SIMULTANEOUS REDUCTION OF GLUCOSE AND ACIDIFICATION OF A MUST OF VERDEJO GRAPE USING THE GLUCOSE OXIDASE-CATALASE ENZYME SYSTEM CO-IMMOBILIZED IN ALGINATE-SILICA BEADS

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Abstract

The aim of this work was to evaluate the capacity of co-immobilized glucose oxidase (GOX) - catalase (CAT) system in alginate-silica beads as an effective biotechnological strategy to simultaneously decrease glucose content and pH in a must of Verdejo grape from the Appellation of Origin Rueda. For this purpose, a response surface design with 3 factors (concentration of immobilized enzymes, initial pH of the must and temperature) was carried out, evaluating glucose consumption and pH variation.

Introduction

Global warming threatens to cause multiple problems in the management of vineyard due to alterations in the cycle of vine and grape that may have repercussions on the normal and adequate production of quality wines.

One of these current problems that is expected to intensify, is the increment in the concentration of sugars in the grapes due to the rise in average temperatures, which leads to wines with a higher alcohol degree. For this reason, several strategies are under research and development to reduce the alcohol degree of the wine without affecting its organoleptic quality.

One of these strategies consists of treating musts enzymatically to reduce their glucose concentration and, simultaneously, to increase their acidity before alcoholic fermentation (Ruiz et al., 2018).

GOX (EC 1.1.3.4) catalyzes the oxidation of β-D-glucose in the presence of oxygen to gluconic acid and hydrogen peroxide. CAT (EC 1.11.1.6) degrades hydrogen peroxide, protecting GOX inactivation and regenerating O_2 for GOX in its microenvironment (Dubey et al., 2017).

Materials and methods

The enzymes used for co-immobilization were GOX (Gluzyme®Fortis, Novozymes®) and CAT (Catazyme® Novozymes®). Fig. 1 shows the protocol for co-inmmobilization of GOX and CAT in silica-alginate gel.

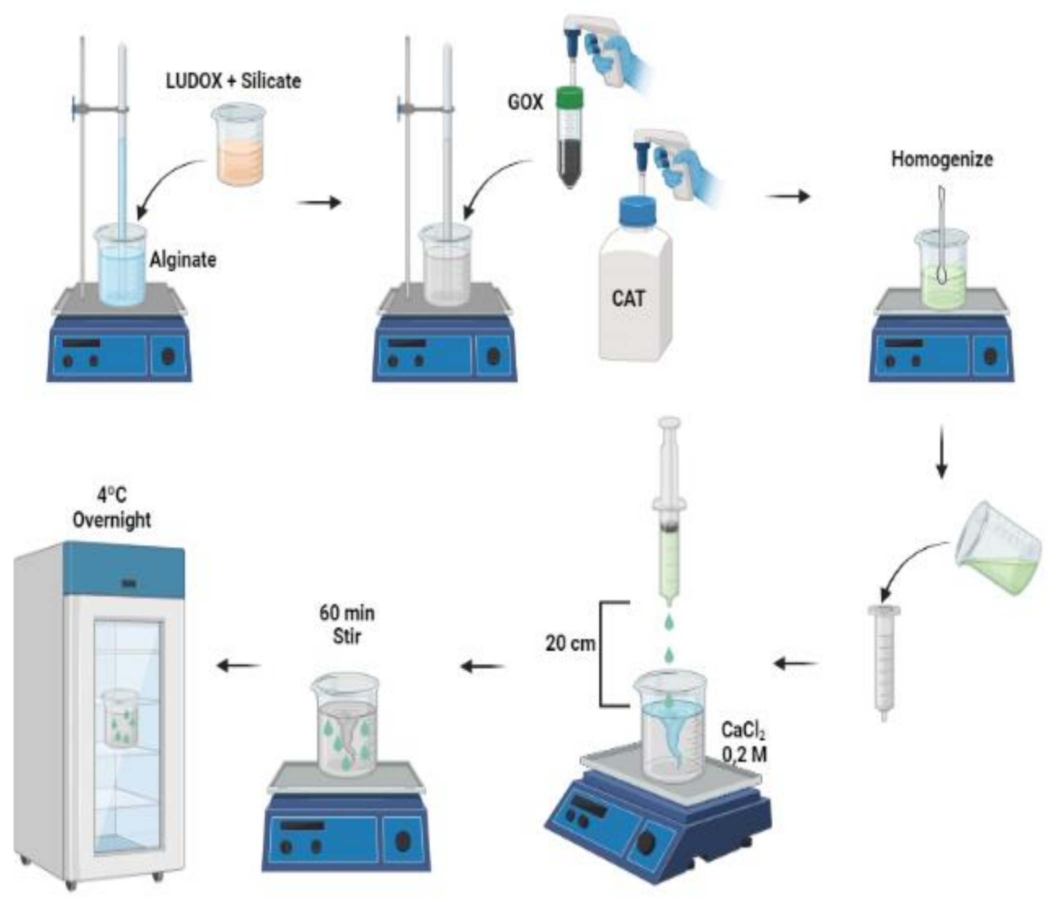


Figure 1. GOX and CAT co-immobilization protocol.

