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# DRYING OF $\alpha$ -AMYLASE BY SPRAY DRYING AND FREEZE-DRYING – A COMPARATIVE STUDY

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**Abstract** - This study is aimed at comparing two traditional methods of drying of enzymes and at verifying the efficiency of each one and their advantages and disadvantages. The experiments were performed with a laboratory spray dryer and freeze-dryer using  $\alpha$ -amylase as the model enzyme. An experimental design in star revealed that spray drying is mainly influenced by the inlet air temperature and feed flow rate, which were considered to be the main factors influencing the enzymatic activity and water activity; the long period of material exposure to high temperatures causes a partial activity loss. In the experiments of freeze drying, three methods of freezing were used (freezer, acetone and dry ice, and liquid nitrogen) and samples subsequently freeze-dried for times ranging between 0-24 hours. The product obtained from the two techniques showed high enzymatic activity and low water activity. For the drying of heat-resistant enzymes, in which the product to be obtained does not have high added value, spray drying may be more economically viable because, in the freeze drying process, the process time can be considered as a limiting factor when choosing a technique.

**Keywords:**  $\alpha$ -amylase; Spray drying; Freeze-drying; Experimental design; Comparative study.

## INTRODUCTION

The use of dehydrated enzymes for industrial applications has become increasingly common, especially in formulations of pharmaceuticals and food (Samborska *et al.*, 2005; Namaldi *et al.*, 2006; Kurozawa *et al.*, 2009; de Jesus and Maciel Filho, 2011). Among the most used enzymes we can mention  $\alpha$ -amylase. Alpha-amylase (1,4- $\alpha$ -D-Glucan glucanohydrolase; EC 3.2.1.1) is an endoenzyme that breaks down starch by hydrolysis to maltose (Lévêque *et al.* 2000). Alpha-amylase is widely used in the food and pharmaceutical industries, laundry detergents and in “desizing” in textiles (Sivaramakrishnan *et al.*, 2006; Biazus *et al.*, 2009; de Jesus and Maciel Filho, 2011).

The drying of enzymes has commonly been carried out by spray drying and freeze-drying (Çakaloz *et al.*, 1997; Maa *et al.*, 1998; Heller, 1999; McLoughlin *et al.*, 2003; Samborska *et al.*, 2005; Kudra, 2008; Santos and Silva, 2008).

Spray drying is a common method for producing powder from a liquid. During the process, the liquid is initially atomized into a chamber of heated air, creating a spray of fine droplets. The solvent quickly evaporates under these conditions, forming dried particles. These particles are then separated, by means of a cyclone, into a collection container attached to the unit (van Deventer *et al.*, 2013). Some advantages of spray drying include the ability to quickly produce a dry powder (e.g., compared to

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lyophilization) and the ability to control the particle size distribution. Some examples of the limitations of spray drying include the efficient particle collection and the potential instability of materials sensitive to high temperatures (McLoughlin *et al.*, 2003). Even so, a growing number of studies have confirmed the promise of spray drying to produce dried biopharmaceuticals (Samborska *et al.*, 2005; Namaldi *et al.*, 2006; Sivaramakrishnan *et al.*, 2006; Kurozawa *et al.*, 2009).

Also known as lyophilization, freeze-drying is a method of preservation of food and biological materials (enzymes, proteins, vitamins, etc.), floral products, drugs, and whole animals. It can also be used for the recovery of water-damaged items from fires and floods. During this process, water is removed from the material by sublimation while the material is still frozen. The frozen material is cooled to approximately  $-29\text{ }^{\circ}\text{C}$ . Freeze-drying requires pressures lower than 5.33 mbar (evaporation from the frozen state) and long drying times, considerably increasing the cost of the process (Drouzas and Schubert 1996). The material is placed on trays in a refrigerated vacuum chamber, and heat is carefully applied by conduction. As a result, any water in the material is changed directly from ice to water vapor without first changing into water (Douglas *et al.*, 2000; Biazus *et al.*, 2009). Freeze-drying has been widely used for the preparation of various types of biological materials, enzymes and foods, because this procedure is believed to be effective to maintain the biological activities of the material over a long period of time (Nakagawa *et al.*, 2013).

