# EVALUATION OF ACTIVITY OF DIFFERENT LAMINATES WITH IMMOBILIZED INVERTASE CANDELILLA WAX

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**Introduction.** The main large-scale application areas of immobilized enzymes are in the food (production of high fructose syrups by using of immobilized invertase and glucose isomerase) and pharmaceutical industries. Invertase is a highly efficient enzyme that has been described as specific for converting sucrose to glucose and fructose. The applications of hydrolyzed sugar include artificial honey, the brewing industry, in jam's production to reduce sucrose crystallization, inter alia (1). Moreover, invertase is applied to treat of sucrose intolerance that also requires to new methods for its immobilization. Entrapment of invertase in a thin polymer layer on the surface of the materials (laminates) applied for handling food can be an alternative to the enzyme application for sucrose level control.

The objective of the present study was to evaluate the behavior of invertase immobilized in Candelilla (*Euphorbia antisyphilitica)* wax in the different laminates.

**Methods.** Invertase (EC 3.2.1.26, Sigma Co.) was introduced in Candelilla wax (Multiceras S.A. de C.V.) hexane solution using inverse micelle system contained 0.05 M AOT en hexane and watery enzyme solution (100:1). The mixture was uniformly distributed on the surface of materials (wood, aluminum, ceramic, plastic and glass) forming laminates after hexane evaporation. The amount of immobilized invertase was estimated as the difference between the amounts of protein applied to the support and recovered in the various washings (2). Enzymatic sucrose hydrolysis was carried out by means of described previously technique at 120 rpm (3). Reducing sugars were measured by dinitrosalicylic acid (DNS) method (3).

**Results.** High percentage of invertase immobilization (between 89.6 and 98.3) was achieved on different obtained laminates (Fig. 1). Lower enzyme retention was observed in the case of laminate formed on the wood surface (Fig. 1). Kinetics of sucrose hydrolysis with free enzyme and immobilized on different laminates (Fig. 2) are demonstrated that in the system with free enzyme the decrease of reducing sugars was observed after 50 min of reaction due to chemical degradation of compounds (4). The maximum concentration was 24.9 mM. In contrast, kinetics of accumulation of reaction products with immobilized enzyme are characterized by the presence of a lag period (approximately 30 min) probably related to the processes of diffusion. After 90 min of reaction, lower level of product reaction was detected with laminate on plastic (14.5 mM). Higher levels were observed in the presence of wood and ceramic contained systems (40.7 and 35.3 mM), while the levels detected with glass and aluminum laminates were similar to detected with free enzyme (23. 6 and 24.4 mM).

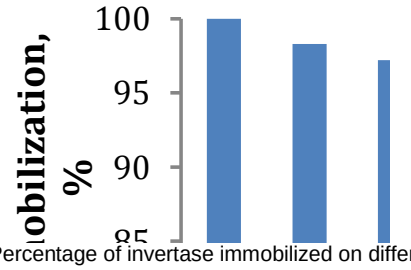


**Fig.1.** Percentage of invertase immobilized on different laminates: (P) plastic, (G) glass, (C) ceramic, (W) wood, and (A) aluminum, as well as en candelilla wax pellet (Pellet).

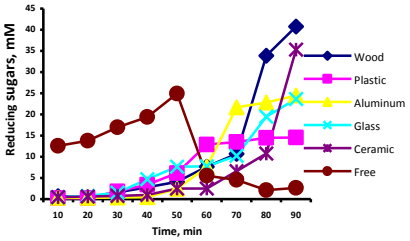


**Fig. 2.** Sucrose hydrolysis by invertase in free form and immobilized on Candelilla wax in different laminates.

**Conclusions.** All obtained laminates allow to invertase immobilization. Sucrose hydrolysis with invertase immobilized on candelilla wax laminates was varied depending the type of applied materials, and in some cases was better than with free enzyme.

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