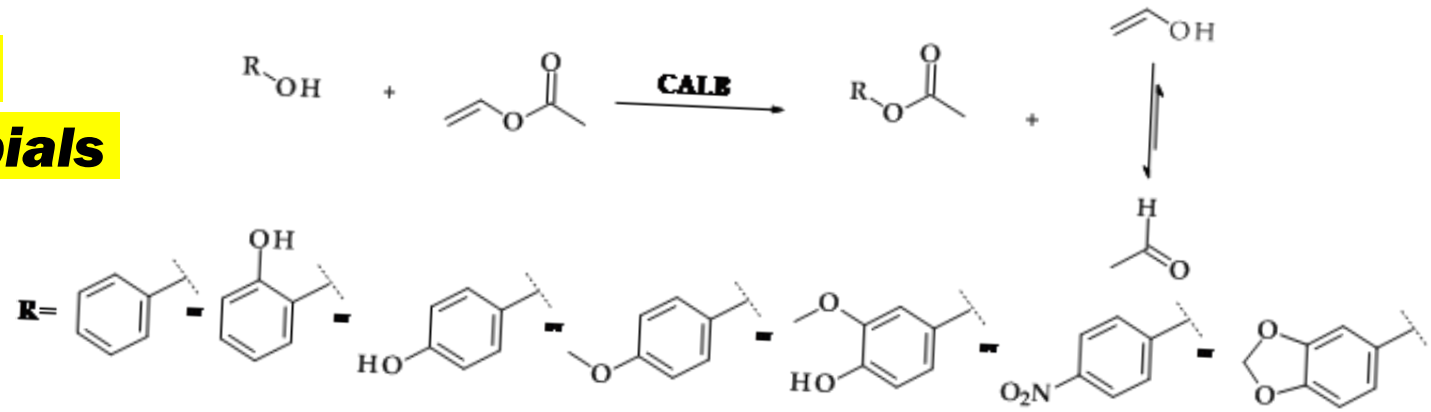
Enzymatic Synthesis of Flavours and Fragrances, Antioxidants and Antimicrobials on the Example of Benzyl Alcohol and its Selected Derivatives



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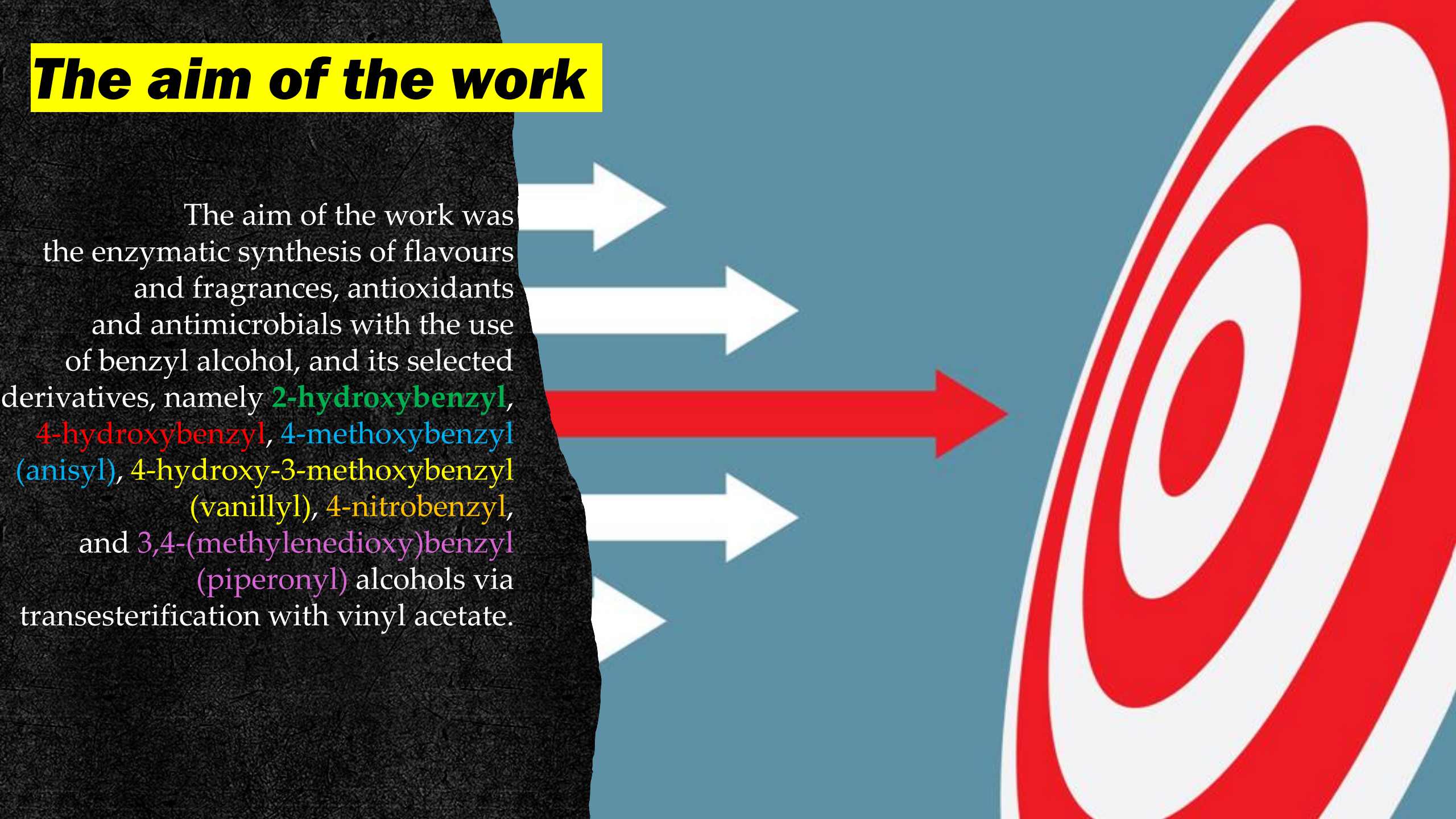
The 3rd international electronic conference on Foods: Food, Microbiome, and Health
- A Celebration of the 10th Anniversary of Foods' Impact on our Wellbeing, 1–15 Oct 2022

