

# Microencapsulation of Lipase and Savinase Enzymes by Spray Drying Using Arabic Gum as Wall Material

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## Abstract

Enzymes have been used in detergents over the years. They can improve the detergent's efficiency due to their activities against hard stains. Nevertheless, enzymes cannot maintain their properties indefinitely, since they are exposed to stress factors, like temperature, pH, mechanical processes and others. Consequently, enzymes lose their structure and they are not functional. For this reason, microencapsulating these proteins is a feasible solution to improve their use in industrial processes and commercial products. Spray drying technology has been selected because a lot of scientific literature proved its useful application in a variety of industries. In particular, savinase and lipase are the two encapsulated enzymes in this work. Savinase attacks proteins and lipase removes fats, so they are suitable enzymes for detergent industry. Arabic gum has been used as wall material. Morphology, size and activity of the obtained microcapsules have been analyzed in order to find the best conditions to produce them. In conclusion, useful microcapsules of lipase and savinase can be obtained with the mentioned technology.

## Keywords

Savinase, Lipase, Arabic Gum, Spray Drying, Microencapsulation

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## 1. Introduction

Clothes can get dirty with inorganic and organic stains. Inorganic dirt can be removed easily, while organic stains are often insoluble in water and they are stuck on clothes. In this type of dirt, enzymes are useful [1].

Enzymes have been used to improve the cleaning efficiency of detergents for more than 35 years. They are catalytic proteins. The action of enzymes in detergents depends

on a variety of factors, like washing temperature, type of stains to be removed, detergent's composition, washing procedure and wash-water hardness. However, all types of enzymes are not acceptable for their use in detergents. In order to use enzymes in detergents, they should have some characteristics [2]:

- Stability at temperature is over a broad range from 20°C to 50°C and even above;
- The optimum pH should be in alkaline range;
- It should be detergent compatible;
- It should have specificity towards different compounds.

Over the years, enzymes have been attractive in detergent industry because they are derived from renewable sources, they reduce energy use and costs and they are highly space-efficient, since a little amount of enzyme can remove a lot of different stains [3]. Moreover, enzymes account for about 6% of the total raw material costs for powder detergents [4].

Lipase and savinase are two types of enzymes which are used in detergents due to their cleaning properties. On one hand, lipase is classified as hydrolase, since it catalyzes the hydrolysis of water-insoluble long chain triglycerides [5]. It can remove tomato, butter, oils, chocolate and cosmetic stains [2]. On the other hand, savinase represents a subgroup of high-alkaline proteases belonging to subtilase enzyme family, so they catalyze the cleavage of peptide bonds in other proteins [6] [7]. Specifically, it is a serine protease [3]. Savinase enhances the cleaning of protein-based soils, such as grass, blood, human sweat and egg [2].

However, the stability of these enzymes is not good. They should add to detergents formulation and be employed in laundering. This is a mechanical process which could break the enzymes. Furthermore, lipase and savinase are protein molecules, so they can be denatured and inactivated under certain conditions, like an improper pH or temperature, so their activity could decline [8]. Concretely, savinase has got an irreversible thermo-inactivation at 70°C, which seems to lead to the auto digestion of that enzyme [9]. Therefore, microencapsulation can be a possible solution for this inconvenient.

Microencapsulation technique has been used in chemical industry for different types of products (fertilizers, pesticides, pharmaceuticals, carbonless paper, scented products, detergents, etc.) since 1930s [10]. For example, this technique is one of the most important used to process food because it preserves food properties [11], [12]. In microencapsulation, a wall material represents the package of different active ingredients, which can be solid, liquid or gaseous [13].

Enzymatic encapsulation in detergents confers a series of advantages among which the followings stand out:

- A) Improvement of the catalytic stability against temperature and pH in the washing environments.
- B) Increased tolerance to organic solvents and other inhibitors present in detergents.
- C) Increased mechanical stability.
- D) Increase in the rate of enzymatic reuse.
- E) Increased profitability and viability of manufacturing processes.

F) Improvement of functionality in bioreactors.

The purposes of using microencapsulation in these products are, to achieve controlled release, to protect sensitive materials from their environment or to make active materials easier and safer to handle [10].

The term “microcapsule” is defined as a spherical particle with a size between 50 nm to 2 mm and it contains a core substance. Microcapsules vary in size and shape and they may also possess additional external wall [14]. Choosing a correct wall material is essential because it must protect the active ingredient [15], [16]. In this case, we have used Arabic gum. It is a natural polysaccharide composed of sugars galactose, arabinose, rhamnose and glucuronic acids. It contains a protein component, too [17]. Most studies have confirmed that Arabic gum is useful in microencapsulation because of its properties [18].

