probiotics due to their effect on the improvement of the intestinal flora resulted from their competition for substrates with pathogens in the gastrointestinal tract. Other benefits from these microorganisms include the activation of the immune system in humans, stabilization of the digestive system, cancer prevention, lactose digestion improvement (Depeint *et al*., 2008; Rodríguez-Huezo *et al*., 2007; Sanz *et al*., 2007; Sato and Iino, 2010). Spray drying is a process that allows the production of a food product in a dry powder form, using atomization of a dispersion or emulsion exposed to hot air in a heating chamber. The water in the treated food is evaporated immediately, allowing the food constituents to entrapped within a thin film of the encapsulating material. The particles generated present spherical geometry, with a diameter ranging from 20 to 200 μm (López Hernández, 2010).

The hot air might achieve temperatures ranging from 100 to 200°C; however, since the evaporation process is instantaneous the dried food is only exposed to moderated temperatures (around 50-60°C) preventing any thermal degradation of the treated product. In this process, the heat from the air is taken away from food constituents in a short period due to water evaporation (Gharsallaoui *et al*., 2007; López Hernández, 2010).

The microencapsulation using spray drying method is very common when encapsulating food ingredients, such as vitamins, folic acid, aromas, essential fats, probiotics, lipids, linoleic acid, vegetal oils, minerals, pigments and milk, among others (Parra Huertas, 2011; Wandrey *et al*., 2010).

Moreover, a spray-drying process has been previously used to test the activity of *B. bifidum* in several food products. In this regard, Rodríguez-Huezo *et al*. (2007) evaluated the activity of *B. bifidum* after

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submitting the microorganism to the spray drying process, using agave nectar as an encapsulating agent. Their results showed that the best condition was achieved with a bacterial counting of 1.26×10^8 CFU/g (determined immediately after the drying process) when the hot air was at 140°C. While after five weeks of storage at 4°C the bacterial count of the target microorganism was reduced to 6.0×106 CFU/g.

MATERIALS AND METHODS

Raw materials: Mango fruits (var. *Magdalena River*) it was were purchased in the municipal market (Monteria, Colombia). Saccharose, whey obtained from the elaboration of fresh cheese (Is manufactured in the pilot plant of the University of Cordoba), maltodextrin (Bell Chem International) and *Bifidobacterium bifidum* ATCC 11863 (MDM Científica) used for the beverage preparation.

Beverage elaboration: Mango fruits were sanitized using sodium hypochlorite (50 ppm). Then, the mango pulp was extracted and mixed in the proportion of 24% w/w with saccharose (7% w/w) and whey (69% w/w). The mixed ingredients were pasteurized at 72°C during 20 sec and immediately refrigerated at 4°C. The probiotic microorganism it was incorporated at a minimal concentration of 10^9 CFU/g.

Microorganism inoculum: The probiotic microorganism (in dry form) was rehydrated using 1% of the inoculum in MRS broth supplemented with 0.05% L-cysteine and incubated at 37±2°C for 24 h in anaerobic conditions. Then, the probiotic was centrifuged at 3000 rpm for 10 min and added to sterile water (Bustamante *et al*., 2006; Rodríguez-Huezo *et al*., 2007). Following this, the whey was added in a proportion of 10% (v/v) to favor the adaptation of the target microorganism. This new inoculum incubates at 37°C for 24 h. After this, the probiotic was inoculated in the whey again and incubated at 37°C, to achieve a final concentration of 10^9 CFU/g. It was determined using MRS agar in anaerobic conditions (Vinderola *et al*., 2009). Also, optical density was measured with a spectrophotometer at 660 nm (Bustamante *et al*., 2006). With this relationship was determined the amount of whey previously inoculated that was necessary to achieve a minimal microbiological count of 10^9 CFU/g de *B. bifidum.*

Spray drying process: The concentrated beverage was elaborated using the spray-drying machine (Vibrasec®, Model PASALAB 1.5). The encapsulating agent is maltodextrin (Factor A), with a concentration ranging from 15 to 35%. The inlet temperature of the hot air (Factor B) it was set from 120 to 160°C. The rotor speed (Factor C) it was used around 20000 to 28000 rpm and the outlet temperature of the hot air (after the concentration process) it was set between 65-74°C.