The aim of this study was to verify the advantages and disadvantages of spray drying and freeze-drying in the drying of enzymes for industrial use, using  $\alpha$ -amylase as the model enzyme.

## MATERIALS AND METHODS

### The Enzyme

The enzyme used was a commercial bacterial  $\alpha$ -amylase (AQUAZYM<sup>®</sup> 120L by Novo Nordisk) from *Bacillus* sp.

### Spray Drying

The experiments were carried out with a borosilicate laboratory spray dryer Lab-plant, SD-04 model (Huddersfield, UK), equipped with a twin fluid

spraying nozzle of 0.5 mm diameter and inside chamber with 50 cm height and outside diameter of 21.5 cm.

Five hundred grams of enzymatic broth were used for each drying experiment with a beak atomizer. During operations, five feed flow rates were used as follows: 0.20, 0.23, 0.31, 0.38, and 0.41 mL.min<sup>-1</sup>, by means of a peristaltic pump to a nozzle, where atomization of the solution was established using a pressurized air stream (Table 1). Air compression was 0.41 bar (vacuum pump operating from 1 to 100 rpm). Drying air entered the drying chamber in the same direction as the descending spray droplets. The main process variable was the dryer air inlet temperature (130, 145, 180, 215, and 230  $^{\circ}\text{C}$ ). The outlet air temperature could not be controlled directly, but it was a function of the air inlet temperature and solution feed rate. Outlet air temperatures were observed as 78, 85, 90, 111, 117, 120, 140, 145, and 156  $^{\circ}\text{C}$  corresponding to the feed solution rate and inlet air temperatures (Table 1).

The spray dryer experiments were optimized through experimental design in star with two variables in order to investigate the effects of feed flow rate and the inlet air temperature upon enzymatic activity and water activity (Table 1).

Response surface methodology (RSM) was used to estimate the main effects (Barros Neto *et al.*, 2007).

The calculation for the two value coded assay in star design is as follows  $\alpha = (2^n)^{1/4}$ , where n is the number of independent variables. A complete 2<sup>2</sup> factorial experimental design was applied to analyze the two variables (four experiments), with axial points for  $\alpha = \pm 1.41$  (four experiments) and three experiments at the central point, giving a total of eleven experiments (Barros Neto *et al.*, 2007). The variables chosen were inlet air temperature (T,  $^{\circ}\text{C}$ ) and feed flow rate (Q, mL.s<sup>-1</sup>) and enzymatic activity and water activity were the response.

Data were analyzed using the software package *Statistica 7.0* (Statsoft, Tulsa, USA).

### Freeze-Drying

The experiments were performed with a vertical freeze-dryer Telstar, Cryodos -80 model (Terrassa, Spain). During the lyophilization process, the chamber temperature was maintained at approximately -90  $^{\circ}\text{C}$  and 0.05 mbar.

Initially, the enzymatic broth was frozen with liquid nitrogen, dry ice and acetone, or in a freezer (refrigerator).

**Table 1: Coded and actual level experimental matrix of the factorial design, enzymatic activity and water activity (response) obtained from the spray drying process.**

Experiment	Coded		Real			Response	
	X <sub>1</sub>	X <sub>2</sub>	T (°C)	T* (°C)	Q (mL.s <sup>-1</sup> )	EA (U.mL <sup>-1</sup> )	a <sub>w</sub>
1	-1	-1	145	90	0.23	0.77	0.47
2	+1	-1	215	145	0.23	0.66	0.32
3	-1	+1	145	85	0.38	0.78	0.57
4	+1	+1	215	140	0.38	0.70	0.36
5	-1.41	0	130	78	0.31	0.89	0.61
6	+1.41	0	230	156	0.31	0.77	0.30
7	0	-1.41	180	120	0.20	0.74	0.34
8	0	+1.41	180	111	0.41	0.72	0.42
9	0	0	180	117	0.31	0.71	0.41
10	0	0	180	117	0.31	0.72	0.38
11	0	0	180	117	0.31	0.71	0.39

T\* is the outlet air temperature, T is the inlet air temperature and Q is the feed flow rate.