The aim of the present research is the microencapsulation of lipase and savinase with spray drying using Arabic gum as shell material and spray drying as a microencapsulation technology in order to:

- Prevent or retard degradation of the enzyme in detergent formulations.
- Separate incompatible substances that can affect the enzyme activity in detergent formulations.
- Facilitate the dosage of detergent powder.

This process would allow us to have these enzymes in a more stable way so they could be used in detergents more efficiently. In this article, we are going to describe the stages of achieving enzymes' microcapsules and a way to verify that they are functional. Parameters such as inlet temperature, suction power and mass relation between Arabic gum/enzyme will be optimized in order to obtain microcapsules with adequate morphology and high encapsulation efficiency and encapsulation yields. Scanning electron microscopy allows identifying the microcapsules morphology and spectrophotometry methods allow to determinate the activity of the enzymes.

## 2. Materials and Methods

### 2.1. Materials

Arabic gum from Acacia tree (Sigma-Aldrich, Spain) has been used as shell material. We have chosen this compound because it is soluble in water, so it will set free the active ingredients during the washing cycle. The core materials are the two enzymes, used separately: commercial lipase (LIPEX 100L) (Univar Iberia, Spain) and commercial savinase (SAVINASE 16L) (Univar Iberia, Spain).

On the other hand different reagents have been used to determine the enzyme activity:

- For lipase analysis: 4-nitrophenyl acetate (PNPA) (Sigma-Aldrich, Spain),
- For savinase analysis: porcine hemoglobin (Sigma-Aldrich, Spain), trichloroacetic acid (Sigma-Aldrich, Spain), sodium hydroxide (Sigma-Aldrich, Spain) and Folin & Ciocalteu's phenol reagent (Sigma-Aldrich, Spain).

In both tests, phosphate buffered saline medium at pH 7.4 (Sigma-Aldrich, Spain)

was used.

## 2.2. Emulsion Preparation

As we had to find the correct conditions of the microencapsulation process, some experimental matrices were designed in order to determine the most suitable values.

Tested proportions of the materials are showed in the **Table 1**.

The emulsion has been completed with distilled water until 100 ml in lipase experiments and 50 ml in savinase tests. In this way, homogeneous emulsions have been obtained.

Emulsions were prepared at a constant agitation speed of  $1200 \text{ min}^{-1}$ , during 15 min at room temperature.

The best proportions were 1:1 for lipase and 1:2 for savinase according the SEM study.

## 2.3. Microencapsulation by Spray Drying

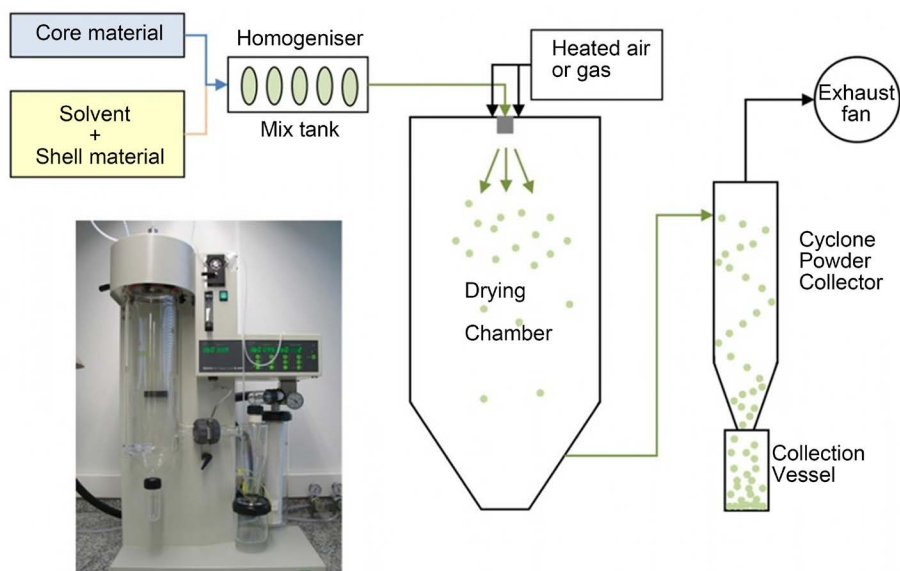
Spray-drying was performed using a spray-dryer BÜCHI B-290 (BÜCHI, Switzerland) with a standard 0.7 mm nozzle. The **Figure 1** is a spray-dryer's diagram [19].

The experimental parameters are showed in **Table 2**.