The survival of *B. bifidum*: Microbiological count of *B. bifidum* was determined in triplicate using MRS agar in anaerobic conditions, incubating the system at 37°C for 48 h (Vázquez and Rojas, 2009; Vinderola *et al*., 2009). The survival percentage of *B. bifidum* was determined using Eq. (1), which considers the relationship between the number of the target microorganism before the drying process (N_o) and the number of target microorganisms in the final product (N):

$$\%\, Survival\ B.bifidum = \frac{N}{N_0} x100 \quad (1)$$

Moisture content and water activity: The moisture content of each treatment was determined gravimetrically. Samples from each treatment (5 g) were dried in a muffle (Memmert®) at 105°C for 24 h. After this time, samples it was weighted in an analytic weighing scale (Anandharamakrishnan *et al*., 2008). The water activity analysis it was done in a hygrometer (NOVASINA®, model LabMaster-aw) at 25°C.

Particle size: An optical microscope (Optika Microscope®, Model B-500 ERGO) provided with a system for data acquisition (Optika Vision Lite 2.0) was used to determine particle size in the final product.

The Angle of repose (α_{rep}): This analysis it was done according to ASTM methodology for Measuring the Angle of Repose of Free-Flowing Mold Powders, which has been described previously by Gallo *et al*. (2011) and Rodas and Rousé (2010). The Eq. (2) used for this determination, in which "d" is the internal diameter of the funnel (0.7 cm), placed at a constant height (H: 3.81 cm), while "Da" is the diameter of the conical pile formed:

$$\alpha_{rep} = tan^{-1}\left[\frac{2H}{(D_a - d)}\right] \quad (2)$$

Product yield and deposit formation: These two variables were determined to utilize the percentage relation of solids in the product (recovered powder) and the solids adhered to the walls of the heating chamber regarding the solids feed at the beginning of the drying process (Fazaeli *et al*., 2012).

Statistical design and data analysis: Theexperiments wereutterly randomized, using the central composite design with six repetitions in the central point and three axial points. Every analysis is in triplicate (Table 1). Statistical design and data analysis it was done using the software Design Expert 8.0.6 and the statistical software S.A.S v.9.2, with 5% of a significant level.

Table 1: Conditions of spray drying and counting *Bifidobacterium bifidum*

Treatment	Maltodextrin (%)	I.T (°C)	S.S (rpm)	O.T (°C)	*B. bifidum* count (CFU×g^{-1}) Defore drying	*B. bifidum* count (CFU×g^{-1}) After drying	*B. bifidum* reduction
1	25	160	24000	71	1.5×10^{11}	1.5×10^{10}	0.99
2	20	150	22000	74	2.3×10^{10}	7.0×10^{9}	0.52
3	25	140	24000	77	$3,1\times10^{11}$	1.3×10^{11}	0.39
4	25	140	20000	71	1.5×10^{11}	5.7×10^{10}	0.42
5	35	140	24000	71	2.2×10^{11}	8.9×10^{10}	0.38
6	30	150	26000	68	6.2×10^{10}	2.0×10^{10}	0.50
7	25	140	24000	71	2.4×10^{11}	1.8×10^{11}	0.12
8	25	120	24000	71	9.0×10^{9}	1.8×10^{9}	0.69
9	25	140	24000	65	7.5×10^{10}	4.4×10^{10}	0.23
10	30	130	26000	74	1.3×10^{11}	3.6×10^{10}	0.54
11	25	140	28000	71	1.8×10^{11}	7.9×10^{10}	0.36
12	25	140	24000	71	5.5×10^{10}	4.2×10^{10}	0.12
13	25	140	24000	71	3.0×10^{11}	2.3×10^{11}	0.12
14	20	130	26000	68	2.1×10^{10}	6.0×10^{9}	0.55
15	30	150	22000	68	8.1×10^{9}	2.7×10^{9}	0.48
16	20	130	22000	68	1.9×10^{10}	5.4×10^{9}	0.53
17	30	130	22000	74	7.5×10^{10}	2.5×10^{10}	0.48
18	20	150	26000	74	4.5×10^{10}	1.2×10^{10}	0.56
19	25	140	24000	71	2.0×10^{11}	1.5×10^{11}	0.12
20	25	140	24000	71	1.0×10^{11}	7.8×10^{10}	0.12
21	15	140	24000	71	8.5×10^{10}	1.7×10^{10}	0.69
22	25	140	24000	71	2.1×10^{10}	1.6×10^{10}	0.12

