O6-1 **Stability of microencapsulated enzymes produced by *Fusarium oxysporum***

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INTRODUCTION AND OBJECTIVES

Different extracellular enzymes produced by fungi have been widely used in food, pharmaceuticals, and cosmetics industry; in biotechnology; and in bioremediation processes (Rao 1998). Using Box–Behnken design for the optimization of formulation and process, the purpose of this study was to investigate the benefit of the microencapsulation process in handling and in stability of lipases and proteases produced by the fungus *Fusarium oxysporum*, aiming for a possible industrial application of these microcapsules.

MATERIALS AND METHODS

To obtain the enzymatic extract (EE), solid state fermentation was carried out using wheat bran and cottonseed meal as media (Bazarzhapov, 2006).

Spray Dryer technique was used to produce the microcapsules. After a previous experiment of drying the EE alone and the EE combined (1:1) with the adjuvants mannitol, dextrin and Aerosil®, the two last adjuvants were selected for the Box-Behnken design. The chosen factors were ratio enzymatic extract/adjuvants (EE/Ad), ratio Aerosil®/Dextrin (Ar/Dx) and drying temperature (T) (Table 1).

The enzymatic activities were evaluated after the microencapsulation process and at 15 and 30 days, after storage under refrigeration (4°C) and room temperature (25°C). Proteolytic and lipolytic activities were measured according to protocols adapted respectively from Bazarzhapov (2006) and Kanwar (2005).

Physical characterization was performed by analysis of Hausner Factor (HF), Carr Index (CI), angle of repose and moisture content (USP 30/NF25, 2007).

RESULTS AND DISCUSSION

Drying experiment of the EE and its combination with mannitol (1:1) produced powders that became a sticky mass in contact with air and during handling. Thereby, new trials were designed using dextrin and Aerosil® as adjuvants.

Table 1 demonstrates that the yields are above the average for such laboratory scale equipment. Even though there is no statistically significant difference among trials using 80 and 100 °C, there is a tendency of positive correlation between temperature and yields.

Table 1. Experimental Design and Yields

Trial	Factor Levels			Factor Levels			Yield (%)
	EE/Ad	Ar/Dx	T (°C)	EE/Ad	Ar/Dx	T (°C)	
1	-1	-1	0	3/1	3/1	80	60.94
2	+1	-1	0	1/1	3/1	80	60.00
3	-1	+1	0	3/1	1/1	80	61.50
4	+1	+1	0	1/1	1/1	80	62.81
5	-1	0	-1	3/1	2/1	60	52.56
6	+1	0	-1	1/1	2/1	60	53.69
7	-1	0	+1	3/1	2/1	100	62.38
8	+1	0	+1	1/1	2/1	100	62.51
9	0	-1	-1	2/1	3/1	60	53.94
10	0	+1	-1	2/1	1/1	60	57.75
11	0	-1	+1	2/1	3/1	100	61.06
12	0	+1	+1	2/1	1/1	100	63.00
13	0	0	0	2/1	2/2	80	62.81
14	0	0	0	2/1	2/2	80	61.31
15	0	0	0	2/1	2/2	80	61.60

Enzymatic Stability

Stability of the EE and microcapsules was evaluated by enzymatic activity quantification. The results are shown as percentage of activity maintained in relation to EE´s initial activity measured before the process.

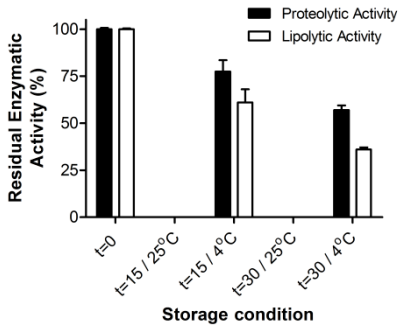

Figure 1. Stability of the enzymatic extract produced by *Fusarium oxysporum*

After 15 days, the EE lost all its activity when stored under room temperature and after 30 days it maintained only 57.90 and 36.45 % of proteolityc and lipolytic activity under refrigeration (Figure 1).

For the microcapsules, there was no effective correlation between EE/Ad and enzymatic activity or between Ar/Dx and enzymatic activity. On the other hand, drying temperature demonstrated an important influence (Figure 2), indicating that high temperatures are not recommended, since they can inactivate the enzymes.