For each drying experiment, a glass tube of 40 mm in diameter containing 5.0 mL of the enzymatic broth was immersed in liquid nitrogen or dry ice and acetone or in a conventional freezer. The freezing temperature was measured by a temperature data logger device (OMRON E5CN model, Illinois, USA) coupled with an entrance K thermocouple. The cooling rates were obtained by the following expression:

$$W = \frac{0 - T}{\Delta t} \quad (1)$$

where: W is the rate of cooling [°C.min<sup>-1</sup>], T is the temperature [°C] and  $\Delta t$  is the freezing time [min].

The samples were dried for different times (from four to twenty-four hours).

The material obtained was analyzed to determine the enzymatic activity (EA), water activity (a<sub>w</sub>) and moisture content.

### Enzymatic Activity

Alpha-amylase activity was tested using the initial solution containing 7% (m/v) soluble starch (substrate) at pH 6, NaCl 0.05% (w/v) and KCl 0.6% (w/v). The solution was incubated at 37 °C for 5 min. Twelve microliters of the sample were added to 2.0 mL of the standard assay reaction mixture (Laborlab S.A., São Paulo, Brazil) and incubated at 37 °C for 10 min. The activity of the alpha-amylase was spectrophotometrically determined by the increase in absorbance at 505 nm (spectrophotometer UV/VIS Sintra 10e by GBC, Australia) (de Jesus and Maciel Filho, 2011).

The dehydrated material was suspended again in distilled water and taken to a water bath for its complete solubility and soon after the enzymatic activity

was determined according to the aforementioned method.

A linear calibration curve was established by analyzing standard solutions of glucose between 0.13 and 0.2  $\mu\text{mol.mL}^{-1}$ .

One unit of enzymatic activity (U) was defined as 1  $\mu\text{mol}$  of glucose produced per minute under the given conditions (37 °C and pH 6).

### Water Activity

Measurements were made using a water activity meter (Aqua Lab Model 3 TE Series, Decagon, USA).

The water activity of all dried samples was measured at 25 °C.

### Moisture Content

The enzyme's moisture content was determined using thermogravimetric analysis, TGA 50 model (Shimadzu, Japan).

## RESULTS AND DISCUSSION

The enzymatic broth initially had an enzymatic activity (EA) of 0.93 U.mL<sup>-1</sup>, moisture content (X<sub>0</sub>) of 4.42 kg-H<sub>2</sub>O.kg<sup>-1</sup> d.b. and water activity (a<sub>w</sub>) of 0.93 at 25 °C and pH 6 (de Jesus and Maciel Filho, 2011).

### Spray Drying

Table 1 presents the results obtained for enzymatic activity and water activity for the 11 experimental runs of spray drying completed during this study. The experimental design showed that the inlet air temperature and feed flow rate influenced the

enzymatic activity. Studies have shown that the loss of activity depended primarily on the inlet flue gas temperature and the temperature distribution within the dryer (Heller 1999, Samborska *et al.*, 2005).

Another critical variable in spray drying is the outlet temperature. A low outlet temperature is necessary to avoid loss of activity. In order to have a suitable operational policy, it is important to consider that the outlet temperature increased with increasing inlet temperature and decreased with increasing liquid feed rate (Table 1) (Maa *et al.*, 1998, Arpagaus 2012). Similarly, the outlet air temperature should be kept low in order to obtain high enzymatic activity in dried  $\alpha$ -amylase. The important observation to be made is related to the fact that, for high inlet air temperatures and low feed flow rates, the outlet temperature compromises the enzymatic activity.