I.T.: Inlet Temperatura; S. S.: Spraying Speed; O.T.: Outlet Temperature

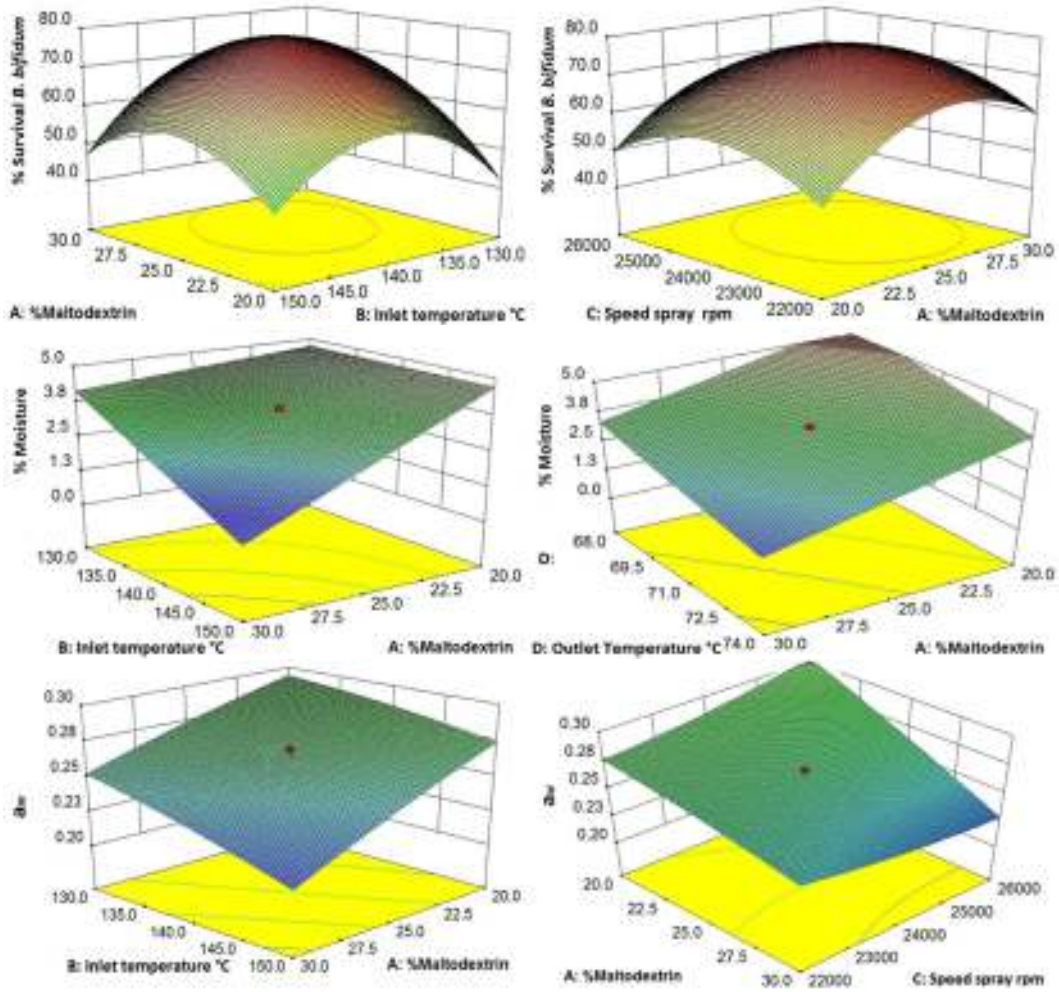

Fig. 1: Survival of *B. bifidum*, moisture content and water activity in the final product