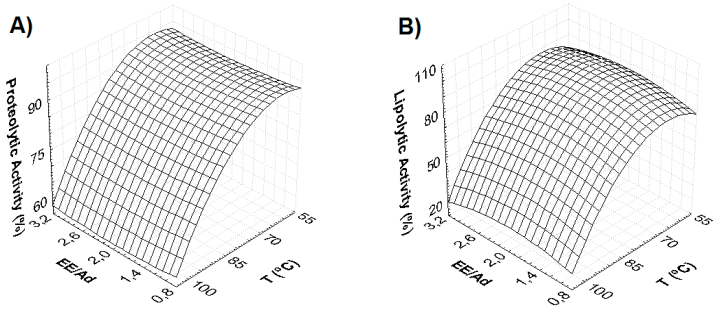

Figure 2. Response surface plots for (A) Proteolytic activity and (B) Lipolytic activity of the microcapsules

Table 2. Enzymatic stability of produced microcapsules after storage under 4 and 25°C

	Trial	t = 0	t = 15 days		t = 30 days	
			4°C	25°C	4°C	25°C
Proteolytic stability (%)	1	86,63	88,45	83,59	86,17***	72,80***
	2	91,19	88,30	88,15	87,54*	80,24*
	3	91,79	89,51	85,87	87,39	77,81
	4	91,19	88,15	85,87	87,23	77,96
	5	94,53	93,62	89,06	91,34	87,39
	6	92,71	91,95	90,12	92,10	88,30
	7	61,55	57,75	55,78	55,93	48,33
	8	59,88	60,18	56,23	59,27*	50,76*
	9	93,31	91,34	87,84	90,43***	86,60***
	10	92,55	93,77	89,06	93,62***	81,16***
	11	64,74	63,83	59,42	62,31**	50,91**
	12	74,62	72,49	69,00	72,64***	60,64***
	13	84,19	83,89	80,09	82,52***	69,91***
	14	81,31	78,88	74,62	79,33***	66,26***
	15	85,71	85,87*	77,81*	84,50***	67,17***

	Trial	t = 0	t = 15 days		t = 30 days	
			4°C	25°C	4°C	25°C
Lipolytic stability (%)	1	79,60	76,00***	69,80***	75,83***	67,16***
	2	75,40	77,60***	68,80***	75,04***	59,36***
	3	82,60	76,80*	72,20*	77,66***	67,84***
	4	77,80	77,60***	69,20***	75,53***	66,75***
	5	84,40	76,00	72,60	74,70***	66,23***
	6	85,40	81,40	79,20	81,00***	69,68***
	7	39,00	35,40	31,80	29,48***	27,45***
	8	43,00	41,20	38,60	39,64***	32,06***
	9	97,20	90,40*	86,00*	85,39***	79,13***
	10	84,40	85,40**	80,00**	86,63***	71,93***
	11	40,20	38,40	37,20	38,96***	30,23***
	12	40,00	39,40	37,60	39,23***	31,99***
	13	86,20	85,40***	77,40***	85,10***	70,65***
	14	89,80	79,40	76,80	80,36***	72,00***
	15	81,40	80,00	77,40	79,84***	71,70***

* p < 0,05; ** p < 0,01; *** p < 0,001

Enzymatic activity data (Table 2) clearly indicate that the technique of microencapsulation increases the stability of the enzymes when compared to the EE not processed (Figure 1). Even though for some samples there was no statistically significant difference between storage temperatures, it can be observed that refrigeration may be the best option for maintaining enzymatic stability.

Physical characterization

Evaluated Hausner Factor range was from 1.09 to 1.17, and Carr Index from 7.14 to 14.98. According to Guo (1985), materials with values of HF and IC below 1.25 and 15, respectively, have good compression characteristics, thus all samples fulfill these requirements. Concerning to angle of repose, since angles below 40° indicate free flow and the measured angles varied from 30.54 to 36.62, all formulations showed good flow.

Moisture content measured after process was low for all samples (about 4 %). Although kept in tightly closed plastic containers, after 30 days, the samples 1, 3, 5 and 7 showed grainy aspect and increased moisture levels to about 11 %, while other samples had less than 1 % of increase and remained as fine powder. Although the enzymatic activity was not greatly affected, these results highlight the importance of the amount of adjuvants in the formulation for greater handling conditions.

CONCLUSIONS

The results demonstrate that microencapsulation by *spray drying* is an important technique to improve handling and stability of enzymes produced by *Fusarium oxysporum*. In addition, the study highlights that the selection of proper process parameters and formulation can provide the most desired characteristics to industrial use of the produced microcapsules. For this study, temperature is the most important variable to stability, while amount of adjuvants influence in handling conditions. With appropriated adaptations, these results can be extrapolated to other experiments with different enzymes.

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