

## Supplementary Materials

### Encapsulation of combi-CLEAs of glycosidases in alginate beads and polyvinyl alcohol for wine aroma enhancement

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## 1 MATERIALS AND METHODS

### 1.1 Particle size measurements

The encapsulated biocatalyst particles of either alginate or lens-shaped polyvinyl alcohol, were arranged in a matrix of 10 x 10 particles equidistantly at 0.5 cm between each. The particles were scanned using a HP Photosmart D110 series Printer/Scanner and their size distribution analyzed using the free open-source software ImageJ (<https://imagej.nih.gov/ij/>).

### 1.2 Statistical analysis

The statistical significance of the encapsulation results was evaluated by an Analysis of Variance (ANOVA). To this aim, the data were arranged according to a two-level full factorial design, and the coded levels for the alginate beads and the Polyvinyl alcohol biocatalysts can be seen in Table S1 and Table S2 respectively. The data were analyzed according to standard ANOVA procedure using the software Microsoft Excel.

**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  $\text{mg}_{\text{combi-CLEA}}/\text{g}_{\text{alginate}}$ , and bead size (S) in mm

Factor	Coded Level	
	-1	1
L	2	20
S	2.8	4.2

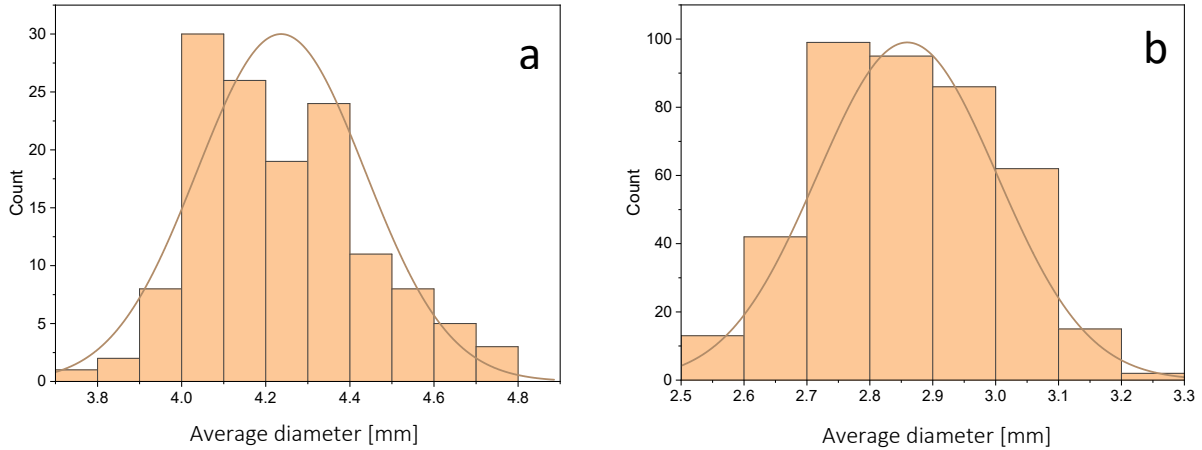
**Table S2.** Coded levels for the two-level full factorial design of the encapsulation of combi-CLEAs of glycosidases in polyvinyl alcohol lens-shaped particles. The factors evaluated were combi-CLEAs loading (L) in  $\text{mg}_{\text{combi-CLEA}}/\text{g}_{\text{alginate}}$ , and particle size (S) in mm

Factor	Coded Level	
	-1	1
L	2	40
S	2.6	6.0

## 2 SUPPLEMENTARY RESULTS

### 2.1 Particle size measurements

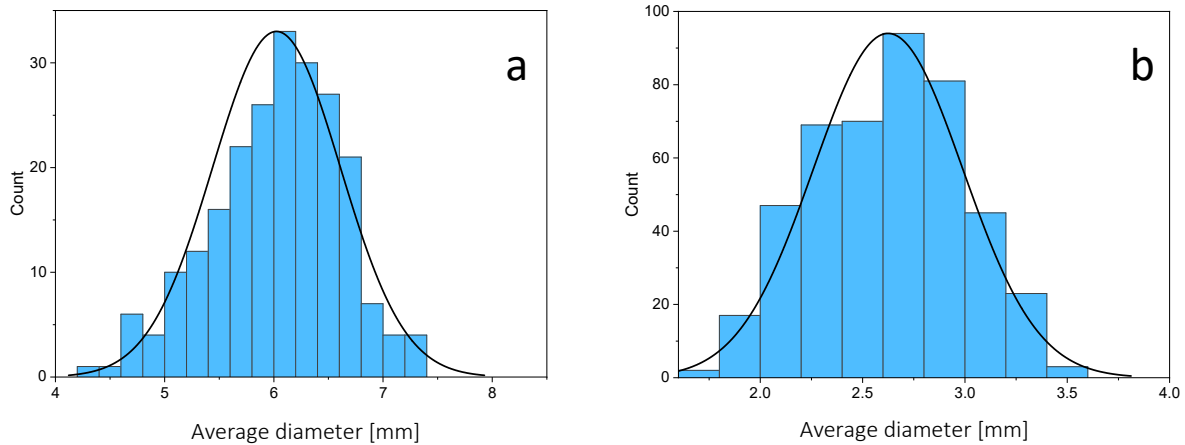
Results of the size measurements of the alginate bead biocatalysts are shown in Figure S1.



