Vol. 16, No. 2 (2017) 347-357
Revista Mexicana de Ingeniería Química

FLUIDIZED BED DRYING OF A GRANULATED PROTOTYPE BASED ON A POTENTIAL PROBIOTIC YEAST *Meyerozyma guilliermondii*: SELECTION OF PROCESS PARAMETERS AND DRYING PROTECTANT

SECADO EN LECHO FLUIDO DE UN PROTOTIPO GRANULADO A BASE DE LA LEVADURA CON POTENCIAL PROBIÓTICO *Meyerozyma guilliermondii*: SELECCIÓN DE PARÁMETROS DE PROCESO Y PROTECTOR DE SECADO

M.L. Chaparro, E. Céspedes, M. Cruz, C.R. Castillo-Saldarriaga*, M.I. Gómez-Álvarez
Department of Bioproducts, Sede Central, Colombian Corporation of Agricultural Research, CORPOICA, Km 14 vía Mosquera, Bogotá, Colombia.
Received June 28, 2016; Accepted February 21, 2017

Abstract

Meyerozyma guilliermondii strain Lv196 has been identified as a native Colombian yeast with a high probiotic potential increasing rumen microbial efficiency at *in vitro* assays when a ruminant digestion system is simulated. Often, these types of products are supplied in a granulated form to facilitate its dosage to ruminants as cows, sheep, among others. To produce granulated bioproducts based on microorganisms, it is required to select suitable drying conditions, protectants and the adequate drying operation, decisive factors to guarantee cell viability and stability during storage. Most granulated products are dried through fluidized bed drying process reducing investment and maintenance costs, and improving mass and heat transfer decreasing operation time and the risk of product overheating. Therefore, in this work a selection of drying protectants through decision matrix was conducted based on parameters as compatibility with Lv196 and cost of protectants. The best two drying protectants were used as functional coadjuvants in the formulation of two granulated prototypes. Drying temperature, chamber pressure and the two prototypes were evaluated in an experimental design to select adequate conditions for a fluidized bed drying process. Finally, the stability of two dried granulated prototypes using selected conditions was determined. Prototype PF2 dried at 45ºC and 0.5 bar during 30 min had a final moisture of 7.84 ± 0.01% and operation yield of 98.54 ± 0.11% guaranteeing a loss of viability lower than 2.09 ± 0.01% after 15 months of storage at room temperature.

Keywords: drying process, formulation prototype, *Meyerozyma guilliermondii*, probiotic, yeast.

Resumen

Meyerozyma guilliermondii accesión Lv196 ha sido identificada como una levadura colombiana con un alto potencial probiótico al incrementar en ensayos *in vitro* la eficiencia de la microbiota ruminal. Por lo general, este tipo de productos son suministrados en forma granular para facilitar su dosificación en animales rumiantes como las vacas, ovejas, entre otros. Para producir bioproductos granulados a base de microorganismos, se requiere seleccionar condiciones, protectores y la operación de secado adecuada, factores decisivos para garantizar la viabilidad celular y estabilidad del producto durante el almacenamiento. La mayor parte de los granulados se secan por medio de una operación de lecho fluido disminuyendo costos de inversión y mantenimiento, favoreciendo los fenómenos de transferencia de masa y calor reduciendo el riesgo de sobrecalentamiento y tiempos de operación. Por lo anterior, en este trabajo se realizó una selección de protectores de secado a través de una matriz de decisión con base en dos parámetros: compatibilidad con la levadura Lv196 and costo de los protectores. Los dos mejores protectores de secado se emplearon como coadyuvantes funcionales en la formulación de dos prototipos granulados. Las condiciones para el secado en lecho fluido se determinaron usando un diseño experimental con tres variables: temperatura de secado, presión de la cámara y los dos granulados. Finalmente, se evaluó la estabilidad de los dos prototipos granulados secados bajo las condiciones seleccionadas. El prototipo PF2 secado a 45ºC and 0.5 bar durante 30 min tuvo una humedad final de 7.84 ± 0.01% y rendimiento de 98.54 ± 0.11%, garantizando una pérdida de viabilidad menor a 2.09 ± 0.01% luego de 15 meses de almacenamiento a temperatura ambiente.

Palabras clave: proceso de secado, prototipo de formulación, *Meyerozyma guilliermondii*, probiótico, levadura.

