

## DEVELOPMENT AND EVALUATION OF FREEZE-DRIED MILK POWDER WITH LOW-LACTOSE CONTENT

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This work aims to develop a freeze-dried milk powder with low lactose content and evaluate the percentage composition, microbiological analysis, and the soy lecithin and temperature effect on the solubility, dispersibility, and wettability of the final product. According to the results, treatments with and without hydrolysis showed significant differences in moisture (3.96% and 2.63%), ash (8.92% and 10.0%), lactose (1.57% and 61.46%), and glucose (33.79% and 0.81%) contents. In both treatments, no microbiological contaminant was observed. For high values of solubility, the most appropriate combination was lecithin 0.6% at 80 °C, for dispersibility was lecithin 0.6% at 94 °C, and for wettability was lecithin 0.65% at 90 °C. Based on the results, freeze-dried milk powder with low lactose content can represent a new technologically and nutritionally viable product. This can be an alternative production process representing potential for marketing as a final product or used as base for the food preparation containing milk with low lactose content.

**Keywords:** biotechnology, dairy products, beta-galactosidase, freeze-drying

Dehydration is one of the most used techniques for storing food due to reduced water activity that prevents the proliferation of microorganisms (FOX & McSWEENEY, 1998; RANKELL et al., 2001; DEETH & HARTANTO, 2009). Moreover, by reducing food weight and volume, there is a cost reduction in the final product transportation and storage (CARIĆ et al., 2009; OLIVEIRA et al., 2009).

Traditionally, dehydrated milk is obtained by partial water evaporation through the concentration process, followed by spray drying, using heating in both steps (CARIĆ et al., 2009; OLIVEIRA et al., 2009). An alternative is the freeze-drying process, in which the milk is dehydrated at low temperature maintaining the sensory and nutritional properties (FELLOWS, 2006; LIAPIS & BRUTTINI, 2006; COSTA, 2007). Especially in the milk powder production with low lactose content, freeze-drying process has advantages over the traditional method, like preventing non-enzymatic browning by the Maillard reaction, facilitating the solubilisation due to the granules porosity in the final product (COSTA, 2007; BARBOSA-CÁNOVAS et al., 2005). This product is aimed at a significant part of the population with lactose intolerance.

Based on this context, the dairy industry has been innovating by offering products with low lactose content, such as UHT milk, yogurts, cheeses, fresh milk, and concentrated milk (PEREIRA et al., 2012). Thus, the objective of the work was to develop a freeze-dried milk powder with low lactose content from the reconstituted skimmed milk standardized through a dehydration curve, as well as evaluate their physicochemical and microbiological characteristics.

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## 1. Materials and methods

### 1.1. Skimmed milk powder and dehydration curve by freeze-drying

The skimmed milk powder obtained by high heat (Molico<sup>®</sup>, Aracatuba, Brazil) was reconstituted at 25 °C, which represents the industrial temperature range, in the following total solids treatment with concentrations of T1: 10% (w/v), T2: 13.3% (w/v), T3: 20% (w/v), and T4: 40% (w/v). The reconstituted skimmed milk powder was hydrolyzed with a commercial  $\beta$ -galactosidase enzyme from *Kluyveromyces lactis* (Maxilact<sup>®</sup> LX 5000, Sao Paulo, Brazil) at a concentration of 0.5% (v/v) at 4 °C for 24 h.

Samples of each T1–T4 treatment (100 ml) were placed in Petri dish of 13 cm diameter and frozen at –18 °C for 48 h, then subjected to freeze-drying (Freeze L101–Liobras, San Carlos, Brazil) until constant weight. For the dehydration curve determination, the Petri dishes were weighed (g) every 3 h. The yield was calculated by gravimetry, using the initial and final weight (g) difference. The experiment was carried out with two replicates and analyses were in triplicate.

