OPERATIONAL STABILITY OF THE ENZYME GLUCOSE OXIDASE ENTRAPPED IN SILICA-CALCIUM-ALGINATE CAPSULES FOR THEIR OENOLOGICAL APPLICATION IN THE CONTEXT OF THE GLOBAL WARMING

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Abstract

Our aim was to obtain a suitable support for the specific immobilisation of an enzyme system capable of degrading β-D-glucose in must keeping an adequate operational stability in oenological conditions. So, our research group has designed a hybrid silica-alginate matrix to co-immobilize glucose oxidase (GOX) and catalase (CAT). This matrix, in the form of spherical capsules, has both the advantages of the organic compound alginate (biocompatibility, flexibility and plasticity) and the inorganic component silica (rigidity, chemical resistance and thermal stability). The application of these capsules in must allowed the conversion of β-D-glucose into gluconic acid, reducing simultaneously its potential alcoholic degree (1.0-1.5 degrees) and pH (0.4-0.5 units). Glucose consumptions have been compared with those obtained in the same conditions by free GOX-CAT system showing a higher stability of the immobilized GOX throughout the reaction time.

Introduction

In order to overcome the effects of global warming that vine growers and winemakers have to address, it is necessary to establish strategies and incorporate technologies that jointly mitigate the changes in the grapevine and grape cycle.

The increase in average temperatures leads to a higher concentration of sugars in musts, which can condition the correct development of the winemaking process, resulting in wines with a higher alcohol content (Röcker et al., 2016) and lower acidity (Botezatu et al., 2021) affecting their organoleptic characteristics and final quality.

The use of enzymes to improve different technological processes in winemaking is becoming a powerful tool in modern oenology, and the immobilization of enzymes in appropriate materials adapted to the technical conditions and legal aspects of oenological production is presented as an innovative technology with great applications.

To ensure the successful application of these immobilized enzymes in oenology, it is necessary to assure their adequate operational stability.

Materials and methods

The enzymes used for co-immobilization were GOX (Gluzyme®Fortis, Novozymes®) and CAT (Catazyme® Novozymes®). The biocomposite capsules consist of an interpenetrating polymeric network of silica-calcium-alginate (del-Bosque et al., 2023) that entraps the enzymes (Figure 1).

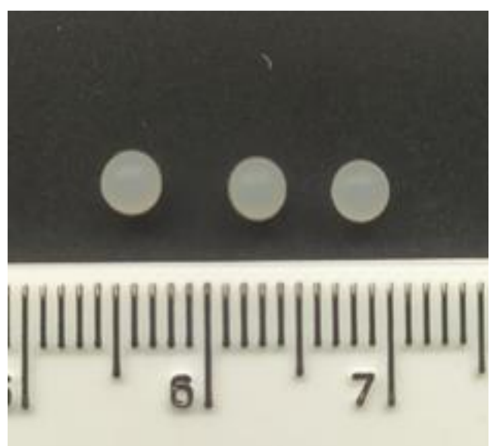
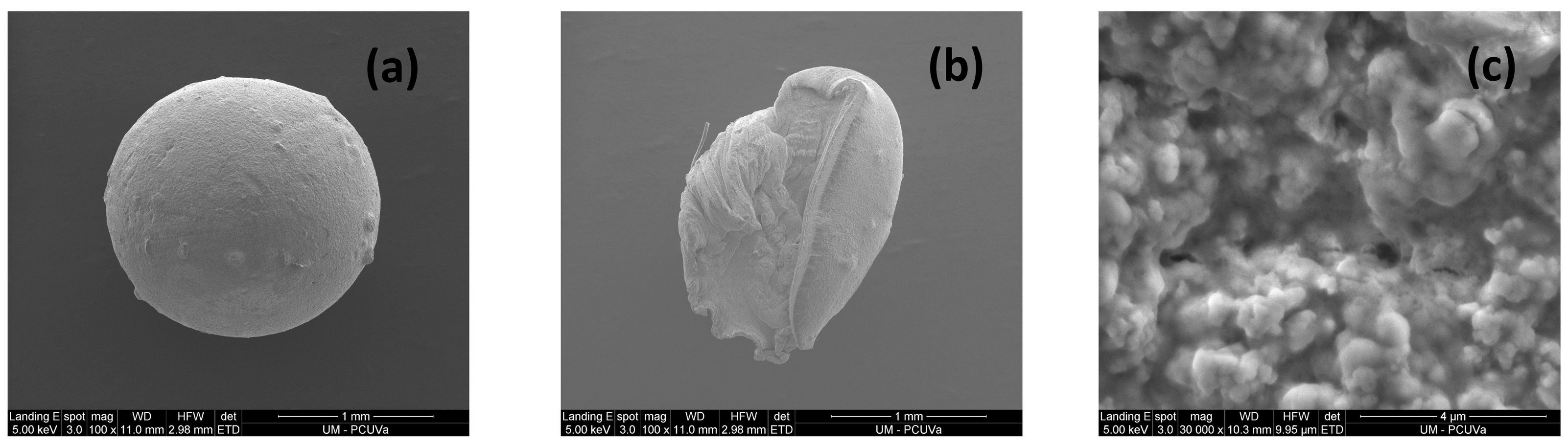


Figure 1. Showing silica-calcium-alginate biocomposite capsules.