* *Corresponding author. E-mail*: mail: crcastillo@corpoica.org.co; crcastillos@unal.edu.co
Tel. 57-1-422-7300 Ext. 1561.

1 Introduction

Yeasts have demonstrated a great potential in the biotechnology industry, especially in the food and beverages sector. In recent years, they have been used as an active ingredient in pre- and probiotic products on diets of ruminants due to their positive effects on the rumen microenvironment. For instance, an intensification of rumen efficiency and activity have increased milk production and quality in cows (Orbera, 2004; Castro *et al*., 2005). However, the search of new yeast strains with probiotic capacity never ends. In Colombia, a new strain of *Meyerozyma guilliermondii* was isolated and evaluated as a probiotic yeast in the diet of monogastric animals. Results showed a significant reduction of methane emissions on animals fed with yeast treatment. Methane emissions reduction is a good indication of a high efficiency gastric system (Rodriguez *et al*., 2015). In the animals fed additives market, a microorganism with high probiotic potential is not enough. A production process technically efficient and economically feasible has to be designed in order to compete in terms of quality, efficacy and price. A high proportion of additives are sold in granulated form using selected coadjuvants providing stability of active ingredients during shelf life at uncontrolled conditions. Consequently, unit operations such as formulation and additive drying became critical stages of the process.

All commercial products based on yeasts require a formulation design to provide a suitable application form and to guarantee stability of the product during the time it will be stored before used (Rhodes, 1993). As fed additives are granulated products, which need a previous drying process, coadjuvants with drying protectant capacity have to be selected to prevent loss of viability of yeast cells. Sugars, albumin, milk, polyols, trehalose, sucrose and amino acids have been reported as drying protectants; however, a compatibility test before they are used is necessary to determine the absent of negative effects over cells of a new yeast strain (Abadias *et al*., 2001; Hubalek, 2003; Morgan *et al*., 2006). Same considerations are followed for diluent coadjuvants. After the selection of coadjuvants and their concentration as a function of yeast dried biomass weight and cellular concentration (CFU/g), a drying process is conducted. Most of the granulated products based on yeast are dried through a fluidized bed drying process (FBD) due to its low energy consumption (in comparison with other drying process as drying rooms or drying oven), increase process productivity, optimal utilization of space and easy scalability to industrial processes (Grabowski *et al*., 1997; Domínguez-Niño *et al*., 2016). Before drying, a critical selection of operation parameters should be done to avoid the loss of viability commonly reported in microbial-based bioproducts (Labuza *et al*., 1972; Luna-Solano *et al*., 2000; Santivarangka *et al*., 2008). A drying process as FBD has two main parameters: one is an intrinsic parameter -chamber temperature-, and the other is an extrinsic one -formulation composition-. In this work, a drying protectant for a granulated product based on probiotic potential yeast *M. guilliermondii* is selected using a decision matrix, and a subsequent FBD evaluation is conducted to determine the drying operation parameters that guarantee the product´s stability during shelf time.

2 Materials and methods

2.1 Microorganism

Meyerozyma guilliermondii strain Lv196 was provided by the Germoplasm Collection of the Biological Control Laboratory (CORPOICA). Yeast was cultured on MYM Agar (yeast extract, 3 g/L; malt extract, 3 g/L; peptone, 5 g/L; sucrose, 10 g/L; agar, 25 g/L) at 28 ± 0.5 ºC for 72 h. Cultures were maintained at 5 ± 0.5ºC and purity was verified with Gram staining before use.

2.2 Selection of drying protectant, temperature and time

2.2.1. Yeast biomass production

Yeast biomass was produced on MYM medium using a 250-mL Erlenmeyer flask with a working volume of 150 mL. Initial cellular concentration is adjusted to 1×10^7 CFU/mL adding 1.5 mL of a yeast cells concentrated suspension. Production conditions were 25 ± 2ºC and 150 rpm for 144 h in a thermo-controlled rotary shaker (LSI-1005P, Daihan Labtech, Namyangju-City, Korea). Aerobic conditions were guaranteed using sterile cotton plugs. Culture purity was verified with Gram staining.

2.2.2. Compatibility test of drying protectants

A compatibility test was carried out to evaluate the effect of different drying protectants on yeast cell viability of *M. guilliermondii* Lv196.