RESULTS AND DISCUSSION

The survival of *B. bifidum*: The survival of *B. bifidum* after the spray drying process is it so in Table 1 and Fig. 1. The concentration of maltodextrin presented a significant difference ($p<0.05$) in the survival of the probiotic, as well as the temperature of the hot air at the end of the process (outlet temperature). Also, the interaction of the factors AB and their quadratic interactions were statistically significant (Table 2). The effect of maltodextrin on the survival of *B. bifidum*it evidenced by the changes in the survival percentages values obtained: from 59.16% when the beverage it incorporated with 20% maltodextrin, changed to 76.36% when with a 25% incorporation the maltodextrin. However, the survival percentage was reduced to 69.60% when this incorporated with 30%.

A similar effect it was observed with the quadratic interaction of factor A, factor D and all other interactions that were statistically significant. Thus, increased concentration of maltodextrin (from 20 to up to 25%) allowed and increased encapsulating effect and a higher survival percentage of the probiotic.

Moreover, the exposition of the beverage to the hot air at 130-140°C (inlet temperatures) and outlet temperatures (68-71°C) improved the probiotic survival, due to rapid water evaporation, which allows the formation of a protective film on the particle surface favoring the product preservation.

However, higher maltodextrin concentration (ranging from 25 to 30%) with higher temperatures at the entrance (140-150°C) and the exit of the hot air (71-74°C) resulted in minimal survival of *B. bifidum*, with values of 31.94 for treatment 6 and 33.15% for 15. Thus, the treatment 8 showed the lowest microbiological counts of *B. bifidum* (1.8×10^9 CFU/g), while the 13 presented the highest viability for this microorganism (2.3×10^{11} CFU/g).

In the treatments of the central point, the survival percentage of *B. bifidum* was 76.21±0.246%, with a mean value of 1.2×10^{11} CFU/g. Rodríguez-Huezo *et al*. (2007) reported similar results, indicating that inlet temperature of 130°C resulted in a significant reduction in the viability of *B. bifidum*. Is due to a combination of factors, such as the formation of the protective matrix generated after a slow drying process and the exposition to relative high outlet temperatures. At 155°C, the drying process could be faster, inducing to crack formation in the protective matrix, in which the oxygen can diffuse and might be toxic for microbial cells. Moderate drying temperatures (140°C) is considered as appropriate for the microencapsulation of microorganisms, with a microbiological count of 1.26×10^8 CFU/g, immediately after drying. Is due to the typical configuration of holes in the protective matrix that made it resistant to the mechanical cracking and solute diffusion. Outlet temperatures around 71°C

Table 2: Mean squares from analysis of variance of the experiment

Source of variation	DF	Survival of *B. bifidum*		Aw		Particle size	
A	1	217.98	*	5.2×10^{-3}	*	4.8×10^{-3}	*
B	1	50.58		1.7×10^{-3}	*	$1,0\times10^{-5}$	
C	1	0.22		$b.6\times10^{-5}$		0.0360	*
D	1	161.22	*	1.5×10^{-3}	*	8.9×10^{-7}	
A2	1	3710.52	*	9.4×10^{-5}		3.0×10^{-4}	*
B2	1	6555.78	*	1.3×10^{-4}		3.3×10^{-7}	
C2	1	2294.50	*	2.9×10^{-4}		4.8×10^{-7}	
D2	1	1352.08	*	2.5×10^{-4}		6.6×10^{-9}	
AB	1	69.79	*	9.0×10^{-5}		1.2×10^{-5}	
AC	1	0.25		1.1×10^{-3}	*	1.5×10^{-5}	
AD	1	37.57		9.0×10^{-5}		5.8×10^{-6}	
BC	1	0.19		9.2×10^{-4}		2.2×10^{-6}	
BD	1	57.73		1.4×10^{-4}		2.3×10^{-4}	
CD	1	2.32		1.0×10^{-3}		5.8×10^{-5}	
Error	5	0.061		3.0×10^{-5}		6.2×10^{-6}	