Microstructural characterisation of silica-calcium-alginate capsules was carried out by environmental scanning electron microscopy (ESEM) (Figure 2a-c) and their composition was analysed by energy dispersive X-ray spectroscopy (EDS) (Figure 2d).



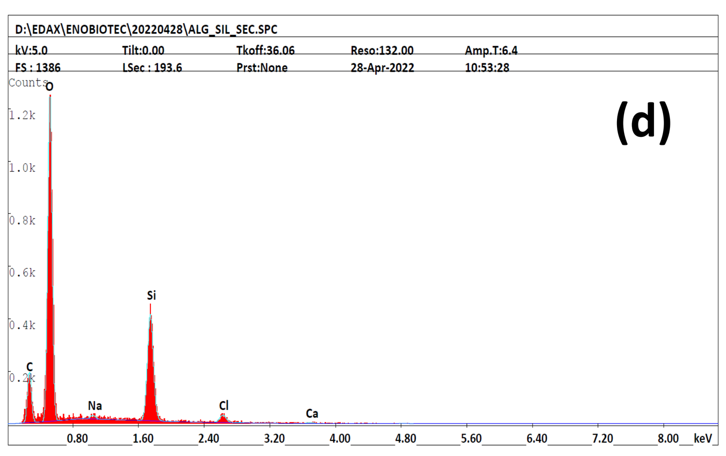


Figure 2. (a) Image (100X) of the outer surface of a silica-calcium-alginate capsule. (b) Image (100X) of a sectioned silica-calcium-alginate capsule. (c) Image (30,000X) of the inner surface of a silica-calcium-alginate capsule showing its porous structure. (d) Graph showing the elemental composition of silica-calcium-alginate capsules by EDS.

Results

Operational stability was evaluated in Verdejo grape musts with an initial pH of 3.8 and 4.0, reusing the capsules in 8 batches of must (Figure 3 and 4).

