P-076 **Microencapsulation of enzymes produced by *Eupenicillium javanicum***

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INTRODUCTION AND OBJECTIVES

Peptidase is a very important enzyme for several forms of life on earth. It is used in food industry, detergent, pharmaceutical, diagnostic and waste management, representing worldwide sale at about 60% of total enzyme market (Rajmalwar 2009).

Studies from Nakadate (2008) demonstrated that the extract of *Eupenicillium javanicum* showed strong antifungal activity against *Aspergillus fumigatus*. Antibacterial tests against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli*, and *Salmonella enteritidis*, also showed activity. Thus, this enzymatic extract may be very useful as a raw material for antimicrobial product.

Since microparticles can isolate, stabilize and protect its contents from exposure to destructives environmental factors, microencapsulation is an interesting technique to maintain enzymatic activity. Spray drying is considered a dehydration process that can also be used as an encapsulation method. This technique can be applied on heat-sensitive foods, pharmaceuticals and others substances. (Ré 2007). The aim of this study was to optimize the microencapsulation process of the enzymatic extract (EE) produced by the fungus *Eupenicillium javanicum*, using Box-Benkhen as an experimental design tool.

MATERIALS AND METHODS

The fungus *E. javanicum* belongs to the mycology collection of the Enzymology Laboratory from FCFRP/USP. It is stored under refrigeration (4°C) on inclined tubes containing Sabouraud medium.

The EE was obtained through solid state fermentation, using 5 g of wheat bran as culture media. The preparations were placed into 250 mL Erlenmeyer flasks with a volume of saline corresponding to 60% humidity. After been autoclaved, these flasks were inoculated with the mycelium's suspension and were incubated under 30°C during 140 hours. The extraction was performed by adding 40 mL of water, macerating and then filtering.

The microparticles were produced by spray drying. The adjuvant used was maltodextrin, due to a previous experiment that demonstrated a better yield and enzyme protection than mannitol and Aerosil®. The feed rate (FR), drying temperature (T) and ratio enzymatic extract/adjuvant (EE/Ad) were factors chosen to vary on the Box-Benkhen design. The initial condition of the spray dryer is shown in Table 1.

Table 1. Spray Dryer initial condition

Parameters	Values
Drying air flow rate	2,75 m^3/min
Nozzle air flow	40 L/min
Atomization air pressure	5,0 kgf/cm^2

Proteolitic activity assay was performed using the protocol described by Sarath *et al.*, (1996) with modifications.

RESULTS AND DISCUSSION

Table 2 demonstrates that the yields were consistent to laboratory scale equipment.

Table 2. Assay yield

Trial	Factor Levels			Factor Levels			Yield (%)
	T (°C)	FR	EE/Ad	T (°C)	FR	EE/Ad	
1	-1	-1	0	50	3	1	43,75
2	+1	-1	0	100	3	1	63,12
3	-1	+1	0	50	9	1	66,12
4	+1	+1	0	100	9	1	61,00
5	0	0	0	75	6	1	52,87
6	0	0	0	75	6	1	54,44
7	0	0	0	75	6	1	55,94
8	-1	0	-1	50	6	0,5	32,58
9	+1	0	-1	100	6	0,5	59,87
10	0	-1	-1	75	3	0,5	45,00
11	0	+1	-1	75	9	0,5	42,71
12	-1	0	+1	50	6	1,5	35,80
13	+1	0	+1	100	6	1,5	57,03
14	0	-1	+1	75	3	1,5	52,46
15	0	+1	+1	75	9	1,5	49,00

Statistic analysis indicated that temperature and EE/Ad were significant to yield. It raised with increasing temperature and similar amounts of EE and Ad (Figure 1). Feed rate, however, had no significant effect.

In Figure 2, the enzymatic activity (EA) is shown as the percentage of maintained activity after the process, comparing to the EE before the process.

Feed rate had no effect on the EA. For the other hand, middle temperatures and EE/Ad trials demonstrated lower loss of activity of the microencapsulated EE.