Source of variation	Angle of repose		Product yield		Deposit formation	
A	123.09	*	203.41	*	204.22	*
B	81.86	*	104.84	*	135.30	*
C	10.47		47.59		17.19	
D	25.96	*	70.92	*	126.25	*
A2	26.46	*	76	*	134.63	*
B2	8.32		3.50		12.99	
C2	0.12		6.46		1.20	
D2	0.023		9.37		23.17	
AB	2.58		27.65		56.57	*
AC	0.011		1.11		0.55	
AD	0.46		0.17		1.75	
BC	0.50		4.86		3.41	
BD	12.04	*	6.6×10-3		1.43	
CD	1.12		0.78		1.44	
Error	0.78		0.81		1.98	

A, maltode×trin; B, inlet temperature; C, speed spray; D, outlet temperature; *, significant at $p<0.05$

resulted in higher survival percentage of 76.21±0.11%, which was diminished (with all other variables kept as constants) with increasing concentration of this temperature. Thus, the survival it was reduced to 40.58±0.11%. Ananta *et al*. (2005), who reported a percentage of survival around 70% for *L. rhamnosus* during the spray drying process, at 70°C outlet temperature, presents similar results. The microbiological count was reduced to 60% and 10%, when the outlet temperature was 75°C and 100°C, respectively.

The reduction in the viability of *B. bifidum* was between 0.99 Log CFU/g and 0.12 Log CFU/g for the treatments at 160°C and 140°C. Similar results reported Golowczyc *et al*. (2011), when testing *L. plantarum, L. kefir* 8321 and *L. kefir* 8348. Reporting a decrease in their viability of 0.11, 0.29 and 0.70 Log CFU•g^{-1} respectively, after the drying process at 160°C (inlet temperature) and 70°C as an outlet temperature. Similarly, Paéz *et al*. (2012) indicated a reduction in the survival of *Lactobacilli* between 0.16 and 0.95 Log CFU/mL after spray drying.

Moisture content: Moisture context presented statistical significance for the factor A, B, C and D, as well as for the interaction AB at a significant level of 5% (Fig. 1). The humidity of the product diminished with increasing concentrations of maltodextrin, with the inlet temperature, the rotor speed and the outlet temperature. When the maltodextrinit was increased from 20% to 30%, the moisture content ranged from 4.7% to 2.52%. Similarly, Mishra *et al*. (2014) reported that the increased of maltodextrin resulted in low values of moisture content in the final powder product based on gooseberry juice (5.6% to 3.8% with percentages of 5 to 9 maltodextrin).

In a system treated in a spray drying, the initial water content has a significant effect on the final moisture content of the obtained powder. The addition of maltodextrin before the drying process increases the concentration of total solids and reduces the amount of available water for evaporation (Abadio *et al*., 2004). Means that the powder with less moisture content might be obtained with increasing concentrations of maltodextrin (Quek *et al*., 2007). Similar results have been reported previously (Bustos-Garza *et al*., 2013; Fang and Bhandari, 2012; Pang *et al*., 2014).

Increasing inlet (130-150°C) and outlet temperatures diminish the moisture content of the product from 4.41% to 2.80% and from 4.40% to 2.85%, respectively. Indicates that increasing temperatures result in increasing mass and heat transfer rates. Krishnaiah *et al*. (2012) found that for powder extract of Morindacitrifolia obtained by spray drying, the moisture content diminished with the increasing inlet and outlet temperatures of the process. They indicated that higher heat transference rates were achieved, providing a steeper higher temperature gradient favoring water evaporation.