### 1.2. Central composite design of lecithin effect of freeze-dried milk powder with low lactose content

In order to develop a freeze-dried milk powder with low lactose content, the most appropriate treatment of total solids was selected for the study of the lecithin and temperature effects in the final product. Therefore, the lecithin (w/w) and temperature effect on the solubility (% RI), dispersibility (%), and wettability (seconds) was evaluated by the Central composite design (CCD) with two variables in five levels each (–1.41, –1, 0, 1, +1.41), as shown in Table 1.

Table 1. Levels of the independent variables for the composite central design for milk powder with low lactose content

Stand- ard run	Independent variables				Dependent variables		
	Lecithin (% w/v) X1		Temperature (°C) X2		Solubility (% RI)	Dispersibil- ity (%)	Wettability (s)
	Coded	Decoded	Coded	Decoded			
1	–1	0.55	–1	70	4	91	194
2	–1	0.55	+1	90	9	93	75
3	+1	0.65	–1	70	10	90	333
4	+1	0.65	+1	90	7	94	36
5	– $\alpha$	0.53	0	80	7	92	68
6	+ $\alpha$	0.67	0	80	6	94	104
7	0	0.6	– $\alpha$	66	7	92	525
8	0	0.6	+ $\alpha$	94	11	100	92
9	0	0.6	0	80	4	93	149
10	0	0.6	0	80	4	93	149
11	0	0.6	0	80	4	93	149
12	0	0.6	0	80	4	93	149
13	0	0.6	0	80	4	93	149
14	0	0.6	0	80	4	93	149

$\alpha$ : the distance of each axial point (also called star point) from the center

The analysis for solubility consisted of mixing 20.0 g sample in 150 ml of distilled water at 25 °C in a glass bottle. The mixture was filtered through a 32-mesh opening sieve and quantified gravimetrically. The retention index percentage (*RI*), which is the ratio of the insoluble and soluble mass, was calculated (VISSOTTO et al., 2006). Analyses were done in triplicate.

$$\% \text{ RI} = 100 \times (170 - M_{\text{Filtered}}) / M_{\text{Filtered}}$$

where:

% RI: retention index percentage

$M_{\text{filtered}}$ : filtrate weight (g)

Dispersibility was determined according to SKANDERBY and co-workers (2009) as follows; 25 g of lyophilized milk powder were mixed in 250 ml of distilled water at a temperature of 25 °C. The samples were shaken for 20 sec and filtered through a 35-mesh sieve. Analyses were performed in triplicate.

For the wettability analysis, 2.0 g of sample were added in 400 ml of distilled water at 25 °C in a glass bottle, and the time required to wet all particles was determined visually (HLA & HOGKAMP, 1999). The determinations were carried out in triplicate.

### 1.3. Percentage composition and microbiological analysis

For the best treatment determination of the freeze-dried milk powder with and without hydrolysis, samples were analysed for protein, fat, and total ash contents according to AOAC (1998) method numbers 16.036, 16.064, and 16.035, respectively. The moisture was determined by gravimetric method according to SKANDERBY and co-workers (2009). The acidity was done according to ADMI Bulletin, 915 (1965). Lactose was determined by lactose–methylamine method (NICKERSON et al., 1975). The determination of glucose was carried out using a glucose oxidase kit (Bioliquid®). All analyses were performed in triplicate.

Microbiological analyses for the freeze-dried milk powder with low lactose content were as follows: aerobic mesophilic microorganism count (CFU ml<sup>-1</sup>) determination was done according to APHA (2005), total coliforms and *Escherichia coli* by Petrifilm EC method (3M), *Staphylococcus* by the method of Petrifilm STX (3M), *Salmonella* by Petrifilm TM SALX method (3M), yeasts were counted with Petrifilm TM method (3M), and *Bacillus cereus* count was done according to MOSSEL and co-workers (1967) and STADHOUDERS (1992). The analyses were performed in duplicate and expressed as mean.

### 1.4. Statistical analysis

The results of the lecithin and temperature effect optimization on the solubility, dispersibility, and wettability of freeze-dried milk powder with low lactose content were analysed using the program Statistic 6.0 (Statsoft Inc., Tulsa, USA). Percentage composition analysis of the freeze-dried milk with and without enzymatic hydrolysis was performed by the Student's *t*-test.

