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Encapsulation of Combi-CLEAs of Glycosidases in Alginate Beads and Polyvinyl Alcohol for Wine Aroma Enhancement

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Abstract: The aromatic expression of wines can be enhanced by the addition of specific glycosidases, although their poor stability remains a limitation. Coimmobilization of glycosidases as cross-linked enzyme aggregates (combi-CLEAs) offers a simple solution yielding highly stable biocatalysts. Nevertheless, the small particle size of combi-CLEAs hinders their recovery, preventing their industrial application. Encapsulation of combi-CLEAs of glycosidases in alginate beads and in polyvinyl alcohol is proposed as a solution. Combi-CLEAS of β-D-glucosidase and α-L-arabinofuranosidase were prepared and encapsulated. The effects of combi-CLEA loading and particle size on the expressed specific activity (IU/$g_{biocatalyst}$) of the biocatalysts were evaluated. Best results were obtained with 2.6 mm diameter polyvinyl alcohol particles at a loading of 60 $mg_{combi\text{-}CLEA}/g_{polyvinyl\ alcohol}$, exhibiting activities of 1.9 and 1.0 IU/$g_{biocatalyst}$ for β-D-glucosidase and α-L-arabinofuranosidase, respectively. Afterwards, the stability of the biocatalysts was tested in white wine. All the encapsulated biocatalysts retained full activity after 140 incubation days, outperforming both free enzymes and nonencapsulated combi-CLEAs. Nevertheless, the alginate-encapsulated biocatalysts showed a brittle consistency, making recovery unfeasible. Conversely, the polyvinyl-encapsulated biocatalyst remained intact throughout the assay. The encapsulation of combi-CLEAs in polyvinyl alcohol proved to be a simple methodology that allows their recovery and reuse to harness their full catalytic potential.

Keywords: combi-CLEAs; glycosidases; wine aroma; alginate; polyvinyl alcohol

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1. Introduction

Research over the last decades has revealed that much of the aromatic expression of wines is due to the enzymatic-mediated release of flavor compounds, namely monoterpenes [1,2]. Monoterpenes occur naturally in fruits and plant tissues mostly as flavorless glycoconjugates composed, on one extremity, of a β-D-glucopyranose nucleus bound to the aromatic aglycon compound, and to sugars like α-L-rhamnopyranose, α-L-arabinofuranose, and β-D-apiofuranose on the other [2,3]. The enzymatic hydrolysis of these glycosides involves a two-step cascading reaction process where the diglycosidic bond is cleaved first by enzymes such as α-L-arabinofuranosidase (ARA), α-L-rhamnosidase (RAM) and β-D-apiosidase (API), resulting in the release of the monoterpenyl β-D-glucoside moiety which is then hydrolyzed in a second step by β-D-glucosidase (βG) releasing the aromatic monoterpene [4,5]. This enzymatic process is extensively applied nowadays in industrial winemaking during the maturation stage, using commercial preparations of soluble enzymes that contain mainly ARA, RAM and βG activities [6]. Nevertheless, the application of soluble enzymes in winemaking has important drawbacks mostly due to their fast inactivation under operational conditions (such as low pH and medium/high ethanol concentration) and because of the impracticality of their recovery. The latter limitation

prevents the reuse of the enzymes, which restricts the concentration of enzymes that can be used, and causes protein remnants in the final product that make necessary additional removal stages [6]. Enzyme immobilization provides solutions to these limitations by linking or containing the enzymes into a carrier, allowing to recover the biocatalyst and increase its stability [7,8].