Water activity (aw): The water activity obtained is shown in Fig. 1. This parameter presented significant difference for the factors A, B and D, as well as for the AC interaction. The water activity of the final product diminished with increasing concentrations of maltodextrin, with the inlet and outlet temperature. The lower values of water activity it observed when incorporating 30% maltodextrin, with outlet temperatures of 150°C (aw = 0.2133) and 74°C (aw = 0.2289). In this regard, Şahin-Nadeem *et al*. (2013) reported that the increase in the inlet temperature diminishes the water activity and the moisture content of the product. However, the increase in the concentration of encapsulation agents reduced the water activity slightly, without being significant. Similar results were reported by Fang and Bhandari (2012) and Fazaeli *et al*. (2012).

The interaction AC shows that with increasing concentration of maltodextrin and higher spraying speed, the water activity diminishes in the final product. The lowest water activity (0.228) was obtained at the conditions of 30% maltodextrin and 26000 rpm, resulting in a product with 2.25% moisture content and 0.214 mm particle diameter.

On the other hand, low concentration of maltodextrin resulted in increased values of water activity, which was also related to higher spraying speed. In this condition, water activity with a value of 0.302 it was observed when using 20% factor A and 26000 rpm. Also, this resulted in a reduced particle size (0.162 mm) and higher moisture content (4.305%). Finally, in the central point, the mean value of water activity was 0.263±0.005.

Particle size: The particle size (Fig. 2) presented significant difference ($p<0.05$) as a function of the factors A, C and A2. This parameter increased (0.2110 mm to 0.2603 mm) with increasing concentration of maltodextrin (20-30%). The availability of maltodextrin in the product increased the particle structure generating bigger particles. Pang *et al*. (2014) described the increased size of particles of the Orthosiphon extract similarly, during the spray drying with increasing concentration of maltodextrin. Likewise Depeint *et al*. (2008) reported that increased of maltodextrin (10-30%) resulted in bigger particles in açaí powder (13.27 µm a 21.35 µm) when using the spray drying process. The presence of higher concentration of maltodextrin in the feeding formulation resulted in higher viscosity and consequently in more significant drops during the spray drying process. Moreover, Carneiro *et al*. (2013) reported a wide range of particle size (0.02-160 µm) produced with maltodextrin and concentrated whey. Contributed to a better stability of the emulsions of animal feed produced with these encapsulating agents. This range in the particle size is similar to particle size