**Figure S1.** Average diameter distribution of biocatalyst beads consisting on combi-CLEAs of glycosidases encapsulated in alginate. (a) big size distribution, (b) small size distribution

The mean diameter of the big size distribution of the alginate bead biocatalysts was  $4.23 \pm 0.19$  mm. The mean diameter of the small size distribution was  $2.86 \pm 0.14$  mm.

Figure S2 shows the size measurement results of the polyvinyl alcohol (PVA) lens-shaped biocatalysts.



**Figure S2.** Average diameter distribution of the lens-shaped biocatalyst consisting on combi-CLEAs of glycosidases encapsulated in polyvinyl alcohol. (a) big size distribution, (b) small size distribution

The mean diameter of the big size distribution of the PVA lens-shaped biocatalysts was  $6.02 \pm 0.58$  mm. The mean diameter of the small size distribution was  $2.62 \pm 0.36$  mm

## 2.2 Statistical analysis

### 2.2.1 ANOVA of the alginate biocatalyst beads

The resulting experimental design data are shown in Table S3 and Table S4.

**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  $\text{mg}_{\text{combi-CLEA}}/\text{g}_{\text{alginate}}$ , and bead size (S) in mm. The results are expressed in terms of specific activity of  $\beta\text{G}$  ( $\text{SA}_{\beta\text{G}}$ ), and specific activity of ARA ( $\text{SA}_{\text{ARA}}$ ) in units  $\text{IU}/\text{g}_{\text{biocatalyst}}$ , replicates  $n = 2$

Factor		$\text{SA}_{\beta\text{G}}$		$\text{SA}_{\text{ARA}}$	
L	S	a	b	a	b
-1	-1	0.05	0.05	0.04	0.03
1	-1	0.24	0.33	0.25	0.22
-1	1	0.03	0.03	0.04	0.04
1	1	0.13	0.13	0.12	0.11

**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-CLEA loading (L) in  $\text{mg}_{\text{combi-CLEA}}/\text{g}_{\text{alginate}}$ , and particle size (S) in mm. The results are expressed in terms of specific activity of  $\beta\text{G}$  ( $\text{SA}_{\beta\text{G}}$ ), and specific activity of ARA ( $\text{SA}_{\text{ARA}}$ ) in units  $\text{IU}/\text{g}_{\text{biocatalyst}}$ , replicates  $n = 2$

Factor		$\text{SA}_{\beta\text{G}}$		$\text{SA}_{\text{ARA}}$	
L	S	a	b	a	b
-1	-1	0.46	0.13	0.27	0.22
1	-1	1.70	1.84	0.87	0.82
-1	1	0.14	0.13	0.16	0.15
1	1	0.96	0.99	0.75	0.66

The analysis of effects and percent distribution of  $\beta\text{G}$  and ARA are presented in Tables S5 and S6 respectively.

**Table S5.** Analysis of effects and percent contribution of loading (L) ( $\text{mg}_{\text{combi-CLEA}}/\text{g}_{\text{alginate}}$ ), and bead size (S) (mm) on the specific activity of  $\beta\text{G}$ , as a result of the encapsulation of combi-CLEAs of glycosidases in alginate beads

Factor	Effects	Sum of squares	Percent contribution
L	0.16	0.0543	66.1
S	-0.08	0.0141	17.1
LS	-0.07	0.0098	12.0

Note: Negative effects are shown in red font color

**Table S6.** Analysis of effects and percent contribution of loading (L) ( $\text{mg}_{\text{combi-CLEA}}/\text{g}_{\text{alginate}}$ ), 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

Factor	Effects	Sum of squares	Percent contribution
L	0.14	0.0382	72.4
S	-0.06	0.0066	12.5
LS	-0.06	0.0075	14.2

Note: Negative effects are shown in red font color

Tables S5 and S6 show a major effect and contribution of combi-CLEA loading, being a positive effect, that is, the higher the loading the higher the specific activity. Particle size and the combined effect of loading and particle size, show a negative impact on the specific activity with a minor contribution in relation to loading alone.

The ANOVA results for  $\beta\text{G}$  and ARA can be seen in Tables S7 and S8 respectively.