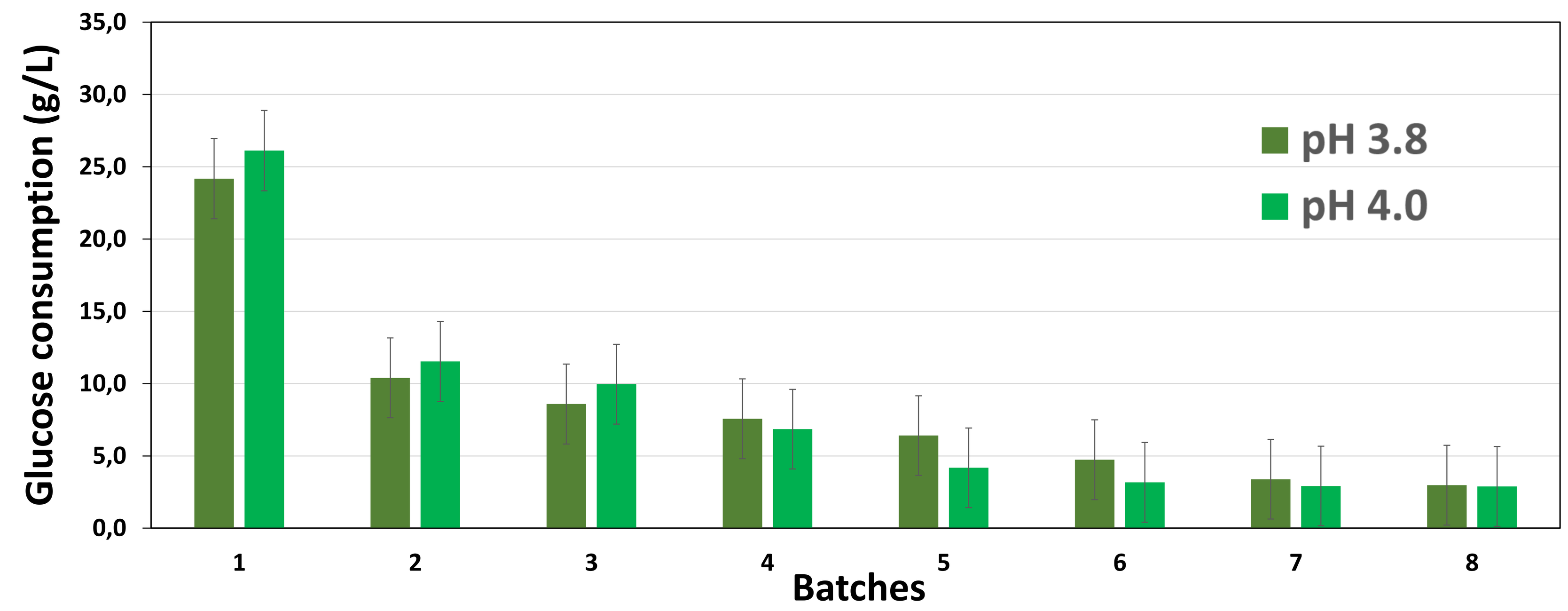


Figure 3. Operational stability: glucose consumption in each batch at pH 3.8 and 4.0.

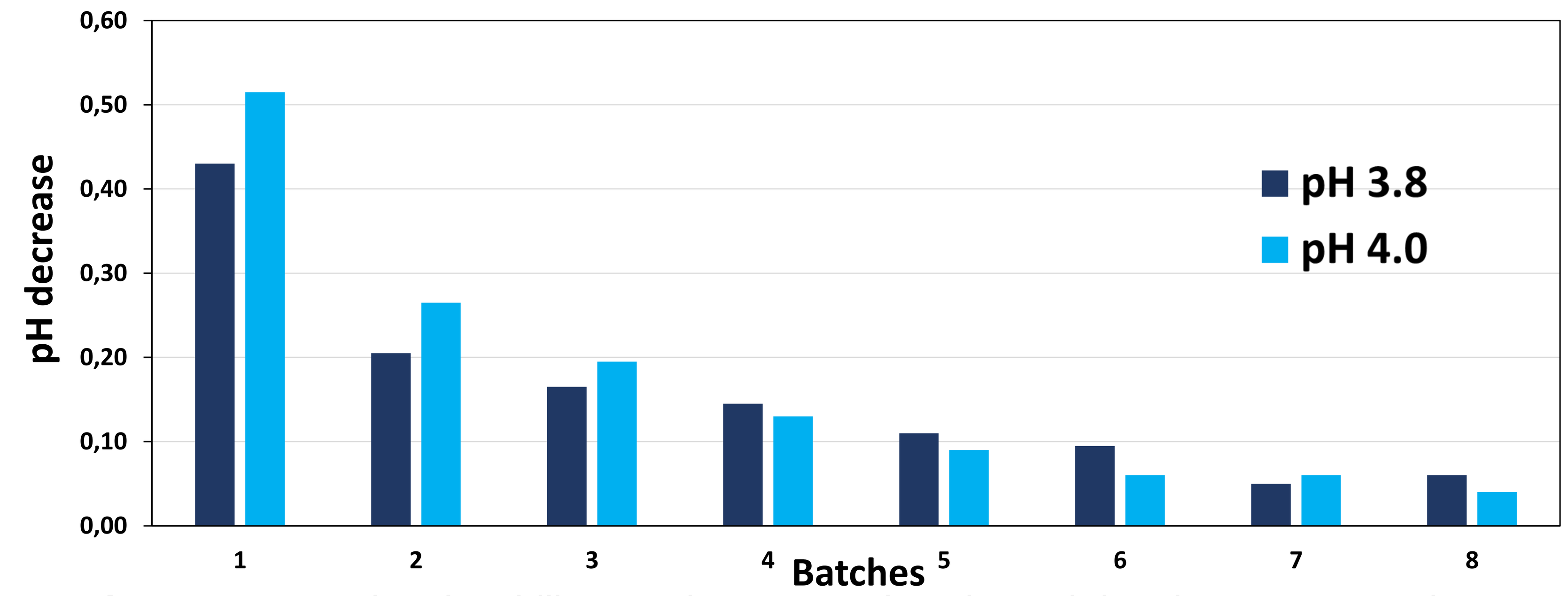


Figure 4. Operational stability: pH decrease values in each batch at pH 3.8 and 4.0.

Results show that although the operational stability of the enzymes decreases with each cycle, the co-immobilised GOX-CAT system is reusable.

Glucose consumption was determined during the first and second 24 h of the enzymatic reaction at different Units per ml of must (U/mL) of co-immobilised and free GOX enzyme (Figure 5).