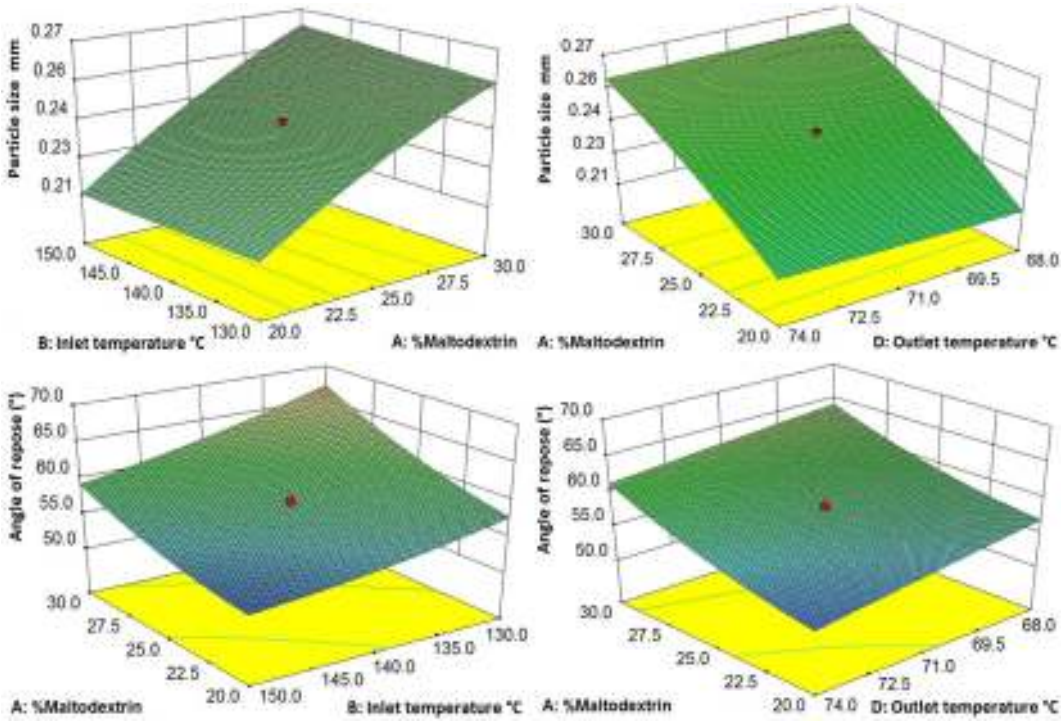

Fig. 2: Particle size and angle of repose of the final product

values reported in this study. On the other hand, the size of the particle diminished (0.2862 to 0.1916 mm) when the spraying speed it was increased. Is due to the smaller drop diameter generated when the spraying wheel was faster. Another factor might also affect the particle size, such as mass flux of the liquid, viscosity, solid content and superficial tension. However, none of them has an impact as high as the one caused by the speed of the spraying wheel (Orna, 2012).

The angle of repose: The angle of repose presented significant difference ($p<0.05$) regarding the factors A, B, D, the quadratic effect of A2 and the interaction of factors BD. Regarding maltodextrin concentration, results showed that increasing maltodextrin concentration (20-30%) resulted in a higher angle of repose of particles, increasing from 54.8° to 62.6°. Is due to the increased amount of maltodextrin, which produces in an increased particle size, diminishing the cohesion among them and therefore obtaining a higher angle of repose. In this regard, Peng *et al*. (2013) reported a mean value of 51.36° for treatments with 30% of maltodextrin as the encapsulating agent.

On the other hand, increasing inlet temperatures (130-150°C) and outlet temperatures (68-74°C) resulted in a diminished angle of repose, going from 61.48° to 55.09° and from 59.5° to 55.89°, respectively. The resulted from the increase of the factors B and D, which resulted in low moisture content and therefore the angle of repose of particles. The highest impact it was produced by the inlet temperature, which resulted in a decrease in the angle of repose of 6.39°, is this the lowest value for this parameter. The interaction BD showed a similar behavior, in which increasing values of these factors reduced the angle of repose of the product. Is useful for indicating the fluidity of any material influenced by the filling height and the material flux. It means that while the angle of repose is low, it is easier for the particles to slide through the system in which they are.

The particle size, the shape, the volume, the surface, the moisture content and the orientation of the particles that constitute the mass of the powder product affect this parameter. The angle of repose varies with the moisture content that surrounds each particle and with the effects of the superficial tension that are predominant in granular products (Ospina, 2002).

Product yield and deposit formation: En la Fig. 3 shown the results of the product yield and the deposit formation in the drying chamber. This parameter has a significant difference ($p<0.05$) concerning the factor A, B, D and the A2 interaction. Increasing maltodextrin concentrations, inlet temperature and outlet temperatures resulted in higher product yield.

In this regard, the product yield increased from 59.33% to 69.41% when the factor A went from 20 to 30%. Is due to increasing the solid content in the initial formulation, which also resulted in an increased particle size, diminishing fine particles. However, the interaction A2 indicated that high maltodextrin concentration increases the product yield although in