The aim of the work

The aim of the work was the enzymatic synthesis of flavours and fragrances, antioxidants and antimicrobials with the use of benzyl alcohol, and its selected derivatives, namely **2-hydroxybenzyl**, 4-hydroxybenzyl, 4-methoxybenzyl (anisyl), 4-hydroxy-3-methoxybenzyl (vanillyl), 4-nitrobenzyl, and 3,4-(methylenedioxy)benzyl (piperonyl) alcohols via transesterification with vinyl acetate.



Methodology

- Enzymatic Synthesis of Acetate Esters of Benzyl Alcohol and its Derivatives
- Column Chromatography and ^{1}H NMR of the Obtained Esters
- Evaluation of Antioxidant Activity by Means of the DPPH· assay
- Evaluation of Antimicrobial Activity with the Disc Diffusion Method

Enzymatic Synthesis of Acetate Esters of Benzyl Alcohol and its Derivatives

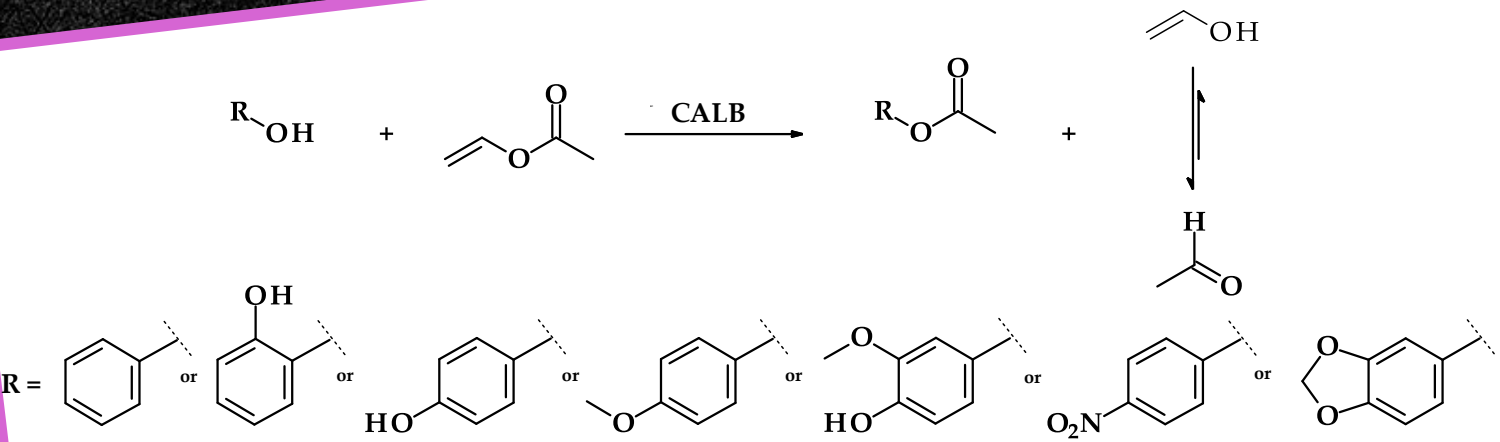


Figure 1. Reaction scheme for benzyl alcohol derivatives esters syntheses via transesterification with vinyl acetate and *Candida antarctica* lipase B as a biocatalyst.