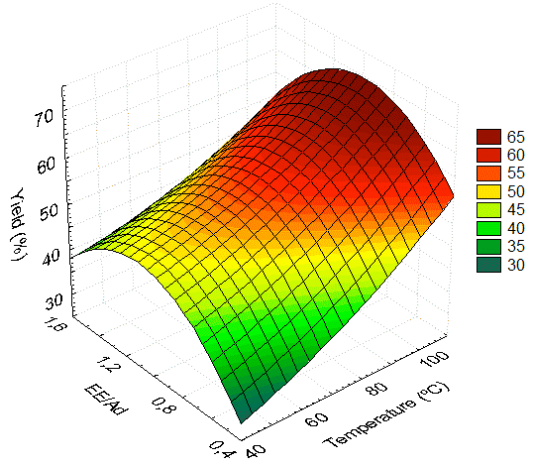

Figure 1. Response surface plot for yield of the micro-encapsulation process

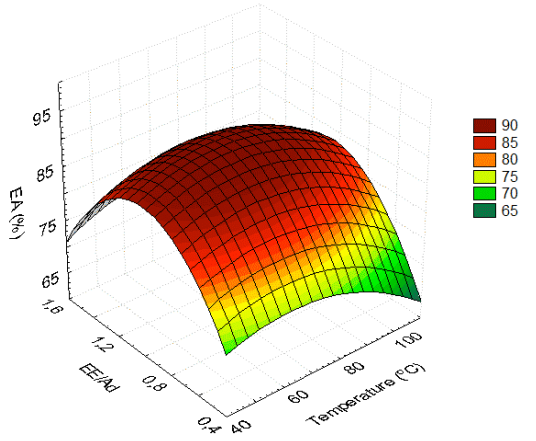

Figure 2. Response surface plot for enzymatic temperature of microencapsulated enzymatic extract

Low concentration of adjuvants was not suficient to protect the enzymes for possible damage resulting from the drying process. The effect of high concentration of adjuvants agrees with the study of DePaz (2002) that says that high concentration of some additives can affect the secondary structure of enzymes by steric hindrance during drying. According to Tzannis (1999) "at high carbohydrate concentrations, preferential sugar-sugar interactions prevailed […] As a consequence, the protein could not be effectively protected during spray drying".

Although there was no statistically significant impact of the drying temperature in the enzymatic activity, there is a tendency of better results with middle temperatures. High temperatures are not recommended, since they can inactivate the enzymes, but low temperatures may not remove all the moisture, and water content can affect enzymatic activity.

The size and form of microparticles are demonstrated in Figure 3. The particles are spherical, with low surface roughness.

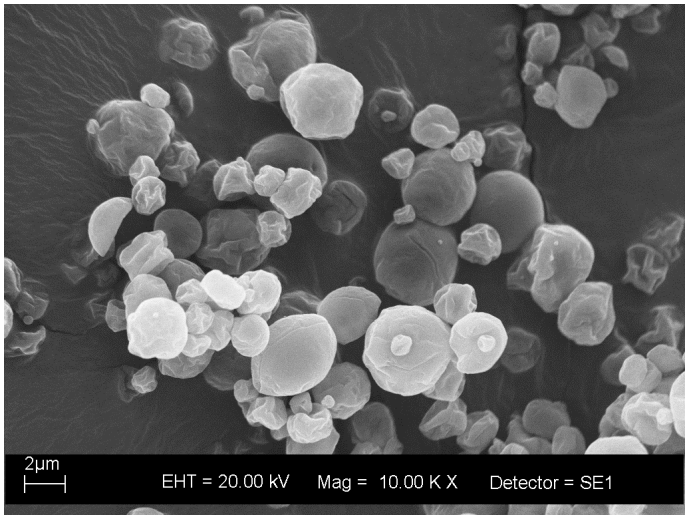

Figure 3. Scanning Electron Microscopy of the microparticles

CONCLUSIONS

The results demonstrate that for microencapsulation by spray drying of the enzymatic extract produced by *Eupenicillium javanicum*, the selection of proper process parameters and formulation can provide the most desired characteristics to the produced microcapsules. For this study, temperature and adjuvant concentration were the most important variables to enzymatic activity and yield. With appropriated adaptations, these results can give directions to experiments with other enzymes.

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