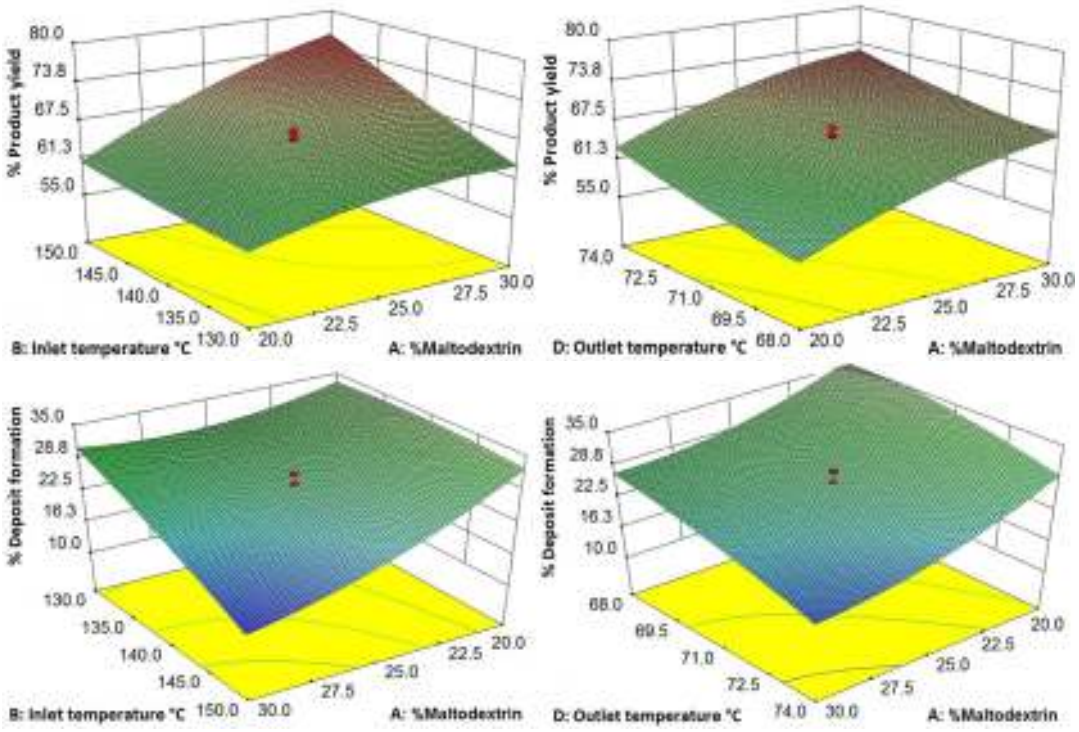

Fig. 3: Product yield and percentage of deposit formation in the drying chamber

low extent. Previous reports have shown similar effects. Igual *et al*. (2014) they have stated that the incorporation of maltodextrin and Arabic gum improved the product yield of Solanumquitoense concentrated by the spray dry, although these solutes had a negative quadratic effect. Peng *et al*. (2013) observed that the increase in maltodextrin concentration (20-30%) improved the yield of purple sweet potato flour significantly. Moreover, when the encapsulating agent used at 40%, there was no significant difference. Also, Caliskan and Nur (2013) have indicated that increased maltodextrin concentrations result in increased product yield since this agent prevents the adhesion of particles to the internal walls of the drier. However, they also indicated that the increased concentration of the encapsulating agent does not improve the product yield after achieving an optimal condition. Bustos-Garza *et al*. (2013), Fang and Bhandari (2012), Fazaeli *et al*. (2012) and Şahin-Nadeem *et al*. (2013), described similar results.

The percentage of deposit formation presented significant difference ($p<0.05$) for the factors A, B, D, the quadratic interaction D2 and the interaction of the factors AB. In this regard, when maltodextrin concentration, the inlet and outlet temperatures have increased the percentage of deposit formation was reduced. The mean value of this variable in the central point was 26.33±1.407%.

Optimal drying conditions: The best drying conditions obtained were 27.5% maltodextrin, 138.2°C inlet temperature, 68.6°C outlet temperature and spraying speed of 24908 rpm for achieving the maximal survival of *B. bifidum* (75.95%). The of water activity of 0.261, the moisture content of 3.49%, the particle size of 0.228 mm and a product yield of 66.92%.The discussion should scrutiny of the results rather than the reiteration of the Results section.

CONCLUSION

The processing variables directly influenced the survival of B. bifidum during the spray drying. In this way, the surviving of this microorganism increased with increasing maltodextrin concentrations and inlet temperature. However, when the inlet temperature was higher than 140°C, the amount of B. bifidum decreased. The water activity of the final product declinewith increasing maltodextrin concentrations, as well as heighten inlet and outlet temperatures, while the particle size and angle of repose presented an opposite behavior regarding maltodextrin concentration.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this study.

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