Table 1 Design of experiments for compatibility test of different drying protectants with yeast *M. guilliermondii* Lv196

Treatment	Coadjuvant[a]	Concentration of drying protectant (% w/v)
T0	Control[b]	0
T1	Glutamate	2
T2	Skim milk	10
T3	Lactose	10
T4	Maltose	10
T5	Corn starch	10
T6	Sucrose	10
T7	Sorbitol	2
T8	Glycerol	4.6

[a]All coadjuvants were evaluated in a suspension with yeast cells. [b]Yeast suspension with no coadjuvants.

Assays were performed in 50 mL-plastic flask containing 30 mL of fermented broth as a working volume. A specific quantity of each drying protectant was added and mixed with yeast cells at 200 rpm and 25 ± 2ºC in a thermo-controlled rotary shaker (Unimax 1010 coupled to Inkubator 1000, Heidolph, Chicago, IL, United States). Table 1 presents the different drying protectants evaluated with their respective concentrations (% w/v) selected based on reported literature (Gómez-Alvarez, 1997; Quiroga *et al*., 2011; Ruiz *et al*., 2015). Each treatment was conducted in triplicate.

After 5 days, the loss of cell viability of each treatment (in relation to control) was determined with viable plate count method in MYM Agar (Eq. 1). Where FCT is final cellular concentration of treatment *i*, and FCC is final cellular concentration of control, all in terms of CFU/mL. A statistical analysis was conducted through non-parametric Kruskal-Wallis test with a 95% of confidence using Statistix ® software version 8.

$$\text{Loss of cell viability (\%)} = \left[1 - \frac{\log_{10}[FCT]_i}{\log_{10}[FCC]}\right] \times 100\% \quad (1)$$

2.2.3. Decision-matrix method

Drying protectants with no loss of cellular viability or an increase of cellular concentration in relation to control were analyzed through decision-matrix method (Baheti *et al*., 2010; Ronowicz *et al*., 2015). Two indexes were used as decision factors: compatibility with yeast cells and protectant prices. Compatibility index was determined as a relation of final cellular concentration of yeast after contact with drying protectant in relation to control (Eq. 2). Price index was determined as a relation of the price of each drying protectant suspension (based on concentrations expressed in Table 1) in relation to diluent cost selected to formulation section (Eq. 3). To design these prototypes rice flour was used as the diluent. Prices from different protectants were supplied by local distributors (Colombia) and converted into US Dollars.

$$\text{Compatibility index} = \frac{\log_{10}[FCT]}{\log_{10}[FCC]} \quad (2)$$

$$\text{Price index} = \frac{\text{Price of drying protectant suspension}}{\text{Price of diluent}} \quad (3)$$

In this case, contaminant load (bacterial and fungi) was not used as decision criteria because its concentration was under critical value (< 100 CFU/g). Data is shown in Table 2.

Table 2 Drying protectant prices and contaminant load characterization.

Drying protectant	Price (USD/kg)[a]	Contamination		
		Bacteria (CFU/g)	Fungi (CFU/g)	Yeasts (CFU/g)
Glutamate	2.48	< 100	< 100	< 100
Skim milk	5.16	< 100	< 100	< 100
Lactose	3.26	< 100	< 100	< 100
Maltose	32.64	< 100	< 100	< 100
Corn starch	1.37	< 100	< 100	< 100
Sucrose	0.98	< 100	< 100	<100
Sorbitol	1.73	< 10	< 10	< 10
Glycerol	1.14	< 10	< 10	< 10

[a]Prices were converted to US Dollars using the average for the first half of 2016.

Table 3 Temperature and time values evaluated to determine the effect on yeast cells viability.

Treatment	Temperature (ºC)	Time (h)
1	25ºC	1
2	30ºC	
3	35ºC	
4	40ºC	
5	25ºC	2
6	30ºC	
7	35ºC	
8	40ºC	

Each decision criteria was assigned with the same valuation percentage and a combined index was determined (price index / compatibility index). Drying protectants were ranked and the best two were used for the next experiments. In case of an equal value of the combined index, the tiebreaker criteria was the value of the compatibility index.