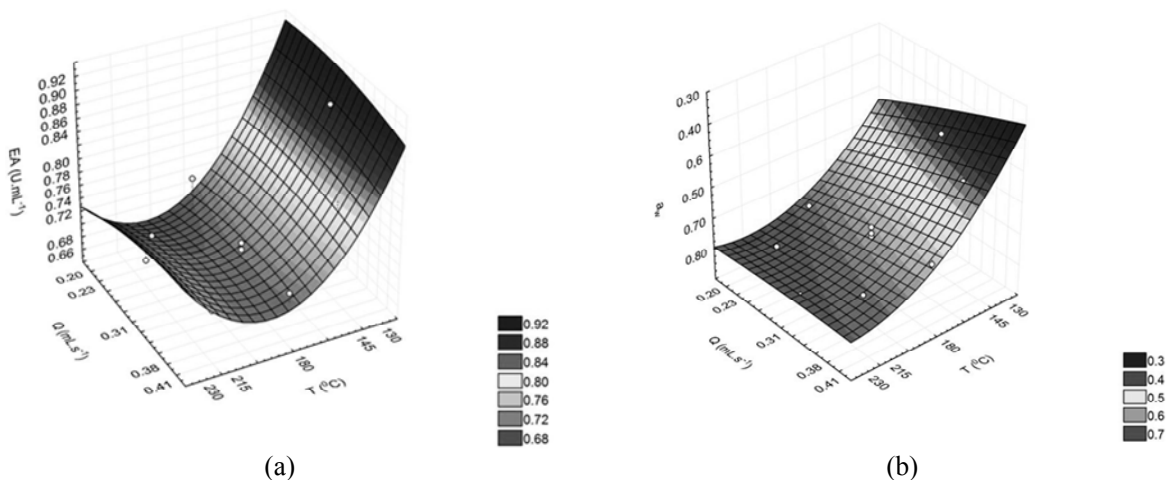
The surface response shows that, for high flow rate and low inlet air temperature, there is a significant increase in the enzymatic activity (Figure 1). When the process was carried out at the central point ( $T = 180\text{ }^{\circ}\text{C}$  and  $Q = 0.31\text{ mL}\cdot\text{s}^{-1}$ ), there was a considerable loss of the original enzymatic activity, on the order of 24%; however, when the process was carried out at lower temperatures, there was a greater preservation of enzyme activity, mainly in experiment number 5 (Table 1). For very high inlet air temperature and low feed flow rate, there is noted a drastic reduction in enzymatic activity (Figure 1a).

By measuring water activity, it is possible to predict which microorganisms will or will not be potential sources of spoilage. Lower water activity of a dried product implies better potential for storage. Small  $a_w$  values were obtained when the experiments were conducted with low flow and, mainly, high

temperatures (Figure 1b). In this case, the residence time of the enzymatic broth can be considered as the main factor for the decrease of water activity, as well as the increase of the temperature of the inlet gas, resulting in improved temperature distribution inside the dryer, so that the material has a larger dehydration. Furthermore, the increased temperature dramatically decreases the enzymatic activity (Figure 1a); finding a good enzymatic activity/water activity relationship is one of the major problems involving the drying process of enzymes. By analyzing the response surface (Figures 1a and 1b), the best results are obtained when the process is performed at temperatures around  $145^{\circ}\text{C}$  and flows of  $0.23\text{ mL}\cdot\text{s}^{-1}$ . In this case there was a preservation of 83% of the initial activity and a water activity of 0.47. The increase in the temperature of the inlet air results in increased temperature of the outlet air due to the increased supply of thermal energy; with increased feed flow rate, the temperature of the outlet air drops significantly due to the increased rate of water evaporation (Samborska *et al.*, 2005).

### Freeze Drying

The freezing methods used before lyophilization can substantially affect the structure of the ice formed, the water-vapor flow during primary drying and the quality of the final dried product. Controlling how a solution freezes can shorten lyophilization cycles and produce more stable formulations (Santana *et al.*, 2010). Some proteins undergo cold denaturation during slow freezing or annealing, which can have deleterious effects on product quality upon reconstitution.