To evaluate the capacity of the encapsulated enzyme system, a response surface design (Box-Behnken) was carried out, with three factors: concentration of enzymes expressed as number of beads (10-15-20, 30 U of GOX and CAT per g of beads), initial pH of the must (3.6-3.8-4.0) and temperature (10-15-20°C), generating 19 experimental conditions.

In each experimental condition, the consumption of glucose and pH were measured in Verdejo must after 48 h, with agitation at 150 rpm. Glucose was measured using an enzymatic kit.

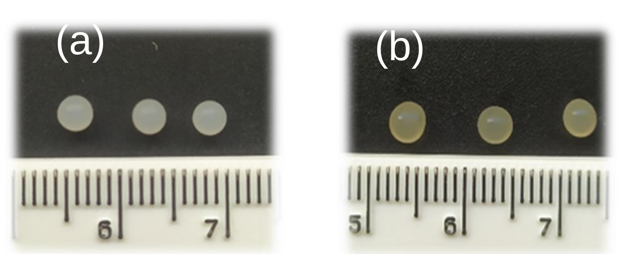


Figure 2.
(a) Image of alginate-silica beads prior to assay.
(b) Image of alginate-silica beads after testing

Results

An adjusted model (α= 0.05) was obtained from the data of the 19 experimental conditions. The following results for the must treated with the immobilized GOX-CAT were obtained:

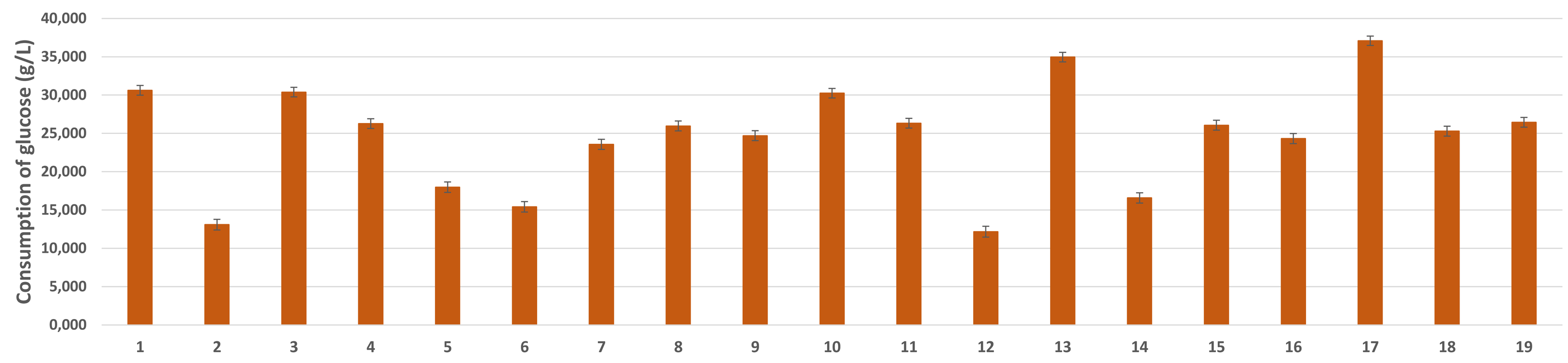


Figure 3. Graph of mean values of glucose consumption with 95% confidence level.

- The consumption of glucose, up to 37 g/L, achieved levels that would allow a decrease between 1-2 alcoholic degrees in wine. The R^2 statistic indicated that the model used was adequate, since it was able to explain 93.36% of the variability of the data.

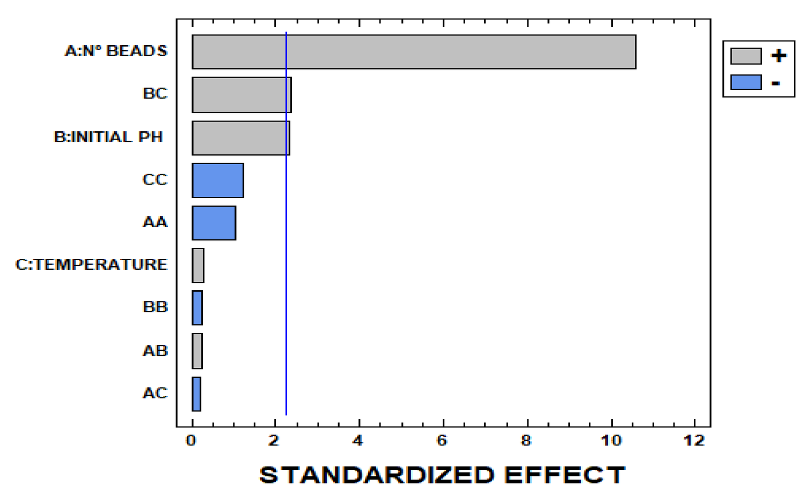


Figure 4. Standardized Pareto plot, showing that the factors enzyme concentration, initial pH of the must as well as the interaction between initial pH and temperature were significant in glucose consumption.