Low temperatures were selected because proteins can be denatured at temperatures

**Table 1.** Emulsion preparations of lipase and savinase microcapsules.

<b>Mass ratio (lipase: Arabic gum)</b>	1:0.8; 1:1; 1:1.3; 1:1.4; 1:1.6; 1:2
<b>Mass ratio (savinase: Arabic gum)</b>	1:1; 1:1.25; 1:2; 1:2.5; 1:2.8; 1:3



**Figure 1.** Diagram of spray drying process. Feed is atomized; it gets in contact with spray-air; drying is produced in the drying chamber; and the dried product is separated from the drying air in a cyclone powder collector.

**Table 2.** Experimental spray dried conditions for lipase and savinase.

Enzyme	Inlet temperature (°C)	Feed flow rate (%)	Suction power (%)	Air flow rate (cm)
LIPASE	60	20	75	4
	65	20	75	4
	65	10	75	4
	60	10	75	4
	55	10	75	4
	55	10	70	4
	55	10	65	4
SAVINASE	55	10	65	4
	65	10	65	4
	60	10	65	4

around 70°C [20]. 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 suitable experimental conditions and proportions are showed in **Table 3**:

### 3. Characterization Tests

#### 3.1. Determination of Microcapsules Morphology. Scanning Electron Microscopy (SEM)

For microcapsules surface observation, a PHENON scanning electron microscope (FEI Company, United States) was used. Each sample was fixed on a standard sample holder and sputter coated with gold. Samples were then examined with suitable acceleration voltage and magnification [21].

#### 3.2. Determination of Encapsulation Yield (EY%)

The encapsulation yield was calculated as ratio of the weight of the resultant powder after spray drying from the collecting bottle and the weight of all solids (including wall and core materials) in the emulsion, expressed as percentage.

Some microcapsules adhering to the walls of the drying chamber or cyclone were not considered, so this yield will be only approximate.

Equation 1 allows calculating the enzymes encapsulation efficiency:

Equation 1—Process EY (%)

$$EY (\%) = 100 \times \frac{W_f}{W_s}$$

$W_f$ : final weight collected (g).

$W_s$ : weight of initial solids (g).

**Table 3.** Best experimental conditions.

Enzyme	Enzyme (mass ratio)	Gum arabic (mass ratio)	Inlet temperature (°C)	Outlet temperature (°C)	Feed flow rate (%)	Suction power (%)	Air flow rate (cm)
LIPASE	1.0	1.0	55	36	10	65	4
SAVINASE	1.0	2.0	60	37	10	65	4

### 3.3. Particle-Size Distribution (Mastersizer)

Mastersizer E 3000 (Malvern Instruments Ltd., UK) is a particle-size analyst. It measures the particle-size distribution due to the measurement of dispersed light intensity. This fact occurs when a laser goes through the dispersed sample of particles. So, microcapsules of savinase and lipase were dispersed in 15ml of ethanol, due to the insolubility of Arabic gum in this medium. Then, they were analyzed by this method.

### 3.4. Study of Enzyme's Activity

The protein activity was analyzed by spectrophotometry using a spectrophotometer UV-vis (Thermo scientific, Spain). The protein content will be determined by comparing the absorbance of enzyme samples before and after microencapsulation process.

In the case of lipase, it has got esterase activity too. So, lipase can hydrolyze 4-nitrophenyl acetate (PNPA) and the product of the reaction, 4-nitrophenol, has got a maximum absorbance at 400 nm. However, savinase is a protease, so the method of Anson was used [22].

Different concentrations of commercial enzymes were prepared: 0.2, 0.5, 1, 1.5 and 2 (mg/ml). In all of them, the phosphate buffer at pH 7.4 was used.

First, 50 µl of each concentrations of lipase were dissolved in the buffer and then 30µl of PNPA 30 mM were added and shaken. The solution was left in repose, for 1 hour, for obtaining a stable reaction. Finally, the absorbance was measured at 400 nm for all samples. With the obtained results, was realized the calibration curve for lipase.

For savinase, the same concentrations of enzymes were used. 5 ml of hemoglobin 20 mg/ml and 1 ml of enzyme were mixed during 10 minutes at 35.5°C. Next, 10 ml of TCA 0.3 M were added and the samples were centrifuged during 30 minutes at 4000 rpm. 5 ml of the supernatant was taken and mixed with 10 ml of NaOH 0.5 M and 3 ml of Folin & Ciocalteu's phenol reagent. In addition, samples were diluted at a ratio of 1:10 and the absorbance was measured at 750 nm. Thus, a calibration curve was realized for savinase.

The same protocol was followed for the microcapsules using two concentrations of the microcapsules: 2 mg/ml and 4 mg/ml. Three replicates were done for each concentration.