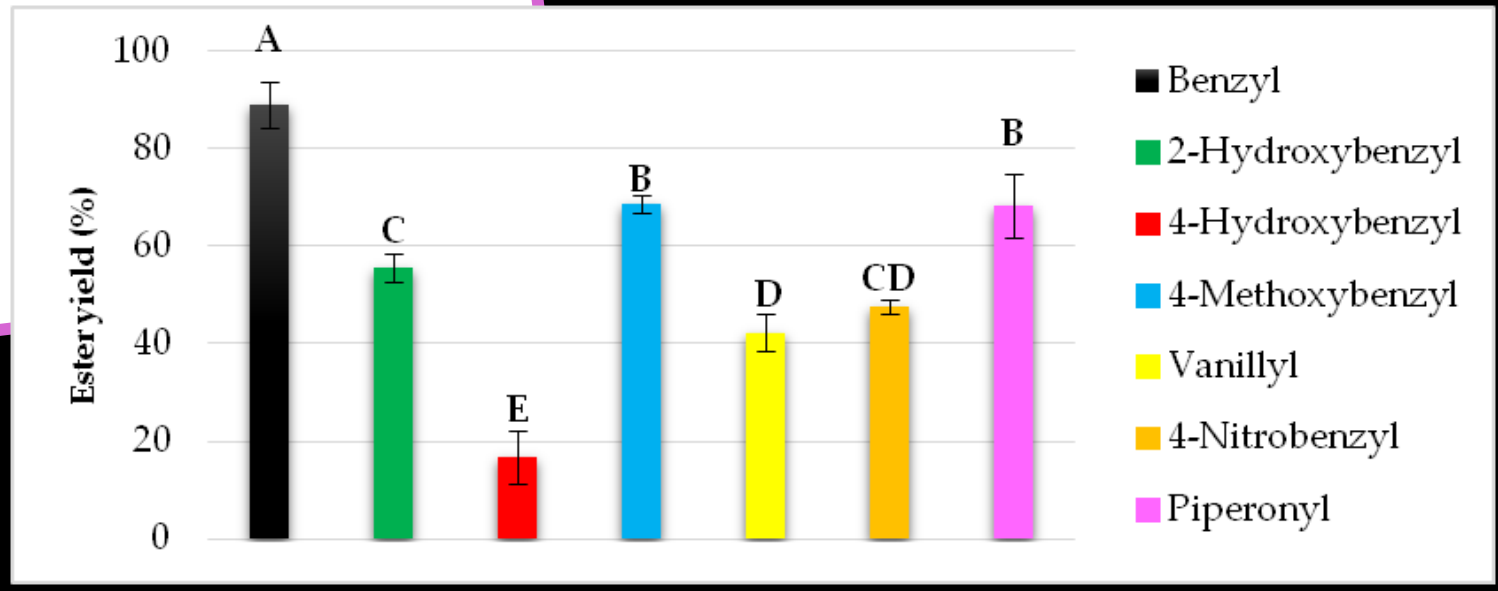


Figure 2. The yields of enzymatic syntheses of esters of benzyl alcohol and its derivatives. The bars present the means ± SD of isolated yields. Yield: 100 × (actual quantity received/ideal quantity calculated). Means with the same capital letter (A-E) did not differ significantly ($\alpha = 0.05$).

^{1}H NMR of the Obtained Esters

Spectra were recorded on Bruker AVANCE 300 MHz spectrometer (Bruker, Billerica, MA, USA) with $CDCl_3$ as a solvent. Proton chemical shifts are reported below in ppm (δ) and were relative to tetramethylsilane (TMS) as an internal standard.

Benzyl acetate: ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.10 (3H, s), 5.11 (2H, s), 7.28 – 7.44 (5H, m)

2-Hydroxybenzyl acetate: ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.14 (3H, s), 5.15 (2H, s), 6.88 – 7.02 (2H, m), 7.24 – 7.37 (2H, m), 7.78 (1H, s)

4-Hydroxybenzyl acetate: ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.11 (3H, s), 5.06 (2H, s), 5.16 (1H, s), 6.78 – 6.91 (2H, m), 7.20 – 7.37 (2H, m)

4-Methoxybenzyl acetate: ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.10 (3H, s), 3.83 (3H, s), 5.07 (2H, s), 6.86 – 6.97 (2H, m), 7.27 – 7.38 (2H, m)

Vanillyl acetate: ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.11 (3H, s), 3.92 (3H, s), 5.05 (2H, s), 5.72 (1H, s), 6.89 – 6.91 (3H, m)

4-Nitrobenzyl acetate: ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.16 (3H, s), 5.20 (2H, s), 7.47 – 7.57 (2H, m), 8.18 – 8.28 (2H, m)

Piperonyl acetate: ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.08 (3H, s), 5.00 (2H, s), 5.96 (2H, s), 6.76 – 6.88 (3H, m)

Flavouring Properties of the Synthesized Esters

Table 1. Flavouring esters permitted for use in food with benzyl alcohol moiety.