## 2. Results and discussion

### 2.1. Dehydration curve by freeze-drying

The dehydration speed rate calculated from the dehydration curve with linear regression for different treatments were  $T1=3.33$ ,  $T2=4.24$ ,  $T3=4.61$ , and  $T4=4.71\% \text{ h}^{-1}$ . It can be seen that the dehydration rate ranged from 3.33 to  $4.71\% \text{ h}^{-1}$ . This indicates that every hour during freeze drying process, the dehydration rate is influence proportionally by the total solids (BARBOSA-CÁNOVAS et al., 2005). There is a relationship between the water content of food and its capacity to change, and it is precisely at this factor that lyophilisation aims to act, reducing the food water content, concentrating the solutes, thereby reducing the risk of changes. The dehydration treatment T1 lasting 30 h resulted in 1.92% of moisture in the final product. Thus, the dehydration time T4 treatment with 40% powdered milk was only 15 h and the final moisture content was 3.98%, see Figure 1.

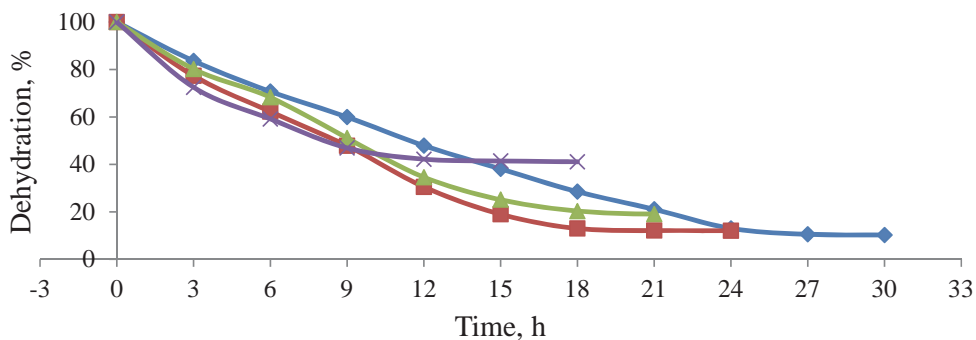


Fig. 1. Dehydration kinetics for the skimmed milk treatments with hydrolyzed lactose

—◆—: T1 - 10%; —■—: T2 - 13.3%; —▲—: T3 - 20%; —×—: T4 - 40%

The final moisture percentages of lyophilized milk powder were T1 (1.92%), T2 (1.77%), T3 (3.28%), and T4 (3.98%). The moisture obtained in the four treatments is in accordance with the current legislation that establishes 5% for lyophilized products and 4% for skimmed milk powder (BRASIL, 2006). The freeze-dried milk yields in the different treatments were T1=10 g, T2=12 g, T3=20 g, T4=40 g in 27, 21, 18, and 12 h, respectively. Taking into account the freeze-drying time and the yield obtained (Table 2), the treatment 4 (T4) that had the lowest freeze-drying time with higher yield was chosen for the study of the effects of temperature and addition of lecithin by central composite design.

### 2.2. Central composite design (CCD) of temperature and lecithin effect of freeze-dried milk powder with low lactose content

Table 1 shows experimental conditions from the CCD and the results (response) obtained in terms of corresponding solubility/dispersibility percentage and time in seconds of wettability.

There was variation in all the responses tested regarding the levels tested for lecithin and temperature.

In Figure 2, the results are shown for solubility, dispersibility, and wettability of the milk powder with low lactose content that have been submitted to statistical analysis. The solubility was determined indirectly by RI, as a result, the higher product solubility is associated with lower RI values. So, solubility reflects the retention index percentage (RI) of the product in a solvent, in which the RI determines the dissolution rate of the milk powder particles. According to the results obtained, the solubility of freeze-dried milk powder with low lactose content varied from 4 to 11% RI. The coefficient of determination,  $R^2$ , was 0.93127, which means that 93.13% of the response variation is explained by the statistical design proposed. According to Figure 2A, lecithin between 0.55 and 0.6% and temperature between 70 and 80 °C reach a maximum solubility of 4% RI. VISSOTTO and co-workers (2006) found that lecithin was effective in improving milk powder solubility at 0.5%. The use of higher quantities did not contribute to the increase in product solubility.