Recent developments have led to the coimmobilization of ARA, RAM and βG as carrier-free biocatalysts in the form of cross-linked enzyme aggregates (combi-CLEAs) [9,10]. The cross-linked enzyme aggregates (CLEA) technique endows enzymes with some salient features such as high specific activity, high stability, and insolubility [11,12]. Furthermore, the technique is simple, inexpensive and allows the immobilization of nonpurified enzymes [11,12]. Combi-CLEAs and CLEAs in general, display a broad particle size distribution ranging from 1 to 1000 μm allowing easy recovery by centrifugation. However, in industrial winemaking, this would involve process modifications that can be a major hurdle for their application [11,12]. One way to unravel this difficulty, is the entrapment or encapsulation of combi-CLEAs in polymeric carriers. The inclusion of CLEAs into a carrier is still preferable to the direct encapsulation of free enzymes, since individual enzymes can easily leach out from the polymeric matrix due to their smaller size. Furthermore, immobilization conditions can cause considerable inactivation [13]. Inactivation is even more severe in the case of the immobilization of multiple enzymes, since appropriate immobilization conditions for one enzyme may result harsh for the others, and a compromise must be sought to prevent major activity loss [14]. Conversely, combi-CLEAs are highly concentrated enzyme clusters that have been stabilized at mild conditions, securing a high retention of the enzymatic activities. Furthermore, the larger size of combi-CLEAs reduces the risk of leaching [15].

The present work consisted of the encapsulation of combi-CLEAs of glycosidases in alginate beads and in polyvinyl alcohol (PVA) lens-shaped particles for wine aroma enhancement applications.

Alginate is a quite workable anionic polymer that has been widely used in the biomedical and food industries due to its biocompatibility, low cost, and mild gelation conditions suitable for enzyme entrapment [16]. PVA is a water-soluble polymer that has been widely used as a carrier for enzyme immobilization due to its mechanical and biocompatible properties that make it suitable for biomedical and food applications [17]. PVA can be shaped into various morphologies, including beads, hydrogel particles, hybrid films, PVA fibers, and nanofibrous mats, providing geometries that facilitate mass transfer and separation [18].

The immobilization of glycosidases to produce removable biocatalysts has great potential for the wine industry, allowing a better control of the reaction times to tailor the aromatic qualities of wines as per specific requirements, and also making the use of the enzymes more efficient.

2. Results and Discussion

2.1. Entrapment of Combi-CLEAs in Alginate Beads

Combi-CLEA loading was evaluated in the range from 2 to 40 $mg_{combi\text{-}CLEAs}/g_{alginate.}$ However, the biocatalyst beads at 40 $mg_{combi\text{-}CLEAs}/g_{alginate}$ exhibited a very weak structure, disintegrating even during the activity assays. Therefore, the results shown in Figure 1 correspond to those obtained only up to 20 $mg_{combi\text{-}CLEAs}/g_{alginate}$. The weakening of the biocatalysts at higher combi-CLEAs loadings is caused by reduced areas for ionic cross-linking between the guluronate blocks of the alginate polymeric chains, preventing the formation of a robust gel structure [16].