**Table S7.** Analysis of variance of the effect of loading (L) ( $\text{mg}_{\text{combi-CLEA}}/\text{g}_{\text{alginate}}$ ), and bead size (S) (mm) on the specific activity of  $\beta\text{G}$ , as a result of the encapsulation of combi-CLEAs of glycosidases in alginate beads. Replicates  $n = 2$

Factor	Sum of squares	Degrees of freedom	Mean square	$F_0$	$p$ -value
L	0.054341	1	0.05434	54.63158	< 0.0001
S	0.014089	1	0.01409	14.16423	0.0070
LS	0.009841	1	0.00984	9.894078	0.0163
Error	0.003979	4	0.00099		
Total	0.082250	7			

Note: Significant  $p$ -values are marked in light blue, considering a significance level of  $\alpha = 0.05$

**Table S8.** Analysis of variance of the effect of loading (L) ( $\text{mg}_{\text{combi-CLEA}}/\text{g}_{\text{alginate}}$ ), 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  $n = 2$

Factor	Sum of squares	Degrees of freedom	Mean square	$F_0$	$p$ -value
L	0.038198	1	0.038198	333.0616	< 0.0001
S	0.006619	1	0.006619	57.71161	< 0.0001
LS	0.007472	1	0.007472	65.15356	< 0.0001
Error	0.000459	4	0.000115		
Total	0.052747	7			

Note: Significant  $p$ -values are marked in light blue, considering a significance level of  $\alpha = 0.05$

The ANOVA results in Tables S7 and S8 show that all factors (combi-CLEA loading and bead size) exert a significant effect on the specific activity of  $\beta\text{G}$  and ARA.

### 2.2.2 ANOVA of the Polyvinyl alcohol biocatalysts

Tables S9 and S10 show the analysis of effects and percent distribution of  $\beta$ G and ARA respectively.

**Table S9.** Analysis of effects and percent contribution of loading (L) ( $\text{mg}_{\text{combi-CLEA}}/\text{g}_{\text{alginate}}$ ), and particle size (S) (mm) on the specific activity of  $\beta$ G, as a result of the encapsulation of combi-CLEAs of glycosidases in polyvinyl alcohol lens-shaped particles. Negative effects are shown in red font color

Factor	Effects	Sum of squares	Percent contribution
L	1.15	2.663	78.7
S	-0.48	0.453	13.4
LS	-0.32	0.203	6.0

Note: Negative effects are shown in red font color

**Table S10.** Analysis of effects and percent contribution of loading (L) ( $\text{mg}_{\text{combi-CLEA}}/\text{g}_{\text{alginate}}$ ), and particle size (S) (mm) on the specific activity of ARA, as a result of the encapsulation of combi-CLEAs of glycosidases in polyvinyl alcohol lens-shaped particles. Negative effects are shown in red font color

Factor	Effects	Sum of squares	Percent contribution
L	0.58	0.662	94.9
S	-0.12	0.028	4.0
LS	-0.03	0.002	0.2

Note: Negative effects are shown in red font color

Analogously to the case of the alginate beads, there is a major effect and contribution of combi-CLEA loading (Tables S9 and S10), being a positive effect. Particle size and the combined effect of loading and particle size, show a negative impact on the specific activity with a minor contribution in relation to loading alone.

The ANOVA results for  $\beta$ G and ARA can be seen in Tables S11 and S12 respectively.

**Table S11.** Analysis of variance of the effect of loading (L) ( $\text{mg}_{\text{combi-CLEA}}/\text{g}_{\text{alginate}}$ ), and particle size (S) (mm) on the specific activity of  $\beta$ G, as a result of the encapsulation of combi-CLEAs of glycosidases in polyvinyl alcohol lens-shaped particles. Replicates  $n = 2$

Factor	Sum of squares	Degrees of freedom	Mean square	$F_0$	$p$ -value
L	2.6629	1	2.663	168.18	< 0.0001
S	0.4535	1	0.453	28.64	0.0011
LS	0.2032	1	0.203	12.83	0.0089
Error	0.0633	4	0.016		
Total	3.3829	7			

Note: Significant  $p$ -values are marked in light blue, considering a significance level of  $\alpha = 0.05$

**Table S12.** Analysis of variance of the effect of loading (L) ( $\text{mg}_{\text{combi-CLEA}}/\text{g}_{\text{alginate}}$ ), 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  $n = 2$

Factor	Sum of squares	Degrees of freedom	Mean square	$F_0$	$p$ -value
L	0.6621	1	0.662	412.22	< 0.0001
S	0.0279	1	0.028	17.35	0.004
LS	0.0015	1	0.002	0.93	0.366
Error	0.0064	4	0.002		
Total	0.6979	7			

Note: Significant  $p$ -values are marked in light blue, considering a significance level of  $\alpha = 0.05$

Both combi-CLEA loading and particle size show a significant effect on the encapsulation of combi-CLEAs of glycosidases in PVA (Tables S11 and S12). The combined effect of both factors has a minor impact as compared to the results with alginate, being non-significant for ARA (Table S12).