## 4. Results and Discussion

### 4.1. Microcapsules Morphology. Scanning Electron Microscopy (SEM)

The morphology of the enzymes microcapsules was studied by scanning electron mi-

croscopy (SEM). **Figure 2** shows the micrographs of microcapsules obtained in the best conditions.

Inlet temperature must not be high because enzymes could lose their activity. Both enzymes require similar conditions.

#### 4.2. Determination of Encapsulation Yield (EY%)

**Table 4** shows encapsulation yields of the most suitable microcapsules. They were obtained using the mass ratio 1:1 (enzyme:Arabic gum) for lipase and 1:2 for savinase. Although yields are not high, they represent good results. Some trials revealed higher yields, but the morphology of the microcapsules was not correct. This may be due to the mass ratio, considering that we employed similar experimental conditions in all trials.

#### 4.3. Particle-Size Distribution (Mastersizer)

The results of this analysis are showed in **Figure 3**.

These diagrams indicate the size of the microcapsules. Lipase microcapsules have a size between 2 and 30  $\mu\text{m}$  approximately. Savinase microcapsules have similar size. The majority of microcapsules dimensions are around 9  $\mu\text{m}$ , so they have an appropriate size.

#### 4.4. Results of Study of Enzyme Activity

The enzymes calibration curves obtained are the next (**Figure 4**).

The absorbance of two enzymes samples with different concentrations, 2 and 4 mg/ml, were measured. To achieve 2 mg/ml sample 50 mg of microcapsules were weighed, and 100 mg for 4 mg/ml sample. Then, 25 ml of phosphate buffer were added.

The results are shown in the next **Table 5**:

The next stages allowed us to calculate theoretical and experimental concentrations:

-Theoretical concentrations (**Table 6**)

1) Relation (mass ratio) between enzyme: Arabic gum. It is 1:1 for lipase and 1:2 for savinase.

2) Amount of enzyme and gum according to their mass ratio.

3) Concentration of enzyme in dissolution.

4) Calculate the enzyme amount in 50  $\mu\text{l}$  that were used for tests.

5) Amounts of enzyme and gum considering the yield of microencapsulation process.

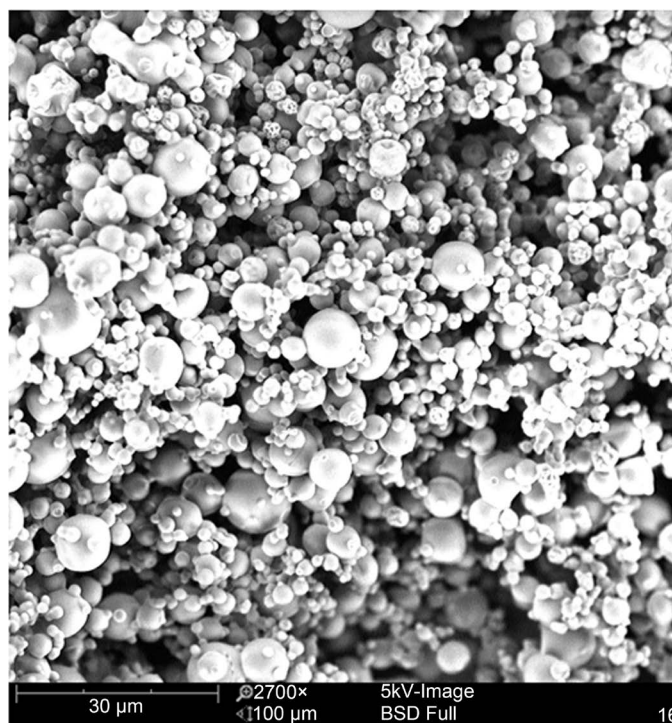
-Experimental concentrations (**Table 7**)

1) Calculate concentrations with absorbance and calibration straights. They are concentrations in 50 $\mu\text{l}$  of dissolution.

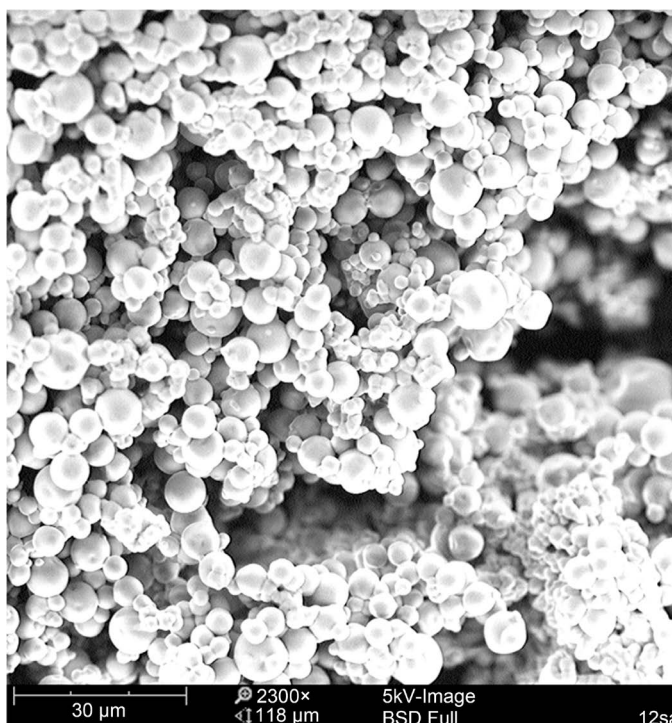
2) Calculate amount of enzyme in weighted microcapsules.