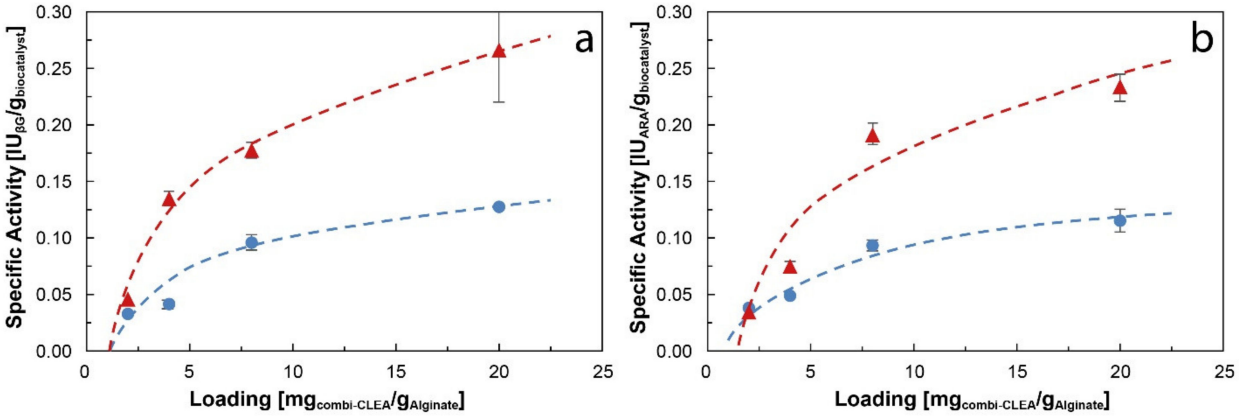

Figure 1. Effect of combi-CLEAs loading in alginate beads on the expressed specific activity of (**a**) βG and (**b**) ARA, in beads of two sizes: (▲) 2.8 mm average diameter and (●) 4.2 mm average diameter. Results are presented as mean ± margin of error, $n = 2$.

The specific activity (SA) of βG and ARA increased with combi-CLEAs loading, as expected given the greater amounts of combi-CLEAs per unit mass of biocatalyst. Nevertheless, this increase in SA is not linear and decreases at higher loadings due to diffusional limitations. At higher loadings the diffusion of substrates and products in and out of the beads becomes the limiting rate, which renders a portion of the encapsulated activity unexpressed.

Remarkable activity differences were obtained with respect to bead size (Figure 1). Larger beads (4.2 mm) displayed overall lower SA than the smaller beads (2.8 mm). Diffusional limitations are responsible for this behavior, since larger particles impose higher mass transfer limitations.

The statistical analysis confirms that both combi-CLEAs loading and bead size have a significant impact on SA, with loading having a positive effect, meaning a direct correlation between loading and activity, and bead size being inversely correlated to activity, as discussed above. Results of the statistical analysis are available in Supplementary Materials, Sections 1.2 and 2.2.

2.2. Entrapment of Combi-CLEAs in Polyvinyl Alcohol Lens-Shaped Particles

Figure 2 shows the results of the encapsulation of combi-CLEAs in PVA. The PVA-encapsulated biocatalysts exhibited a robust consistency at all loadings (2 to 60 $mg_{combi-CLEA}/g_{PVA}$). Specific activity increased with loading for βG (Figure 2a) and ARA (Figure 2b), with the SA/loading rate slowly decreasing. As in the case of alginate, this behavior is the consequence of diffusional limitations. Overall, higher specific activities were achieved in the smaller particles.

Combi-CLEAs loading and particle size were determined to be significant factors affecting the SA in the PVA-encapsulated biocatalysts as the statistical analysis shows (in Supplementary Materials Sections 1.2 and 2.2).

By comparing Figures 1 and 2, it can be seen that higher specific activities were obtained in the PVA particles. This could be the outcome of two main factors, namely, porosity and geometry. It has been reported that alginate forms beads with pore diameters around 12–16 nm [19], whereas LentiKats® have been reported to exhibit pore diameters ranging from 4 to 28 μm [20], that is, approximately three orders of magnitude higher. The lens-shaped geometry of LentiKats® also favors activity over spherical structures due to their comparatively lower thickness that allow higher diffusion rates of substrates. PVA lens-shaped particles of 2.6 to 6 mm diameter display thicknesses from 200 to 500 μm [21], significantly lower than the section length of the alginate beads (2.8 to 4.2 mm). On the other hand, the extended loading range (in comparison with the alginate-encapsulated bio-

catalysts) allowed a higher SA (1.9 and 1.0 IU/$g_{biocatalyst}$ for βG and ARA, respectively, at 60 $mg_{combi\text{-}CLEA}/g_{PVA}$ in the 2.6 mm particles), showing no signs of structural weakening.