The results for the low lactose milk powder dispersibility subjected to statistical analysis are presented in Figure 2B. Dispersibility of a product is the powder's ability to disperse in water as a single particle, or, in other words, is the agglomerate's ability to separate. According to the results obtained, the dispersibility of freeze-dried milk powder with low lactose content varied from 90 to 100%, reaching a complete dispersion in 20 seconds. LEE and co-workers (2014) verified that for dairy powders, when subjected to different temperature conditions, the complete dispersion occurs in up to 10 min. The coefficient of determination,  $R^2$ , was 0.7382, which means 73.82% of the response variation is explained by the model proposed. According to Figure 2B, the dispersibility was 100% under the following conditions: 0.6% lecithin at 94 °C. The effective temperature in the product was between 80 and 94 °C.

The surface wettability, one of the most crucial properties of the material surface, is a key parameter for understanding the material interactions with their surrounding environment and their applications (GALET et al., 2010; KIRDPONPATTARA et al., 2013). Wettability is the powder's ability to absorb water on its surface. According to the results obtained, the solubility of the freeze-dried milk powder with low lactose content varied from 36 to 525 seconds. The coefficient of determination,  $R^2$ , was 0.95915, which means that 95.91% of the response variation was explained by the model proposed. According to Figure 2C, lecithin between 0.50 and 0.60% (w/v) and temperature below 65 °C provided the maximum wettability in 36 seconds. The product with the lecithin addition needed less time (s) for the particles to get wet, an improvement that can be seen up to a maximum of 0.60% lecithin and below 65 °C. This is explained by the properties 'wetting' and 'emulsifier' that lecithin has on the product. According to the results, lecithin at 0.65% and 90 °C was most effective in improving the wettability. A similar study was carried out by FONSECA and co-workers (2011), which evaluated the wettability influenced by the addition of different lecithin levels (0.4, 0.5, 0.8 g lecithin/100 g total solids) in goat milk. The authors observed that the wetting time ranged from 6 minutes for non-lecithin milk to 73 s for milk with 0.8% of lecithin. VISSOTTO and co-workers (2006) verified that lecithin powders at a concentration of up to 0.5% needed shorter times for the particles to wet than those submitted to the simple mixing process, and higher contents did not significantly improve the wettability of powder particles. The Brazilian legislation allows a maximum concentration of 0.5% of lecithin in milk powder (BRASIL, 1996).