2.2.4. Effect of temperature and time on yeast cell viability

Once the drying protectant was selected, it was necessary to evaluate the effect of temperature and time of exposure on yeast cell viability. This introduced notions about process' conditions to evaluate in a fluidized bed drying process. Fresh yeast cells were cultured on MYM medium at 28 ± 0.5 ºC for 72 h. These cells were harvested using an inoculating loop and suspended in 100 mL of 1 %w/v Tween® 80. This suspension had a cellular concentration of 7×10^8 CFU/mL (determined by plate count method). From this suspension, 1.5 mL were transferred to 2 mL-tubes. The tubes were placed in a thermostatic water bath (SWB1122A, Lindberg Blue M, Asheville, NC, United States). Four temperatures (25ºC, 30ºC, 35ºC and 40ºC) and two exposure times (1 h and 2 h) were evaluated (Table 3). Each treatment was conducted in triplicate. Yeast suspension without temperature exposition was used as a control.

Cell viability (expressed as percentage of survival) was computed to determine the positive or negative effect of experimental variables (See equation 1). A statistical analysis was done through non-parametric Kruskal-Wallis test with a 95% of confidence interval using Statistix ® software version 8.

Table 4 Experimental matrix for fluidized bed drying assay.

Experiment	IAT (ºC)	CP (bar)	GP
1	35	0.5	FP2
2	45	0.5	FP1
3	45	0.5	FP2
4	35	1	FP1
5	35	0.5	FP1
6	45	1	FP2
7	35	1	FP2
8	45	1	FP1

2.3 Evaluation of a fluidized bed drying process for a prototype based on yeast M. guilliermondii

2.3.1. Formulation of two prototypes

Yeast biomass was produced as in section 2.2.1. Fermented broth was centrifuged at 4500 rpm for 15 min (Rotina 480, Hettich Lab Technology®). Wet yeast biomass was mixed with each selected drying protectant from section 2.2.3., and a common diluent in a mixer (N50 Mixer, Hobart®) and extruded using an oscillating granulator (YK-160A, Target Pharmatech Co., LTD®) to obtain two wet granulated prototypes, codified as FP1 and FP2.

2.3.2. Fluidized bed drying (FBD) assays

Both prototypes, PF1 and PF2 were dried to evaluate the drying protectant performance in real conditions at pilot scale. The drying process was carried out in a spray dryer configured as a fluidized bed dryer, equipped with inlet air temperature and chamber pressure control, a flap to adjust the inlet airflow and spraying air regulator (D-01277, Glatt® GmbH, Binzen, Germany). Outlet air flap was set at 100% and during the drying process, the pressure drop was kept in a constant value of 5-15 mBar. During the experiments, environmental conditions were 24 ± 4ºC and a relative humidity (HR) of 70 ± 5%. Experiments were conducted by triplicate using a full factorial design, 2^3. The three experimental variables were: (1) inlet air temperature (IAT), (2) chamber pressure (CP), and (3) granulated prototype (GP). IAT interval was selected from the results of section 2.2.4. Experimental matrix is shown in Table 4.

The time of exposure of the prototypes was 30 min and the fluidized bed load was of 50 g of wet granulated prototype. The independent response variables were cell viability (expressed as percentage of survival), final moisture and operation yield (expressed as grams of dried prototype/grams of wet prototype × 100%). An ANOVA analysis using Statgraphics® Centurion v.16 for best drying condition selection was conducted.

2.3.3. Stability at storage conditions

Dried prototypes, PF1 and PF2, with the best drying conditions were stored at 18 ± 2 ºC and 70 ± 5 % RH during 15 months. Loss of cell viability was computed based on plate count method results in MYM Agar.

3 Results and discussion

3.1 Drying protectants compatibility

The results from yeast cell compatibility with drying protectants after 5 days of contact expressed in terms of loss of cell viability are shown in Fig. 1. The average final cellular concentration of all treatments was 1.47×10^{10} CFU/mL, 47 % higher than control. Between drying protectants, no significant difference was observed except for T2 with a loss of cell viability of 15.6 ± 0.7%. This negative effect was attributed to the low capacity of degradation of protein present on skim milk by *M. guilliermondii*.