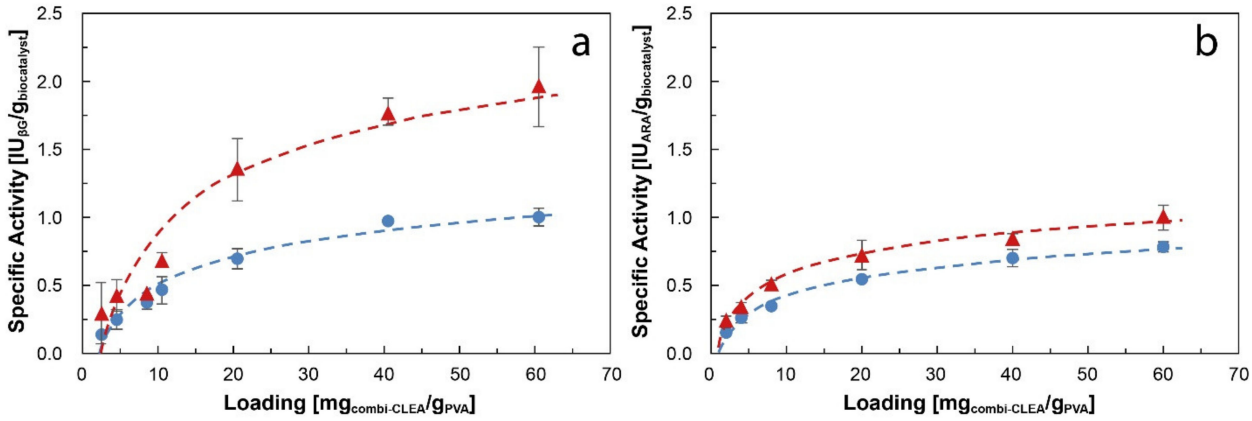

Figure 2. Effect of combi-CLEAs loading in polyvinyl alcohol lens-shaped particles on the expressed specific activity of (**a**) βG and (**b**) ARA, in PVA particles of two sizes: (▲) 2.6 mm average diameter and (●) 6.0 mm average diameter. Results are presented as mean ± margin of error, $n = 2$.

Results obtained in this work with PVA were better than those reported by Tavernini et al. [4] with the same combi-CLEAs preparation entrapped in chitosan and cross-linked with glutaraldehyde. The βG specific activity obtained in this study at 60 $mg_{combi\text{-}CLEA}/g_{PVA}$ is nearly two times higher, while almost the same ARA specific activity was achieved in both works.

The 2.8 mm alginate biocatalyst produced at 20 $mg_{combi\text{-}CLEA}/g_{alginate}$ and the 2.6 mm PVA biocatalyst encapsulated at 40 $mg_{combi\text{-}CLEA}/g_{PVA}$ were selected for evaluating their stability. The latter one was preferred than the 60 $mg_{combi\text{-}CLEA}/g_{PVA}$ particles since the error margins of SA overlap (Figure 2), and the slight increase in SA is not enough to compensate the use of more materials (PVA and combi-CLEAs), especially considering that these biocatalysts are envisioned for industrial use.

2.3. Operational Stability of the Biocatalysts in White Wine

Free enzymes, combi-CLEAs, combi-CLEAs encapsulated in alginate and combi-CLEAs encapsulated in PVA were subjected to stability tests in white wine for comparison. The results can be seen in Figure 3. Soluble βG and ARA have lost about 50% of the initial activity after about 3 and 10 d, respectively. By 20 d of incubation, βG and ARA have lost approximately 90% and 80% of their respective initial activities.

The residual activity of the combi-CLEAs decreased during the first 10 d but remained stable then on until 140 d of incubation. The inactivation behavior exhibited by βG and ARA in the combi-CLEAs is different. Whereas βG stabilizes at around 60% residual activity, ARA stabilizes at 90% residual activity. This behavior can be attributed to the dissimilar quaternary structure of these enzymes. βG from *Aspergillus niger* is known to be constituted by three subunits, as opposed to ARA which is composed of only one unit, making the βG structure more labile to environmental alterations [22–24]. The improved stability of combi-CLEAs as compared to the soluble enzymes is a consequence of aggregation and cross-linking which produce intermolecular bonds that stabilize the structure of the enzymes [11], being one of the most remarkable features of the CLEA technique.