	CAS[a] Number	JECFA[b] Number	CoE[c] Number	FEMA[d] Number	Odour[e]	Flavour[e]
Benzyl acetate	140-11-4	23	2040	2135	sweet, floral, fruity, jasmin, fresh	fruity, sweet, with balsamic and jasmin floral undernotes
2-Hydroxybenzyl acetate	6161-96-2	-	-	-	-	-
4-Hydroxybenzyl acetate	3233-32-7	-	-	-	-	-
4-Methoxybenzyl acetate	104-21-2	873	209	2098	sweet, powdery, balsam, vanilla, fruity, plum, cherry, tonka	sweet, fruity, licorice, cherry, vanilla, coumarin
Vanillyl acetate	57404-55-4	-	-	-	-	-
4-Nitrobenzyl acetate	619-90-9	-	-	-	-	-
Piperonyl acetate	326-61-4	894	2068	2912	sweet, floral, strawberry jam, hawthorn, metallic	floral, soapy, fruity, berry and slightly jammy with a powdery nuance

[a] – Chemical Abstracts Service; [b] – Joint FAO/WHO Expert Committee on Food Additives; [c] – The Council of Europe; [d] – Flavor Extract Manufacturers Association; [e] – Based on the data of The Good Scents Company Information System (http://www.thegoodscentscompany.com/index.html)

Evaluation of Antioxidant Activity by Means of the DPPH· assay

Table 2. Antioxidant activities of the obtained esters determined by means of DPPH· assay.

	AA (%)	IC_{50} (mM)
Benzyl acetate	3.51 ± 1.56	>100
2-Hydroxybenzyl acetate	10.07 ± 1.04	62.65 ± 7.95
4-Hydroxybenzyl acetate	7.70 ± 0.30	85.72 ± 1.02
4-Methoxybenzyl acetate	3.12 ± 0.41	>100
Vanillyl acetate	**55.15 ± 1.88***	**0.83 ± 0.04**
4-Nitrobenzyl acetate	4.64 ± 0.68	>100
Piperonyl acetate	3.64 ± 0.25	>100

* 1 mM instead of 10 mM; AA – antioxidant activity; IC_{50} - concentration required for 50% reduction of the DPPH radical

The highest antioxidant activity!

Evaluation of Antimicrobial Activity with the Disc Diffusion Method

Table 3. Comparison of antimicrobial activity of the obtained esters.

	Inhibition zone diameter (mm)		**Ester sensitivity***	
	E. coli PCM 2057	*S. aureus* PCM 2054	*E. coli* PCM 2057	*S. aureus* PCM 2054
Benzyl acetate	6.7 ± 0.6 CD	6.0 ± 0.0 C	No effective	No effective
2-Hydroxybenzyl acetate	**19.0 ± 1.0 A**	**20.3 ± 0.6 A**	**Medium**	**Strong**
4-Hydroxybenzyl acetate	6.0 ± 0.0 D	6.0 ± 0.0 C	No effective	No effective
4-Methoxybenzyl acetate	8.7 ± 1.2 BC	6.0 ± 0.0 C	No effective	No effective
Vanillyl acetate	10.7 ± 1.5 B	13.7 ± 0.6 B	Weak	Weak
4-Nitrobenzyl acetate	6.0 ± 0.0 D	6.0 ± 0.0 C	No effective	No effective
Piperonyl acetate	7.7 ± 0.6 CD	6.0 ± 0.0 C	No effective	No effective

* <10 mm - no effective; 10-16 mm - weak; 16-20 mm - medium; >20 mm - strong
Means with the same capital letter (A-E) in the column did not differ significantly ($\alpha = 0.05$).

The highest antibacterial activity!

Conclusion

The presented herein study showed the possibility of modifying benzyl alcohol and its derivatives to acetate esters via enzymatic transesterification of vinyl acetate.

The synthesized esters can be categorized into three groups, i.e., flavouring substances (benzyl, 4-methoxy, and piperonyl acetates), antioxidant agents (vanillyl acetate) and antimicrobial compounds (2-hydroxybenzyl acetate).

The described preliminary research should be extended in order to deepen the knowledge about the activity of the compounds obtained and compare them with their precursors (benzyl and other alcohols)

There should be investigated the antimicrobial activity against a larger amount of food spoilage microorganisms and the antioxidant activity using other known assays, as well as in convenience foods.

Thank you

for your attention!