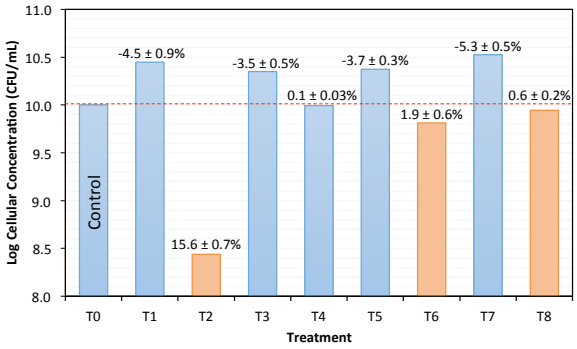

Fig. 1 Compatibility results of different drying protectants on yeast *M. guilliermondii* Lv196. T0 (Control); T1 (Glutamate); T2 (Skim milk); T3 (Lactose); T4 (Maltose); T5 (Corn starch); T6 (Sucrose); T7 (Sorbitol); T8 (Glycerol). Blue and orange bars represent treatment with increase of cellular concentration and loss of cell viability, respectively. Values in upper side of the bars are loss of cell viability magnitude with their standard deviation.

In general, yeasts had a low proteolytic activity, decreasing the possibility of using protein as a nitrogen source to survive in a non-substrate environment (Rodarte *et al*., 2011). Furthermore, *Meyerozyma guilliermondii* is not able to metabolize lactose as a carbon source in complex media (Wrent *et al*., 2015; Stephenie *et al*., 2015). If the coadjuvant is not naturally degraded by yeast, it could be chemically degraded causing cellular autolysis by toxics compounds (Sabaratnam and Traquair, 2002) or affecting physicochemical properties of the suspension; for example pH. Dramatic changes of pH in culture medium can influence modifications on: gene expressions, transport of protons across cellular membrane and degradation of intracellular amino acids. Though T6 (Sucrose) corresponds to a type of sugar, a loss of cell viability of 1.9 ± 0.6% was observed. An initial concentration of 10 %w/v, could have been consumed in less than 24 h based on substrate consumption rates (data not shown). Due to the conditions employed during compatibility test, closed flask and 5 days of contact, CO_2 produced by yeast metabolism, could have reacted with water forming carbonic acid (Slaughter, 1989). Additionally, in absence of carbon source, yeast is able to metabolize intracellular glycogen producing organic acids. Acidification of suspension media could have affected yeast growth and cause cellular death (Chen and Gutmanis, 1976).

The last treatment, in terms of loss of cell viability was glycerol with 0.6 ± 0.2%. Some sugar alcohols could be used as substrate in culture media if a correct supplementation of macro and micronutrients is applied (Díaz *et al*., 2014). For instance, in this work, sorbitol (T7) had a final cellular concentration 6 % higher than glycerol (T8). In compatibility assays is not common to find as a response variable an increase in cell viability but in this case drying protectants as sorbitol (T7), 5.3 ± 0.5%, glutamate (T1), 4.5 ± 0.9%, corn starch (T5), 3.7 ± 0.3%, and lactose (T3), 3.5 ± 0.5%, presented it. In a limiting substrate media as Tween® suspension used for these experiments, without any organic carbon or nitrogen source, yeasts as *M. guilliermondii* can not growth or even enter in a maintenance status.

3.2 Selection of drying protectants based on decision matrix

Based on compatibility and price index, a ranking of compatible drying protectants is shown in Table 5.

As in section 3.1, T7 (sorbitol) and T1 (glutamate)

were the most feasible drying protectants, in terms of the combined index, 0.009 and 0.019. It is important to remember that drying protectants have two main functions: act as a support (organic or inorganic) to confer physical structure to granulated prototypes, and protect yeast cells from damage after a drying process (Lorena *et al*., 2003). In general, polyols have a low protective capacity (Abadias *et al*., 2001; Chen *et al*., 2015a; Chen *et al*., 2015b). Even if sorbitol had a cell viability of 5.3 ± 0.5% higher than control in compatibility test, its protective capacity becomes a drawback in a formulation of a dried granulated prototype in a fluidized bed dryer. Moreover, amino acids, as the one used in T1, have a good protective capacity of cells if they are combined with another protectant. On the experiments conducted in this work, the amino acid was used alone, decreasing the chance of acting as a barrier between yeast cells and heat. This behavior has been reported before on *Trichoderma viride* and *Saccharomyces cerevisiae* (Berny and Hennebert, 1991). Sorbitol and glutamate were used in pre-formulation assays and the mass obtained with both drying protectants did not have good physicochemical characteristics such as consistent particle size, low disintegration and high water retention capacity (data not shown). Those characteristics are important to guarantee yeast cell viability in shelf time and easy manipulation during application by costumers (Marino *et al*., 2004).