βG and ARA remained fully active in the encapsulated combi-CLEAs during the 140 days of assay for both alginate beads and PVA biocatalysts, demonstrating the protective effect exerted by the polymeric carriers. However, the structural properties of the alginate beads were compromised, showing fractures after a few days of incubation and making their recovery unfeasible. On the other hand, the PVA-encapsulated biocatalysts remained fully intact throughout the incubation period.

Release of combi-CLEAs could be attested in the case of the alginate beads after very prolonged incubation times (over 4 months) when the beads started to gradually disintegrate. In the case of combi-CLEAs encapsulated in polyvinyl alcohol and nonencapsulated combi-CLEAs, no enzyme activity was detected in the medium.

The PVA-encapsulated combi-CLEAs developed in this work have shown the highest specific activity obtained to date using the combi-CLEAs encapsulation strategy for these glycosidases [4]. The 2.6 mm average size of the lens-shaped particles is concordant with the minimum particle size required for applications in packed bed or rotating bed reactors (about 0.5 mm) [25], allowing easy recovery and handling. Additionally, their enhanced operational stability makes possible repeated operation cycles to harness the full catalytic potential of these immobilized glycosidases.

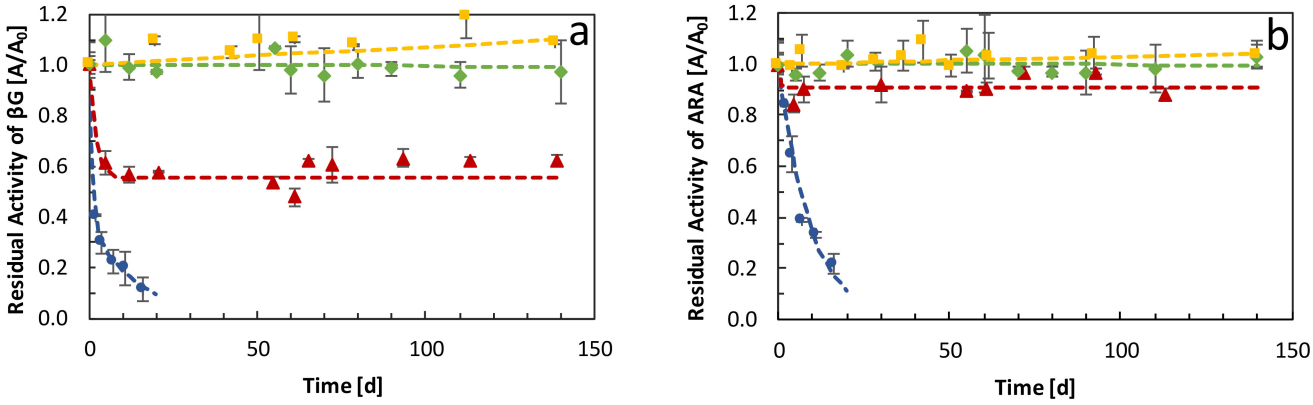

Figure 3. Operational stability of (●) soluble enzymes, (▲) combi-CLEAs of glycosidases, (◆) combi-CLEAs encapsulated in alginate beads, and (■) combi-CLEAs encapsulated in polyvinyl alcohol, carried out in white wine at 16 °C. Results are expressed as residual activity (A/A_0) of (**a**) βG and (**b**) ARA.

3. Materials and Methods

The commercial enzyme preparation Rapidase® Revelation Aroma was purchased from Oenobrands SAS (Montferrier-sur-Lez, OCC, France) containing main activities of βG and ARA from *Aspergillus niger*. Reagents *p*-nitrophenyl-α-L-arabinofuranoside (*p*NPA), and *p*-nitrophenyl-β-D-glucopyranoside (*p*NPG) were from Sigma–Aldrich (St. Louis, MO, USA). Glutaraldehyde grade II (25%, *v*/*v*), ammonium sulfate (AS), and calcium chloride dihydrate were acquired from Merck (Darmstadt, HE, Germany). Bovine serum albumin (BSA) and sodium alginate (sodium polymannuronate) were obtained from Loba Chemie (Mumbai, MH, India). LentiKat® Liquid and LentiKat® Stabilizer were provided by GeniaLab (Braunschweig, NI, Germany). All other reagents were of the highest purity available.