As a result, the theoretical amounts of enzymes are higher than experimental amounts. The next **Table 8** summarizes them.

Studies have shown that the loss of activity depended primarily on the inlet flue gas temperature and the temperature distribution within the dryer [23] [24]. Another



(a) 2700×



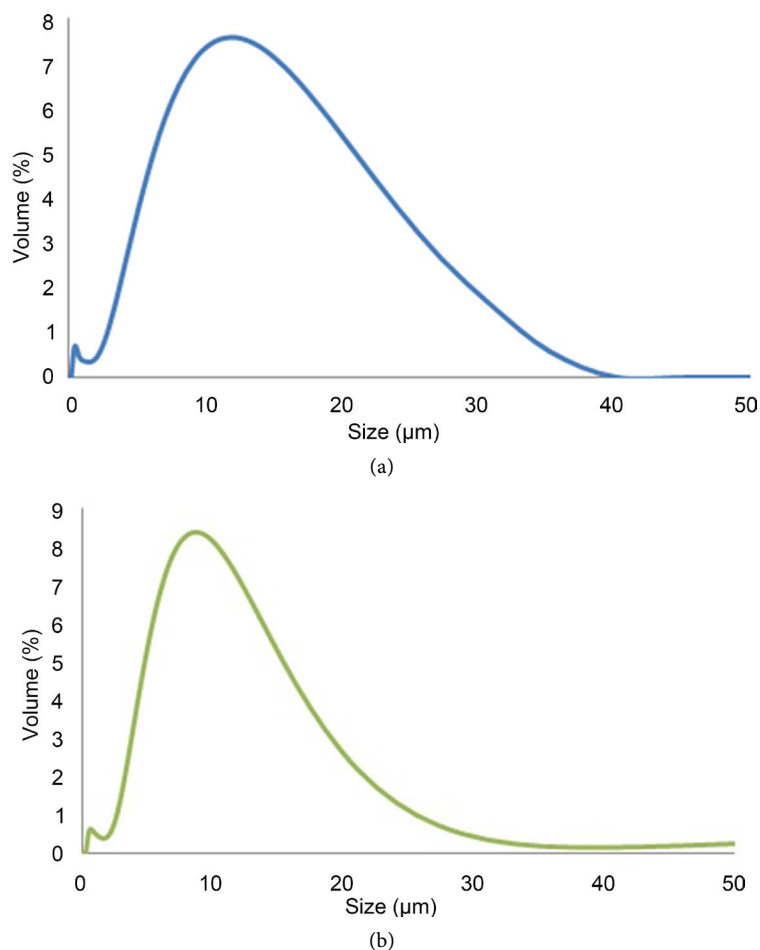
(b) 2300×

**Figure 2.** SEM photographs of Lipase microencapsulated (experimental conditions: inlet temperature (IT) 55 °C, feed flow rate (FFR) 10%, suction power (SP) 65%, air flow rate (AFR) 4 cm, 1:1 mass relation) (a); and savinase microencapsulated (experimental conditions: IT 60 °C, FFR 10%, SP 65%, AFR 4 cm, 1:2 mass relation) (b) in the most suitable experimental conditions.



**Table 4.** Encapsulation yield (%) of the best results.

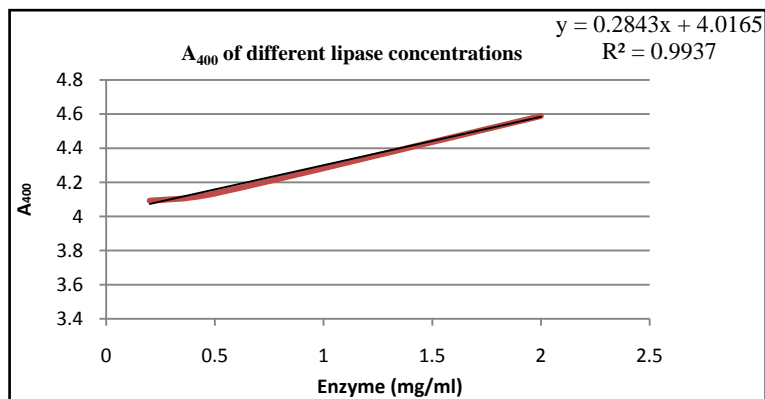
Enzyme	Initial solids (g)	Collected (g)	Yield (%)
Lipase	10	3.99	39.95
Savinase	7.25	2.67	36.86