Lactose (T3) and corn starch (T5) have been reported as membrane-protecting agents for yeast cells in stress conditions such as dehydration in fluidized bed drying process (Chen *et al*., 2006; Acuña *et al*., 2015). For instance, lactose is able to interact with lipids membranes replacing water molecules and avoiding damage during water evaporation. This interaction is proportional to its degree of polymerization (Watson and Preedy, 2016). They also have the capacity of inhibit free radical production, a phenomenon associated with loss of viability of active substances during storage decreasing shelf life (Heckley and Quay, 1983). Due to their protective and antioxidant capacity, lactose and corn starch were selected and used in formulation of two granulated prototypes based on *M. guilliermondii* yeast strain Lv196.

3.3 Evaluation of temperature and time effect on yeast cell viability

The effect of temperature and time of exposure on yeast cells viability was determined to establish a baseline for drying conditions to apply on an FBD process. As is shown in Fig. 2, experimental variables presented non-significant statistical difference ($p > 0.05$) in relation to control except for two conditions: 30ºC for 2 hours, and 40ºC for 1 hour where an unusual increase in cell viability was observed. To 30ºC and 35ºC, the tendency was the same, the cell viability increased up to 8 % in 2 hours of exposure. Nevertheless, a temperature of 40ºC causes an up and down tendency due to the negative effect of higher temperatures of yeast growth. This behavior has been reported by different authors, but due to the variability of the yeast strains, psychrophilic, mesophilic and thermophilic, it has to be explored on each strain.

At 1 hour of exposure, cellular concentration was increased in relation to temperature in the evaluated interval (from 25ºC to 40ºC), describing a linear equation with the form $y = mx + b$ and a lineal regression coefficient of 95% (see Fig. 3).

The positive effect of temperature on cellular concentration could be attributed to yeast lipid structure regulation capacity. In stress conditions, such as high temperature, yeast can regulate cellular membrane lipid composition, maintaining optimal membrane fluidity for normal cellular functions increasing the probability of cells survival.

Table 5. Ranking of drying protectants

	Price (USD)	Cell viability	Comp. Index	Price index	Comb. Index	Rank
T0	-	10.04	1	0	0	-
T1	0.05	10.45	1.04	0.02	0.019	2
T3	3.27	10.35	1.03	0.03	0.029	4
T5	0.14	10.37	1.03	0.02	0.019	3
T7	0.03	10.6	1.06	0.01	0.009	1

T0 (Control); T1 (Glutamate); T3 (Lactose); T5 (Corn starch); T7 (Sorbitol).

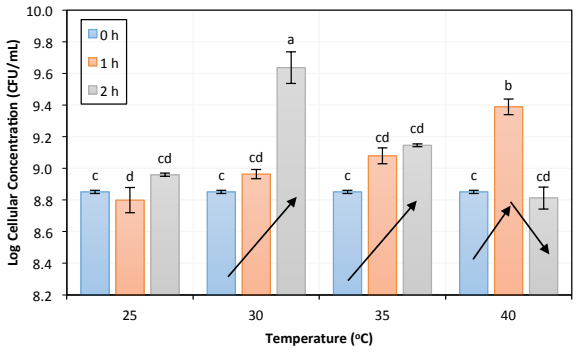

Fig. 2 Cell viability of *M. guilliermondii* Lv196 after different exposition time and temperatures. Means for treatments with the same letter are not significantly different based on Kruskal-Wallis test with a 95% of confidence interval, $p \leq 0.05$.

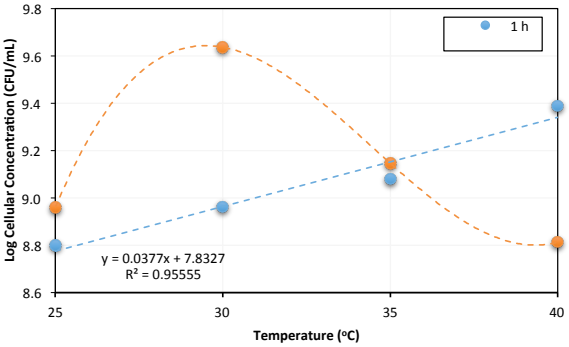

Fig. 3 Tendency line for cellular concentration of yeast strain Lv196 obtained from drying temperature and time of exposure experiments.