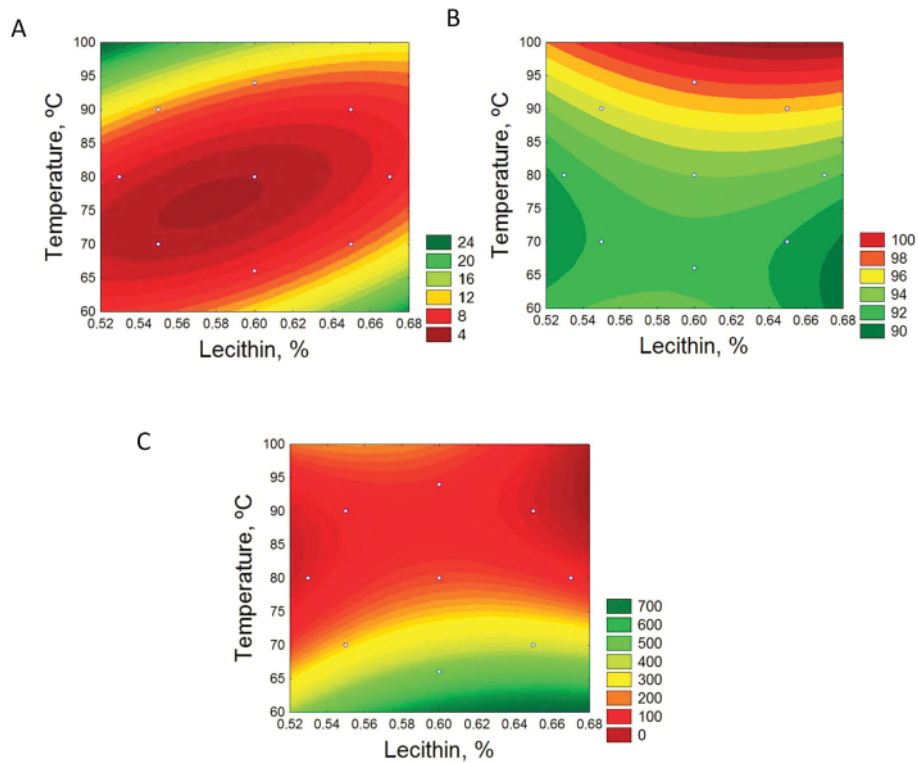


Fig. 2. Response surface for the solubility (A), dispersibility (B), and wettability (C) of freeze-dried milk powder as a function of lecithin, %, and temperature, °C

### 2.3. Percentage composition and microbiological analysis

Table 2 shows the chemical composition of samples obtained with the best lyophilisation time and yield. The results of percentage composition analyses of the freeze-dried milk powder (T4 treatment) with and without hydrolysis were analysed with Student's *t*-test with two samples assuming equal variances (Table 2). According to the results, it can be seen that for acidity percentages, for both treatments (with and without hydrolysis), there were no significant differences at 5% ( $P > 0.05$ ). The milk acidity without hydrolysis was  $1.78 \pm 0.01\%$  and with hydrolysis was  $1.77 \pm 0.03\%$ . Brazilian laws recommend a maximum of 18% milk powder acidity.

Table 2. Chemical composition analysis of T4 milk treatment

Analyses % (w/w)	T4 Milk treatment		P	$t_{calc}$
	With hydrolysis	Without hydrolysis		
Acidity	1.77±0.03	1.78±0.01	0.16233	-1.50855
Moisture	3.96±0.04	2.63±0.24	0.00000	13.41355
Ashes	8.92±0.40	10.00±0.00	0.00005	-6.64302
Protein	31.47±0.05	31.08±0.52	0.04433	2.29893
Lipid	0.00±0.00	0.00±0.00	0.00	0.00
Lactose	1.57±0.01	61.46±0.67	0.00000	-217.54011
Glucose	33.79±1.2	0.81±0.10	0.00000	67.16058

Student's  $t$ -test ( $P>0.05$ ).  $t_{crit} = 2.22813$ .

The results for moisture, ashes, protein, lactose, and glucose by the Student's  $t$ -test demonstrated statistically significant differences among the mean values obtained for the percentage composition determination of freeze-dried milk with and without hydrolysis with a range of 95% confidence (Table 2).

The freeze-dried milk powder with low lactose content showed no microbiological contamination (data not shown), which is in accordance with the Brazilian legislation for milk powder and powder mixture for the dairy based drinks preparation (ANVISA, 2001).

### 3. Conclusions

It was concluded that changing the lecithin content and temperature can improve the solubility, dispersibility, and wettability of freeze-dried milk powder with low lactose content. In order to determine the effect of lecithin addition and varied temperature for the optimum solubility, dispersibility, and wettability, the following conditions were established: lecithin 0.6% at 80 °C led to a maximum solubility of 4% RI; lecithin 0.6% at 94 °C provided a maximum dispersibility of 100%; and 0.65% lecithin at 90 °C gave a maximum wettability of 36 seconds.

Thus, there is a feasibility of producing freeze-dried milk powder with low lactose content as an alternative to heat processes. The lyophilized milk powder with low lactose content has advantages regarding the nutritional value maintenance and escaping Maillard reaction effects, which happen when the milk is submitted to high temperatures for an extended time.

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