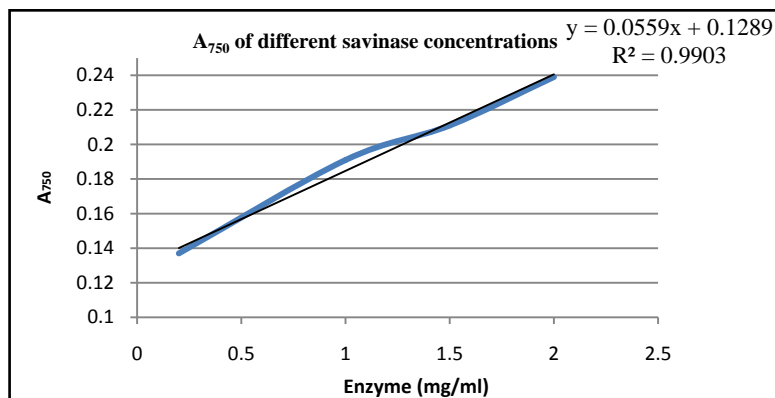
**Figure 3.** Particle-size distribution. (a) Lipase, experimental conditions: inlet temperature (IT) 55°C, feed flow rate (FFR) 10%, suction power (SP) 65%, air flow rate (AFR) 4 cm, 1:1 mass relation; (b) Savinase, experimental conditions: IT 60°C, FFR 10%, SP 65%, AFR 4 cm, 1:2 mass ratio.

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 [25] [26] [27]. In our study we have optimized parameters such as inlet temperature, suction power and mass relation in order to obtain microcapsules with adequate morphology and high encapsulation efficiency and, we calculated the enzyme concentration in microcapsules in order to observe the loss enzyme activity in the best microcapsules.

The outlet air temperature was kept low in both experiments (around 36°C - 37°C)



(a)



(b)

**Figure 4.** Calibration curves obtained measuring absorbance at 400 nm for lipase (a) and 750 nm for savinase (b).

**Table 5.** Absorbance of enzymes samples.

Enzyme	Concentration of microcapsules (mg/ml)	Absorbance
Lipase	2	4.069
	4	4.107
Savinase	2	0.140
	4	0.153

**Table 6.** Theoretical amount of savinase and lipase in microcapsules.

Enzyme	Microcapsules (mg)	Theoretical amount of enzyme (mg)
Lipase (2 mg/ml)	50	9.98
Lipase (4 mg/ml)	100	19.97
Savinase (2 mg/ml)	50	5.72
Savinase (4 mg/ml)	100	11.44

**Table 7.** Experimental amount of savinase and lipase in microcapsules.

Enzyme	Microcapsules (mg)	Concentrations of enzyme (mg/ml)	Experimental amount of enzyme (mg)
Lipase (2 mg/ml)	50	0.19	4.62
Lipase (4 mg/ml)	100	0.32	7.96
Savinase (2 mg/ml)	50	0.27	4.93
Savinase (4 mg/ml)	100	0.63	10.74

**Table 8.** Theoretical and experimental amount of enzymes.

Enzyme	Theoretical amount of enzyme (mg)	Theoretical amount of enzyme considering yield (mg)	Experimental amount of enzyme (mg)
Lipase (2 mg/ml)	25	9.98	4.62
Lipase (4 mg/ml)	50	19.97	7.96
Savinase (2 mg/ml)	15	5.72	4.93
Savinase (4 mg/ml)	30	11.44	10.74

in order to obtain high enzymatic activity in dried microcapsules. 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 loss of activity for each enzyme in the microencapsulation process has been (Table 9).

## 5. Conclusions

In this work, we studied the microencapsulation by spray drying processes for lipase and savinase enzymes. The spray drying conditions were selected based on the influence of different parameters in enzyme activity.

The inlet air temperature and feed flow rate were selected based on the influence of the enzymatic activity. The loss of activity depended primarily on the inlet temperature and the time of the drying.

From the study of morphology and size of the microcapsules, the optimal conditions were selected. The product obtained showed a perfect morphology and size in an efficient way, although encapsulation yields are not high.