This regulation capacity depends on yeast strain characteristics such as maximum growth temperature (Swan and Watson, 1997). Additionally, yeast cells suspension was done using Tween® 80 as a disintegrant agent due to its sprinkling effect. The active principle of Tween® 80, polysorbate, has been reported as a carbon source for different yeast and fungi species (Tsuboi *et al*., 1996; Toaka *et al*., 2011). This could be another reason for *M. guilliermondii* growth at the evaluated temperatures.

After 2 h of exposure, the negative effect of temperature was observed, specifically with values over 30ºC. For example, an increase from 25ºC to 30ºC, final cellular concentration was 8 % higher and specific growth rate (μ) increased from 0.05 h^{-1} to 0.39 h^{-1}. However, when the temperature increased from 30ºC to 35ºC, the specific growth rate decreased 37.6%. Instability of membrane lipid composition regulation could be caused by exposition at high temperatures for a long period of time, increasing the risk of not reestablishing its cellular homeostasis (Beney *et al*., 2000). For instance, at 40ºC, no growth was observed, as an evidence of this instability. Based on the results from this section, drying temperature could be around 40ºC if the time of exposure is equal or less than 1 h. These values were used as a base line to design the experiments for evaluation of fluidized bed drying process from section 3.4.

3.4 *Fluidized bed drying of granulated prototypes*

To determine the best drying conditions of two granulated prototypes, PF1 and PF2, through FBD operation, a full factorial experimental design was conducted as in section 2.3.2. Results of cell viability, final moisture and operation yield are shown in Fig. 4.

According to ANOVA analysis, no process parameter has a significant effect on any response variable (p >0.05) in the evaluated interval. Therefore, the analysis was done identifying the effect of each experimental variable on each one of the three final bioproduct characteristics.

Independently of granulated prototypes (PF1 or PF2) and chamber pressure (0.5 bar and 1 bar), when an air let temperature of 35ºC was used, final moisture was 55.32 % higher than a granulated prototype dried at 45ºC. An increase in air inlet temperature caused an increase in drying rate or diffusion (Joshi and Thorat, 2011). At 45 ºC of IAT, drying rate was 1.09 g removed water/min, 14 % higher than the lowest level evaluated (35 ºC). Operation yield and loss of cell viability had no significant difference (p > 0.05) with temperature changes. However, loss of cell viability was affected by the type of granulated prototype. After the drying operation with a fluidized bed drier, PF1 had a loss of viability of 65.78 % lower than PF2. This shows a high influence of protective capacity of each drying protectant on cell viability of yeast as it has been well discussed in previous sections.

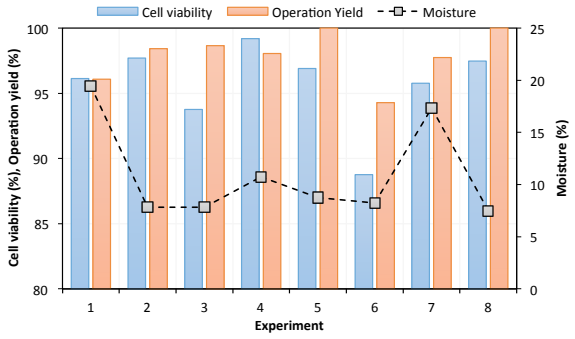

Fig. 4 Response variable values according to experimental matrix. The description of each treatment is on Table 4.

Table 6 Average values of operation yield (OY, %), final moisture (FM, %) and loss of cell viability (LCV, %) in relation to each evaluated experimental factor.

Experimental factors		OY (%)	FM (%)	LCV (%)
IAT (ºC)	35	97.84 ± 2.14	7.86 ± 0.26	5.58 ± 3.62
	45	97.97 ± 1.39	17.59 ± 8.20	3.00 ± 1.33
CP (bar)	0.5	98.29 ± 1.41	10.98 ± 4.92	3.88 ± 1.47
	1	97.52 ± 2.06	10.95 ± 3.87	4.70 ± 3.96
GP	PF1	99.12 ± 0.89	8.71 ± 1.25	2.19 ± 0.85
	PF2	96.69 ± 1.67	13.22 ± 5.24	6.40 ± 2.94