**Figure 1:** (a) Response surface: product enzymatic activity as a function of the variables feed flow rate and inlet air temperature; (b) Response surface: product water activity as a function of the variables feed flow rate and inlet air temperature.

Freezing methods can alter the void–solid interfacial area of a lyophilized cake and, inversely, the thickness of its channel walls. An increased surface area correlates with decreased dried product stability for some proteins (Santana *et al.*, 2010). Freezing by immersing a glass tube into liquid nitrogen can result in increased protein aggregation (Santana *et al.*, 2010) or decreased enzymatic activity (Sadikoglu *et al.*, 2006) when compared with freezer-cooled samples.

The freezing methods used in this study were freezing by liquid nitrogen, dry ice and acetone, and freezer (refrigerator). Table 2 shows the freezing rates obtained for the three freezing methods. A greater rate of cooling was obtained with liquid nitrogen at  $-195\text{ }^{\circ}\text{C}$ . Figure 2 shows mass loss versus drying time and we can observe that, after 16 hours of drying, the residual moisture was constant. When the freezing was performed with liquid nitrogen, it was found that a period of 4 hours of drying was enough to achieve a product with low moisture content. Extensive investigation of primary drying has demonstrated that chamber pressure and shelf temperature are two important parameters (Pirozzi *et al.*, 1996, Alzamora *et al.*, 2003). In this work, the chamber pressure was maintained at approximately 0.05 mbar and a temperature of  $-90\text{ }^{\circ}\text{C}$  was used for all experiments.

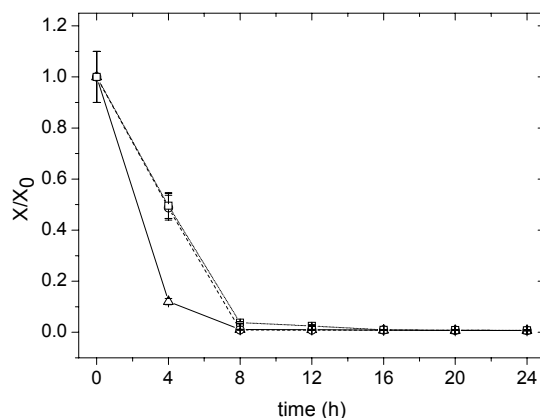
Figure 3 shows the enzymatic activity and water activity as a function of the drying time.

The kinetics of the lyophilization showed that the enzymatic activity (Figure 3a) and water activity (Figure 3b) decreased with drying time; after 4 hours of process, approximately 98% of the initial enzymatic activity was preserved, but water activity was not reduced significantly, indicating a higher residual moisture content, except when the material was frozen with liquid nitrogen. This is evidence that the lyophilization process is favored by higher cooling rates. Good results were obtained after 16 hours of process, since dehydrated material showed water activity of approximately 0.2, except when the material was frozen in freezer, when water activity was higher (close to 0.4). It was observed that the freezing of the material carried out with liquid nitrogen achieved higher enzymatic activity. After 20 hours of the process, it was observed that the moisture content remained constant for freezing with liquid nitrogen. Similar results were obtained after 24 hours of the process for the three techniques. In this study, the freezing method had a significant effect on the enzymatic activity and water activity during and after the drying process.