So, this work demonstrated that it is possible to obtain microcapsules of savinase and lipase with the established conditions even working at low temperatures, giving that spray drying usually needs higher inlet temperatures, although a part of enzymes has been inactivated during the microencapsulation process and, a loss of enzymatic activity was obtained.

The loss of enzymatic activity was considerable in lipase enzyme microencapsulated, but in savinase enzyme microencapsulated this lost was smaller. Therefore for savinase enzyme, the microencapsulation process is technically and economically feasible.

**Table 9.** % of loss activity.

Lipase	55%
Savinase	11%

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## References

- [1] Zhang, J., Zhang, Y., Li, W., Li, X. and Lian, X. (2014) Optimizing Detergent Formulation with Enzymes. *Journal of Surfactants and Detergents*, **17**, 1059-1067. <https://doi.org/10.1007/s11743-014-1626-x>
- [2] Bhatt, S.M. (2008) Amity Institute of Biotechnology. <https://es.scribd.com/doc/11523933/Enzymes-in-Detergents>
- [3] Olsen, H.S. and Falholt, P. (1998) The Role of Enzymes in Modern Detergency. *Journal of Surfactants and Detergents*, **1**, 557-567. <https://doi.org/10.1007/s11743-998-0058-7>
- [4] Edser, C. (2009) Focus on Surfactants. [https://www.researchgate.net/publication/248211794\\_Expanding\\_the\\_Scope\\_of\\_Detergent\\_Enzymes](https://www.researchgate.net/publication/248211794_Expanding_the_Scope_of_Detergent_Enzymes)
- [5] Esteban-Torres M., Reverón, I., Santamaría, L., Mancheño, J.M., de las Rivas, B. and Muñoz, R. (2016) The Lp\_3561 and Lp\_3562 Enzymes Support a Functional Divergence Process in the Lipase/Esterase Toolkit from *Lactobacillus plantarum*. *Frontiers in Microbiology*, **7**, art.1118. <https://doi.org/10.3389/fmicb.2016.01118>
- [6] Lipińska, A., Świerczyńska, D., Tymoszek, Nowakowska, E. and Walusiak-Skorupa, J. (2013) Occupational Asthma in Female Factory Worker Resulting from Exposure to Savinase in Dishwashing Tablets—A Case Study. *Journal of Occupational Health*, **55**, 318- 321. <https://doi.org/10.1539/joh.12-0169-CS>
- [7] Mukherjee, A.K., Adhikari, H. and Rai, S.K. (2008) Production of Alkaline Protease by a Thermophilic *Bacillus subtilis* under Solid-State Fermentation (SSF) Condition Using *Imperata Cylindrical* Grass and Potato Peel as Low-Cost Medium: Characterization and Application of Enzyme in Detergent Formulation. *Biochemical Engineering Journal*, **39**, 353-361. <https://doi.org/10.1016/j.bej.2007.09.017>
- [8] Shuai, W., Das, R.K., Naghdi, M., Brar, S.K. and Verma, M. (2016) A Review on the Important Aspects of Lipase Immobilization on Nanomaterials. *Biotechnology and Applied Biochemistry*. <http://onlinelibrary.wiley.com/doi/10.1002/bab.1515/abstract;jsessionid=9B0797231C25E1ACE6C2258984D27177.f02t01>
- [9] Nasiripourdori, A., Naderi-Manesh, H., Ranjbar, B. and Khajeh, K. (2009) Co-Solvent effects on Structure and Function Properties of Savinase: Solvent-Induced Thermal Stabilization. *International Journal of Biological Macromolecules*, **44**, 311-315. <https://doi.org/10.1016/j.ijbiomac.2008.09.018>
- [10] Konuklu, Y., Unal, M. and Paksoym, H.O. (2014) Microencapsulation of Caprylic Acid with Different Wall Materials as Phase Change Material for Thermal Energy Storage. *Solar Energy Materials & Solar Cells*, **120**, 536-542. <https://doi.org/10.1016/j.solmat.2013.09.035>
- [11] Onwulata, C. (2013) Microencapsulation and Functional Bioactive Foods. *Journal of Food Processing and Preservation*, **37**, 510-532.