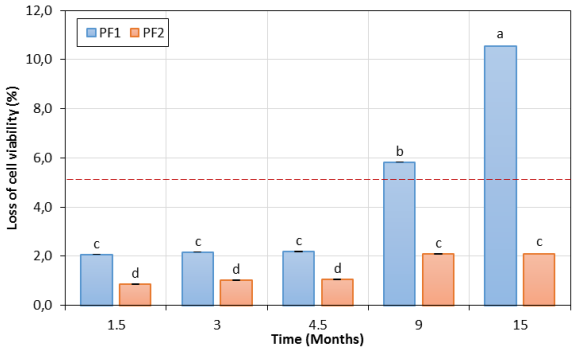

Fig. 5 Loss of cell viability of PF1 and PF2 stored at room temperature for 15 months. Means for treatments with the same letter are not significantly different based on Kruskal-Wallis test with a 95% of confidence interval, $p \leq 0.05$.

Likewise, this loss of viability could be related with molecular weight (MW) and glass transition temperature (T_g) of drying protectants. It has been stablished by different authors that viability decreased when a high MW drying protectant (higher than 2 kDa) is used because of their low capacity of interaction (Perdana *et al.*, 2014; Koster *et al.*, 2003; Tetsuya and Hiroaki, 1996). However, high MW protectants are expected to create a layer of protection over yeast. In this work, drying protectant with the high MW and T_g, guarantee the lower loss of viability after the drying process. PF1 also had the maximum operation yield with 99.12 ± 0.89 %. Chamber pressure had no effect in terms of magnitude on any response variables due to standard deviation of experimental data. PF1 demonstrated a high performance in terms of operation yield and loss of cell viability, response variables related with quality of drying operation and final product.

The best conditions for drying granulated prototype, PF1 and PF2, were: inlet air temperature at 45ºC, chamber pressure of 0.5 bar, during a time of 30 min.

3.5 Stability at room temperature

One of the main problems in probiotic products is loss of viability during storage. Depending on conditions like temperature or humidity and microbiological characteristics of final product such as concentration of contaminants (bacteria and fungi), length of storage could decrease the affecting product response on field (Gennaro, 1995; Labuza *et al.*, 1972). For both granulated prototypes, PF1 and PF2, cell viability was evaluated using plate count method and loss of viability was computed regarding initial cellular concentration. Results are shown in Fig. 5.

No significant differences regarding time were found from 0 to 4.5 months of storage at 18 ± 2 ºC. After 15 months, a decrease of cell viability was observed for both granulated prototypes, PF1 and PF2. Loss of viability for PF1 10.55 ± 0.01%, 5.3 times higher than PF2. Corn starch, a drying protectant used in PF2 formulation, is a biopolymer regularly composed of amylose and amylopectin.

This composition confer to some types of starch a resistance to high temperatures and an enzymatic specificity to withstand amylases degradation, an important characteristic in the design of probiotic products. For example, if a probiotic is provided to an animal and the starch in its formulation had a resistance to gastric amylases, the bioproduct could get further in the gastrointestinal route, guarantee liberation of active substance, yeast *M. guilliermondii*, on rumen and work as a carbon source to beneficial ruminal microorganisms (García-Ceja and López-Malo, 2012).

A loss of cell viability evaluation of PF2 was conducted at 15 months of storage at room temperature with no significant difference with value reported at 9 months. With a shelf life superior than 1 year at storage, PF2 was selected as the best-granulated prototype in relation to PF1 and even some commercial products.

Conclusions

It was possible to design a granulated prototype formulation based on a native yeast, *Meyerozyma guilliermondii*, using corn starch as a drying protectant for a fluidized bed drying process. Selected conditions guaranteed a loss of cell viability of 2.09 ± 0.01% after 15 months of storage at 18 ± 2 ºC, a higher shelf time respect to different commercial products based on other yeast species, which confer a competitive advantage of this product in terms of quality. However, this parameter will be evaluated for more time to determine the maximum shelf time. Future studies with this prototype will focus on (1) evaluating the process alternative to mix an essential oil (EO) to reinforce the effect of the bioproduct on the animal diets avoiding the yeast mortality cause by the antimicrobial characteristic of EO and (2) selection of the packaging material.

Acknowledgements

The authors would like to thank The Ministry of Agriculture and Rural Development (MADR) of Colombia for funding this project.

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