3.1. Specific Activity Analysis

The activity of βG and ARA was determined using *p*NPG and *p*NPA as substrates respectively, measuring the release of *p*-nitrophenol (*p*NP) spectrophotometrically at 405 nm. A volume of 200 µL of 0.6 mM *p*NPG or 0.4 mM *p*NPA was added to 9.8 mL of a 0.1 M acetate buffer solution pH 5.0 containing either soluble enzymes, combi-CLEAs, or 50–100 mg of encapsulated biocatalysts (alginate beads or PVA particles). The reaction was carried out under magnetic stirring at 200 rpm for 10 min and discrete measurements were made at regular time intervals. In these measurements, 0.5 mL of 0.5 M NaOH were added to 0.5 mL of medium sample. The treated samples were then taken to absorbance readings. One international unit of activity (IU) of βG and ARA (IU) was defined as the amount of enzyme producing one µmole of *p*NP per minute at 40 °C pH 5.0. The molar extinction coefficient of *p*NP was 9.214 mM^{-1}.

3.2. Synthesis of Combi-CLEAs of Glycosidases

The procedure for the synthesis of combi-CLEAs of glycosidases was based on that reported by Ahumada et al. [9]. Briefly, an enzymatic solution of Rapidase® Revelation Aroma was prepared in 100 mM phosphate buffer pH 7.0 to a final concentration of 2 $mg_{protein}/mL$. The solution was taken to a bath at 4 °C stirring at 300 rpm. BSA was then added at a ratio of 0.33 $mg_{BSA}/mg_{protein}$. Afterwards, a saturated AS solution was slowly poured into the enzymatic solution at 4 $mL_{AS}/mL_{solution}$, gradually increasing the stirring rate from 300 rpm to 600 rpm leaving the suspension under stirring for 30 min. Then, stirring was stopped and the suspension pH was adjusted to pH 7.0 using NaOH. Stirring was resumed at 600 rpm and glutaraldehyde was slowly added at a 53 $mg_{GA}/mg_{protein}$ mass ratio leaving the suspension under stirring for 1 h. The suspension containing the combi-CLEAs was then centrifuged at 19,460× *g* for 20 min at 4 °C, discarding the supernatant. Finally, the combi-CLEAs were resuspended in 100 mM phosphate buffer pH 7.0 and centrifuged again, repeating the washing procedure three times. The biocatalysts produced were stored at 5 °C until use. Before encapsulation, the combi-CLEAs were vacuum dried for 3 h in a Speedvac SPD121 P centrifugal concentrator (Thermo Fisher Scientific, Waltham, MA, USA) and ground afterwards to be used as powder. The combi-CLEAs powder showed a βG specific activity of 76.8 ± 0.5 $IU/g_{combi\text{-}CLEA}$ and ARA specific activity of 58.5 ± 1.6 $IU/g_{combi\text{-}CLEA}$.

3.3. Encapsulation of Combi-CLEAs in Alginate Beads

Samples of combi-CLEAs powder were mixed with a sodium alginate aqueous solution (4%) at different loadings ranging from 2 to 40 $mg_{combi\text{-}CLEA}/g_{alginate}$ to evaluate the effect of the biocatalyst loading on the expressed activity. The alginate/combi-CLEAs admixture was poured dropwise over a 200 mM calcium chloride solution using a 5 mL plastic syringe and a syringe pump (Model NE-300, New Era Pump Systems Inc., Farmingdale, NY, USA). The beads were cured for 60 min, after which the bead suspension was sieved and the beads were recovered. Washing was performed twice using 0.05 M acetate buffer pH 5.0. The alginate/combi-CLEAs beads were produced in two size distributions averaging 2.8 mm and 4.2 mm diameter for the smaller and larger size respectively. Details about the bead size measurements and the size distribution analysis can be found in Supplementary Materials, Sections 1.1 and 2.1.