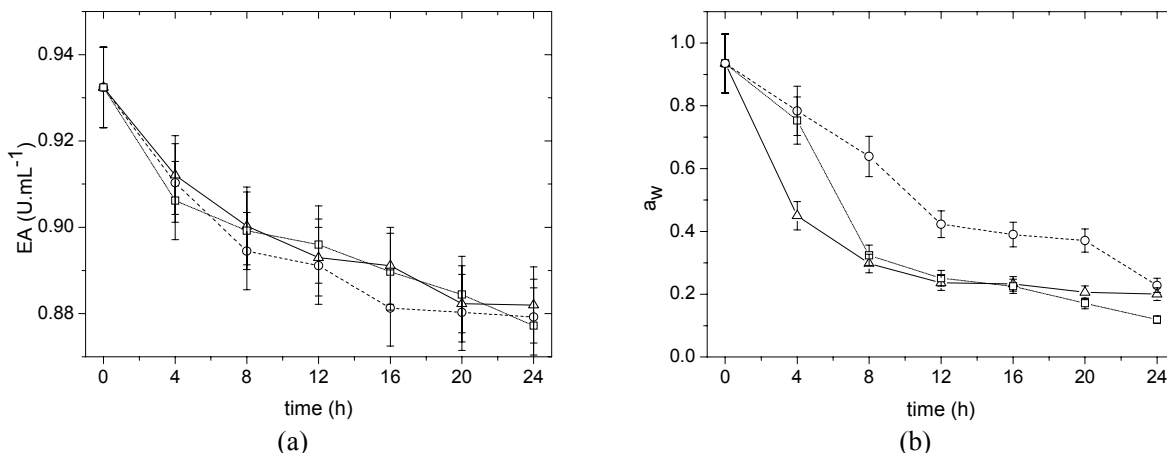
In the freeze drying process with the enzyme  $\alpha$ -amylase, the enzyme activity obtained was greater than that in the atomization process. The activity at the end of the process had a preservation of approximately 95% with an average water activity of 0.25. However, a period of 12 hours was necessary to reach those conditions (Figure 3).

**Table 2: Rate of cooling for contact with liquid nitrogen, dry ice and acetone and in the freezer.**

Technique of freezing	Temperature ( $^{\circ}\text{C}$ )	Freezing time (min)	Freezing rate ( $^{\circ}\text{C}\cdot\text{min}^{-1}$ )
Liquid nitrogen	-195.8	1.89	103.60
Dry ice and acetone	-82.0	7.22	11.36
Conventional freezer	-35.0	243.00	0.14



**Figure 2:** Drying curve of samples lyophilized after freezing with: ( $\Delta$ ) liquid nitrogen; ( $\circ$ ) dry ice and acetone; ( $\square$ ) conventional freezer.



**Figure 3:** Influence of drying time on the (a) enzymatic activity and (b) water activity; initial freezing in ( $\Delta$ ) liquid nitrogen; ( $\circ$ ) dry ice and acetone; ( $\square$ ) conventional freezer.

The process of freeze drying resulted in a more complete drying and preserved more enzyme activity; however, for large scale production it becomes economically unviable, being advisable only for processes which require the use of this enzyme in the dried form and with high catalytic activity, such as in the pharmaceutical industry, where the conversion of a substrate into a desired product cannot generate byproducts and the conversion rate has to be 100%. However, for processes with medium or low added value, in which there is the need to use cheaper reagents and substrates, the use of enzymes dried by spray drying becomes economically more feasible. However, it is noteworthy that the enzyme  $\alpha$ -amylase is a thermotolerant enzyme, being resistant to high temperatures. Thus, in experiments with spray drying, even with the high temperatures of the inlet air and the low feed flow rate, at least 70% of the initial activity was preserved.

### CONCLUDING REMARKS

In this study, we compared the efficiency of the spray drying and freeze drying processes for  $\alpha$ -amylase drying. The material dehydrated by the two techniques was analyzed for its enzymatic activity and water activity. An experimental design in star revealed that spray drying is mainly influenced by inlet air temperature and feed flow rate, which were considered to be the main factors influencing the enzymatic activity and water activity; the long period of material exposure to high temperatures caused a partial activity loss. Finally, it was verified that the freezing method influences the final product quality

after the lyophilization process, and the freezing with nitrogen was considered to be the most appropriate.

The product obtained from the two techniques showed high enzymatic activity and low water activity. For the drying of heat-resistant enzymes, in which the product to be obtained does not have high added value, the spray drying may be more economically viable because, in the freeze-drying process, the process time and the high energy consumption can be considered as limiting factors when choosing a technique. Through this study we also conclude that the process of freeze drying is recommended for the drying of thermolabile enzymes or processes that require the use of dehydrated enzyme with high enzymatic activity, whose end product is of high added value.

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