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## SOLID STATE FERMENTATION AS AN STRATEGY TO MAXIMIZE THE $\beta$ -FRUCTOFURANOSIDASE PRODUCTION

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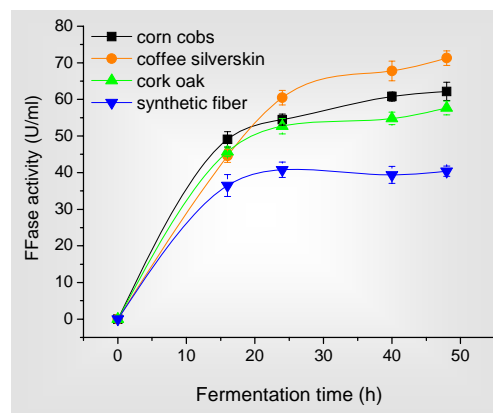
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The production and application of  $\beta$ -fructofuranosidases (FFase; EC.3.2.1.26) has gained tremendous practical importance because these enzymes have transfructosylating activity and are able to convert the disaccharide sucrose to fructooligosaccharides (FOS), which are fructose oligomers of great industrial interest due to their functional properties and physical-chemical characteristics (1). Most of these enzymes are found in fungi such as *Aspergillus*, *Aureobasidium*, and *Penicillium*. Our studies on FFase production revealed *Aspergillus japonicus* (ATCC 20236) as a potentially adequate strain for application on the production of FFase. Assays in submerged fermentation systems with or without immobilized cells of this fungal strain yielded FFase activity values comparable to many literature data, in the order of 40 U/ml (2) (Table 1).

**Table 1.** FFase production by submerged fermentation with *Aspergillus japonicus*, with or without immobilized cells (200 g/l initial sucrose).

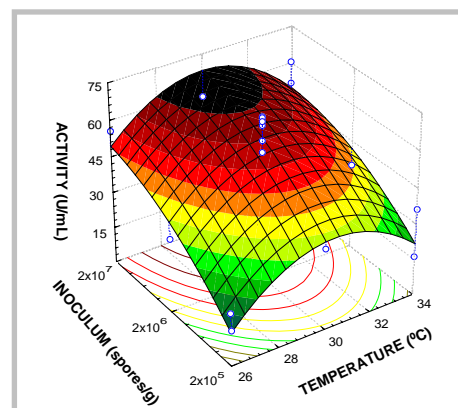
Immobilization carrier	FFase activity (U/ml)
Brewer's spent grains	39.4
Coffee silverskin	41.3
Cork oak	36.3
Corn cobs	44.8
Loofa sponge	26.8
Wheat straw	32.5
Free cells assay	46.4

Aiming to maximize these results, assays under solid-state fermentation (SSF) conditions were proposed. Corn cobs, coffee silverskin, and cork oak were evaluated as support and nutrient source during the FFase production by SSF (Fig. 1). The results clearly revealed an improvement of the enzyme production by SSF when compared to the results obtained by submerged fermentation systems. The highest value, 71.3 U/ml, was obtained after 48 h fermentation using coffee silverskin as solid support and nutrient source (3). This value is favorably comparable to others reported in the literature. The next step of this research consisted in establishing the best operational conditions (temperature, inoculum rate, and moisture content) to be used during the FFase production by *A. japonicus* under SSF conditions using coffee silverskin as solid support and nutrient source. Fermentation assays were performed with the support material moistened to 60, 70 or 80% with the sucrose solution, inoculated with a spore suspension to obtain  $2 \times 10^5$ ,  $2 \times 10^6$  or  $2 \times 10^7$  spores/g dry substrate, and incubated at 26, 30 or 34 °C, during 20 h.



**Fig. 1.** FFase activity during SSF with *A. japonicus* using different materials as solid support. Assays not supplemented with nutrients.

All the studied variables significantly influenced the FFase activity, but the temperature and inoculum rate had the most significant effects (Fig. 2). Maximum FFase (64.1 U/ml) was achieved when using 30 °C,  $2 \times 10^7$  spores/g dry material, and 70% moisture. By establishing the operational conditions the enzyme productivity was significantly increased from 1.5 U/ml.h to 3.2 U/ml.h.



**Fig. 2.** Response surface representing the FFase variations according to the operational conditions used during SSF.

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### Bibliografía.

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