- [12] Li, J., Xiong, S., Wang, F., Regenstein, J.M. and Liu, R. (2015) Optimization of Micro-encapsulation of Fish Oil with Gum Arabic/Casein/Beta-Cyclodextrin Mixtures by Spray Drying. *Journal of Food Science*, **80**, 1445-1452. <https://doi.org/10.1111/1750-3841.12928>
- [13] Dubey, R., Shami, T.C. and Bhasker, K.U. (2009) Microencapsulation Technology and Applications. *Defence Science Journal*, **59**, 82-95.
- [14] Singh, M.N., Hemant, K.S.Y., Ram, M. and Shivakumar, H.G. (2010) Microencapsulation, a Promising Technique for Controlled Drug Delivery. *Research in Pharmaceutical Sciences*, **5**, 65-77.
- [15] De Barros, R.V., Vilela, S. and Alvarenga, D. (2014) Gum Arabic/Starch/Maltodextrin/Inulin as Wall Materials on Thermoencapsulation of Rosemary Essential Oil. *Carbohydrate Polymers*, **101**, 524-532. <https://doi.org/10.1016/j.carbpol.2013.09.083>
- [16] Kim, Y.D. and Morr, C.V. (1996) Microencapsulation Properties of Gum Arabic and Several Food Proteins: Spray-Dried Orange Oil Emulsion Particles. *Journal of Agricultural and Food Chemistry*, **44**, 1314-1320. <https://doi.org/10.1021/jf9503927>
- [17] McNamee, B.F., O'Riordan, E.D. and O'Sullivan, M. (1998) Emulsification and Micro-encapsulation Properties of Gum Arabic. *Journal of Agricultural and Food Chemistry*, **46**, 4551-4555. <https://doi.org/10.1021/jf9803740>
- [18] Mahdavee, K., Jafari, S.M., Ghorbani, M. and Hemmati, A. (2014) Application of Maltodextrin and Gum Arabic in Microencapsulation of Saffron Petal's Anthocyanins and Evaluating Their Storage Stability and Color. *Carbohydrate Polymers*, **105**, 57-62. <https://doi.org/10.1016/j.carbpol.2014.01.042>
- [19] Deis, R.C. (1997) Spray-Drying Innovative Use of an Old Process. *Food Product Design*, **7**, 97-113.
- [20] Alexander, P., Fahnestock, S., Lee, T., Orban, J. and Bryan P. (1992) Thermodynamic Analysis of the Folding of the Streptococcal Protein G IgG-Binding Domains B1 and B2: Why Small Proteins Tend to Have High Denaturation Temperatures. *Biochemistry*, **31**, 3597-3603. <https://doi.org/10.1021/bi00129a007>
- [21] Ferrándiz, M., Capablanca, L., Franco, E. and Mira, E. (2016) Microencapsulation of L-Ascorbic Acid by Spray Drying Using Sodium Alginate as Wall Material. *Journal of Encapsulation and Adsorption Sciences*, **6**, 1-8. <https://doi.org/10.4236/jeas.2016.61001>
- [22] Benítez, R., Ibarz, A. and Pagan, J. (2008) Hidrolizados de proteína: Procesos y aplicaciones. *Acta Bioquímica Clínica Latinoamericana*, **42**, 227-236.
- [23] Heller, M.C., Carpenter, J.F. and Randolph, T.W. (1999) Protein Formulation and Lyophilization Cycle Design: Prevention of Damage Due to Freeze-Concentration Induced Phase Separation. *Biotechnology and Bioengineering*, **63**, 166-174. [https://doi.org/10.1002/\(SICI\)1097-0290\(19990420\)63:2<166::AID-BIT5>3.0.CO;2-H](https://doi.org/10.1002/(SICI)1097-0290(19990420)63:2<166::AID-BIT5>3.0.CO;2-H)
- [24] Samborska, K., Witrowa-Rajchert, D. and Gonçalves, A. (2005) Spray-Drying of  $\alpha$ -Amylase—The Effect of Process Variables on the Enzyme Inactivation. *Drying Technology*, **23**, 941-953. <https://doi.org/10.1081/DRT-200054243>
- [25] Maa, Y.-F., Nguyen, P.-A., Sit, K. and Hsu, C.C. (1998) Spray-Drying Performance of a Bench-Top Spray Dryer for Protein Aerosol Power Preparation. *Biotechnology and Bioengineering*, **60**, 301-309. [https://doi.org/10.1002/\(SICI\)1097-0290\(19981105\)60:3<301::AID-BIT5>3.0.CO;2-L](https://doi.org/10.1002/(SICI)1097-0290(19981105)60:3<301::AID-BIT5>3.0.CO;2-L)
- [26] Arpagaus, C. (2012) A Novel Laboratory-Scale Spray Dryer to Produce Nanoparticles. *Drying Technology*, **30**, 1113-1121. <https://doi.org/10.1080/07373937.2012.686949>
- [27] De Jesús, S. and Maciel Filho, R. (2014) Drying of  $\alpha$ -Amylase by Spray Drying and Freeze-Drying—A Comparative Study. *Brazilian Journal of Chemical Engineering*, **31**, 625-631. <https://doi.org/10.1590/0104-6632.20140313s00002642>



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