The encapsulation results were evaluated in terms of specific activity in $IU/g_{biocatalyst}$.

3.4. Encapsulation of Combi-CLEAs in Polyvinyl Alcohol Lens-Shaped Particles

PVA carriers were made according to the protocol provided by GeniaLab and reported by Jovanovic-Malinovska et al. [26]. LentiKat® liquid was mixed with combi-CLEAs powder at loadings ranging from 2 to 60 $mg_{combi\text{-}CLEA}/g_{PVA}$. The mixture was taken to the hydrogel printer (LentiKat®Printer, Genialab, Braunschweig, NI, Germany) where small droplets were poured over a plastic dish and left to dry at 30 °C until reaching 30% *w*/*w* of the original weight. The biocatalyst lenses were then incubated in 100 mL of LentiKat® stabilizer at room temperature for 2 h. The PVA-encapsulated biocatalysts were produced in sizes averaging 2.6 mm and 6.0 mm diameter. The size measurements and the size distribution analysis are reported in Supplementary Materials, Sections 1.1 and 2.1.

The encapsulation results were evaluated in terms of specific activity measured in $IU/g_{biocatalyst}$.

3.5. Operational Stability of the Biocatalysts

All the biocatalysts were incubated in 100 mL of Gewürztraminer white wine (harvest 2017, Winery Cono Sur, Chimbarongo, Chile) at pH 3.3, 13% of ethanol, and 16 °C at the following concentrations: 1 g/L of soluble enzymes (Rapidase® Revelation Aroma), 1 g/L of combi-CLEA, 50 g/L of alginate biocatalyst beads, and 50 g/L of PVA-encapsulated biocatalyst. Samples were taken periodically measuring βG and ARA activities. The results

were presented as residual activity which corresponds to the ratio between the activity at any given time during the inactivation assay (A) and the initial activity (A_0).