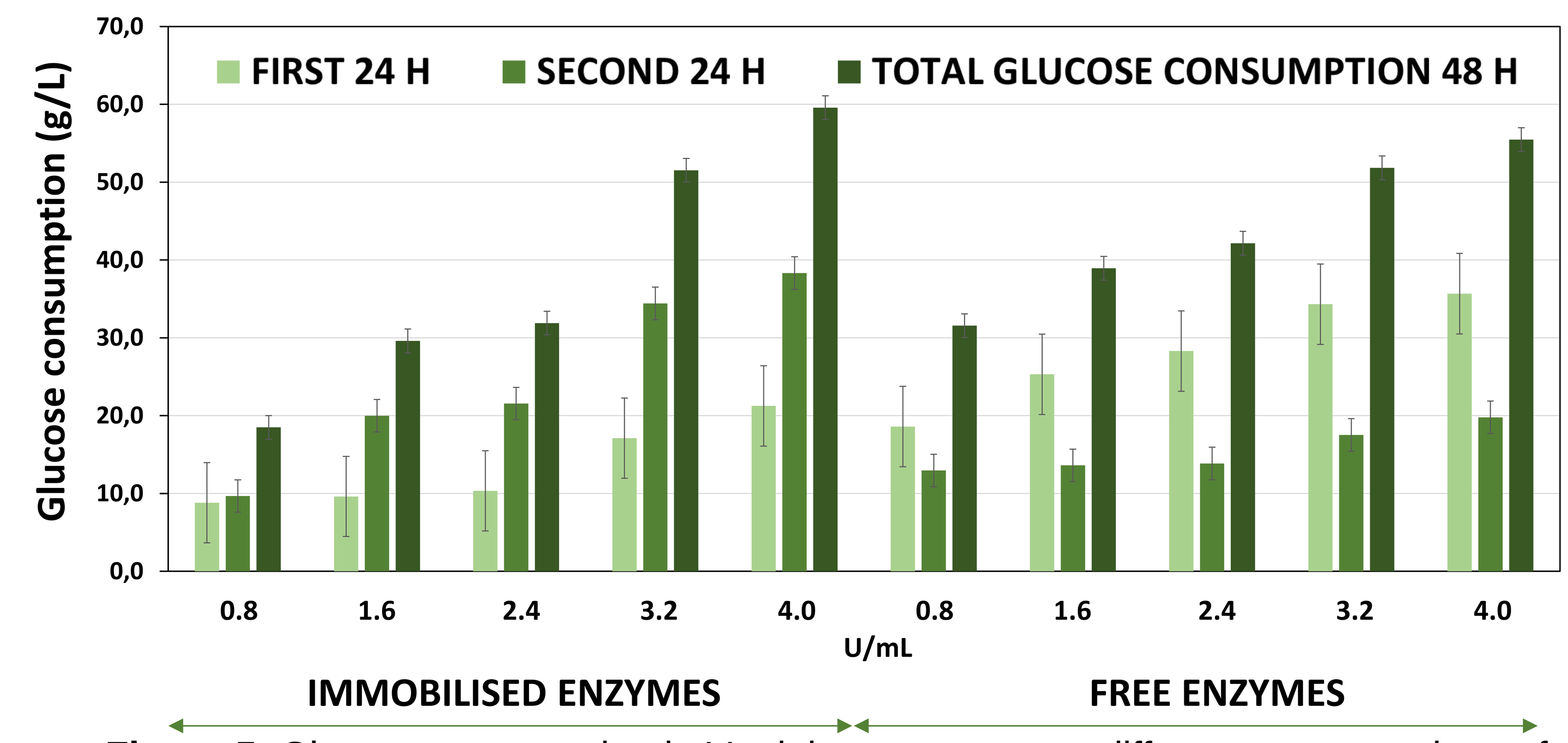


Figure 5. Glucose consumption in Verdejo grape must at different concentrations of entrapped and free GOX.

Conclusions

- During the first 48-hour cycle, in the must with an initial pH of 3.8 and 4.0, a glucose degradation of 24.2 and 26.1 g/L and a pH increase of 0.43 and 0.52 units, respectively, were obtained.
- The ratio of glucose consumed in the second 24 h was higher with immobilized GOX than with free GOX, indicating a higher stability of the immobilized enzyme throughout the reaction time.
- These results show the potential of the alginate-silica co-immobilized GOX-CAT enzymes as a biotechnological strategy to mitigate the effects of global warming on winemaking production and their sufficient activity to reduce the concentration of glucose in must, equivalent to a reduction of 1.0-1.5 alcoholic degrees in wine.

References

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