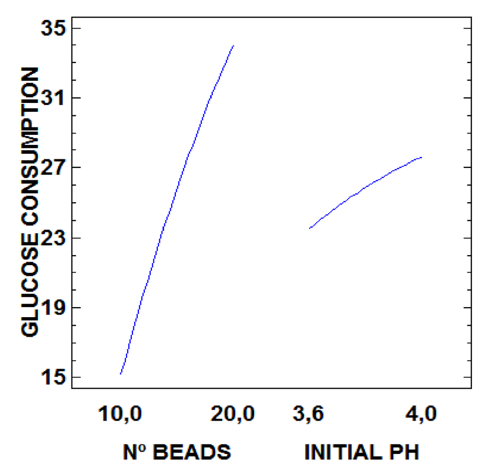
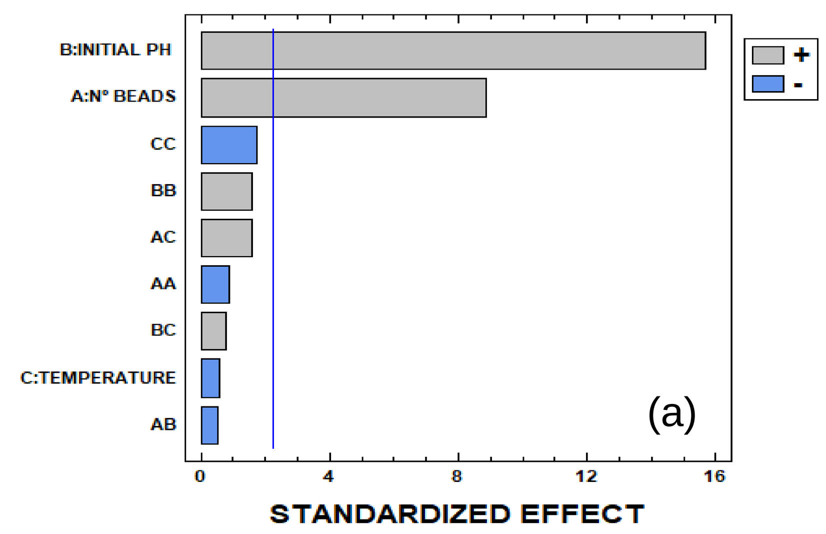


Figure 5. Graph of the significant main effects for glucose consumption, showing as glucose consumption is higher with increasing bead number and initial must pH.

- The decrease in the pH achieved would be in the range of 0.28-0.60. The value of the R^2 statistic was 97.37%.



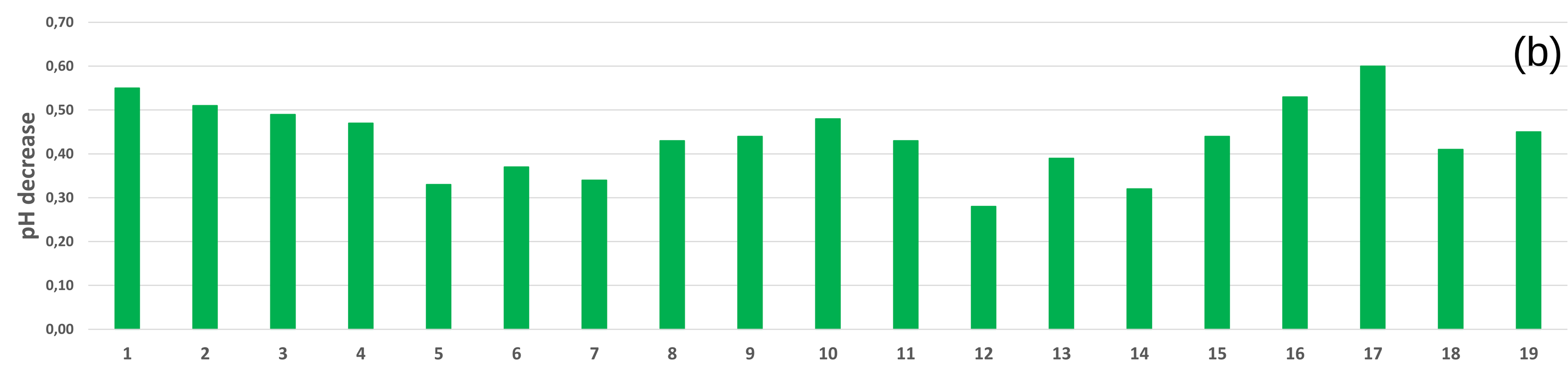


Figure 6. (a) Standardized Pareto plot, showing that the factors initial must pH and enzyme concentration were significant (b) pH decrease of must in the 19 experimental conditions.

Conclusions

- The immobilized GOX-CAT enzymes show sufficient activity to reduce the concentration of glucose in must equivalent to a reduction of 1-2 alcoholic degrees in wine.
- Glucose consumption raised as the number of beads and the initial must pH increased.
- The enzymatic treatment caused a moderate decrease of the initial must pH in the range of 0.3-0.6 units.
- The number of beads and the initial must pH had a significant effect in pH decrease.

These results show the potential of the alginate-silica co-immobilized GOX-CAT enzymes as a biotechnological strategy to mitigate the effects of climate change on winemaking production.

References

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