4. Conclusions

Encapsulation of combi-CLEAs of glycosidases in alginate beads and polyvinyl alcohol lens-shaped particles was successfully accomplished. The methodology is simple and cost-efficient in both cases. Encapsulation in alginate yielded satisfactory results in terms of expressed activity, but the beads suffered from structural weakening when incubated in wine at operational conditions. Conversely, the encapsulation of combi-CLEAs of glycosidases in polyvinyl alcohol allowed to obtain a biocatalyst of 2.6 mm average diameter showing no signs of structural alteration in wine after 140 days of incubation. The specific activity exhibited by this biocatalyst represents a substantial improvement over results achieved with chitosan and alginate immobilized enzymes. The particle size of the developed biocatalyst, as well as their high stability and robustness, allow the recovery and use of immobilized glycosidases in repeated operational cycles to harness the full catalytic potential of these enzymes. Removing the biocatalyst from wine makes possible a better control of the reaction times to tailor the aromatic characteristics of wines as per specific requirements, also avoiding quality and process issues associated to protein remnants, all of which results in higher product quality and cost-efficient operations. The developed biocatalyst represents an important step towards a feasible and convenient alternative to the application of soluble enzymes in industrial winemaking for the aroma enhancement of wines.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/catal11070866/s1. Figure S1: Average diameter distribution of biocatalyst beads consisting on combi-CLEAs of glycosidases encapsulated in alginate. (a) Large size distribution, (b) small size distribution. Figure S2: Average diameter distribution of the lens-shaped biocatalyst consisting on combi-CLEAs of glycosidases encapsulated in polyvinyl alcohol. (a) Large size distribution, (b) small size distribution. Table S1: Coded levels for the two-level full factorial design of the encapsulation of combi-CLEAs of glycosidases in alginate beads. The factors evaluated were combi-CLEAs loading (L) in $mg_{combi\text{-}CLEA}/g_{alginate}$, and bead size (S) in mm. Table S2: Coded levels for the two-level full factorial design of the encapsulation of combi-CLEAs of glycosidases in polyvinyl alcohol lens-shaped particles. The factors evaluated were combi-CLEAs loading (L) in $mg_{combi\text{-}CLEA}/g_{alginate}$, and particle size (S) in mm. Table S3: Two-level full factorial experimental design of the encapsulation of combi-CLEAs of glycosidases in alginate beads. The factors evaluated were combi-CLEA loading (L) in $mg_{combi\text{-}CLEA}/g_{alginate}$, and bead size (S) in mm. The results are expressed in terms of specific activity of βG ($SA_{\beta G}$), and specific activity of ARA (SA_{ARA}) in $IU/g_{biocatalyst}$, replicates $n = 2$. Table S4: Two-level full factorial experimental design of the encapsulation of combi-CLEAs of glycosidases in polyvinyl alcohol lens-shaped particles. The factors evaluated were combi-CLEAs loading (L) in $mg_{combi\text{-}CLEA}/g_{alginate}$, and particle size (S) in mm. The results are expressed in terms of specific activity of βG ($SA_{\beta G}$), and specific activity of ARA (SA_{ARA}) in units $IU/g_{biocatalyst}$, replicates $n = 2$. Table S5: Analysis of effects and percent contribution of loading (L) ($mg_{combi\text{-}CLEA}/g_{alginate}$), and bead size (S) (mm) on the specific activity of βG, as a result of the encapsulation of combi-CLEAs of glycosidases in alginate beads. Table S6: Analysis of effects and percent contribution of loading (L) ($mg_{combi\text{-}CLEA}/g_{alginate}$), and bead size (S) (mm) on the specific activity of ARA, as a result of the encapsulation of combi-CLEAs of glycosidases in alginate beads. Table S7: Analysis of variance of the effect of loading (L) ($mg_{combi\text{-}CLEA}/g_{alginate}$), and bead size (S) (mm) on the specific activity of βG, as a result of the encapsulation of combi-CLEAs of glycosidases in alginate beads. Replicates $n = 2$. Table S8: Analysis of variance of the effect of loading (L) ($mg_{combi\text{-}CLEA}/g_{alginate}$), and bead size (S) (mm) on the specific activity of ARA, as a result of the encapsulation of combi-CLEAs of glycosidases in alginate beads. Replicates $n = 2$. Table S9: Analysis of effects and percent contribution of loading (L) ($mg_{combi\text{-}CLEA}/g_{alginate}$), and particle size (S) (mm) on the specific activity of βG, as a result of the encapsulation of combi-CLEAs of polyvinyl alcohol lens-shaped particles. Negative effects are shown in red font color. Table S10: Analysis of effects and percent contribution of loading (L) ($mg_{combi\text{-}CLEA}/g_{alginate}$), and particle size (S) (mm) on the specific activity of ARA, as a result of the encapsulation of combi-CLEAs of glycosidases polyvinyl alcohol lens-shaped particles. Negative

effects are shown in red font color. Table S11: Analysis of variance of the effect of loading (L) ($mg_{combi\text{-}CLEA}/g_{alginate}$), and particle size (S) (mm) on the specific activity of βG, as a result of the encapsulation of combi-CLEAs of glycosidases polyvinyl alcohol lens-shaped particles. Replicates $n = 2$. Table S12: Analysis of variance of the effect of loading (L) ($mg_{combi\text{-}CLEA}/g_{alginate}$), and particle size (S) (mm) on the specific activity of ARA, as a result of the encapsulation of combi-CLEAs of glycosidases polyvinyl alcohol lens-shaped particles. Replicates $n = 2$.

Author Contributions: Conceptualization, O.R. and L.W.; methodology, O.R.; formal analysis, L.T.; investigation, C.A. and O.R.; data curation, O.R. and L.T.; writing—original draft preparation, L.T.; writing—review and editing, O.R., C.A., L.W. and A.I.; visualization, L.T.; supervision, A.I. and L.W.; project administration, A.I. and L.W.; funding acquisition, L.W. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The data presented in this study are available in the present article and in Supplementary Materials.

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Conflicts of Interest: The authors declare no conflict of interest.

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