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From Waste to Resource: Development of a Sustainable Bacterial Culture Medium from Wine Lees and Toxicity Evaluation in the Zebrafish Model

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Supplementary Materials:

Supplementary Table S1. -Growth kinetic parameters of UNQLp 11 in WP based alternative culture media containing different nitrogen sources.

		Yeast supplementation %w/v	Y_M Log(CFU/ml)	k (day^{-1})
MRS		-	9.69 ± 0.10	3.00 ± 0.70
Control	without salts	-	9.17 ± 0.10	0.38 ± 0.01
	with salts	-	9.07 ± 0.11	0.60 ± 0.01
Commercial YE	without salts	0.5	9.10 ± 0.10	1.20 ± 0.01
		1.0	9.38 ± 0.11*	1.18 ± 0.12
		1.5	9.08 ± 0.14	1.97 ± 0.32*
		2.0	9.09 ± 0.14	1.59 ± 0.30
	with salts	0.5	9.46 ± 0.14*	1.10 ± 0.14
		1.0	9.69 ± 0.11*	1.56 ± 0.19
		1.5	9.47 ± 0.03*	2.00 ± 0.10*
		2.0	9.38 ± 0.21*	1.42 ± 0.34
Autolyzed WL	without salts	0.5	8.65 ± 0.05	1.76 ± 0.15*
		1.0	8.86 ± 0.30	1.26 ± 0.47
		1.5	8.63 ± 0.12	1.63 ± 0.33
		2.0	8.60 ± 0.12	2.40 ± 0.76
	with salts	0.5	9.15 ± 0.10	1.43 ± 0.18
		1.0	9.06 ± 0.10	2.26 ± 0.46*

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		1.5	9.47 ± 0.18*	1.70 ± 0.37
		2.0	9.72 ± 0.30*	1.33 ± 0.46*
Pure WL	without salts	0.5	8.79 ± 0.39	3.99 ± 0.75
		1.0	8.50 ± 0.16	3.20 ± 0.80*
		1.5	9.17 ± 0.15	2.15 ± 0.56*
		2.0	9.46 ± 0.39*	1.55 ± 0.69
	with salts	0.5	8.49 ± 0.07	4.36 ± 0.76
		1.0	8.69 ± 0.18	2.84 ± 0.88*
		1.5	8.94 ± 0.09	1.89 ± 0.28*
		2.0	8.61 ± 0.09	3.12 ± 0.90*

-YM, maximum predicted population (log CFU/mL); K, growth rate constant (day^{-1}) obtained from the Gompertz model fitted using GraphPad 8. The software used does not provide the maximum specific growth rate (μmax). Without significant difference regard MRS (One way ANOVA Two-sided Dunnett's Multiple Comparisons $p<0.05$)

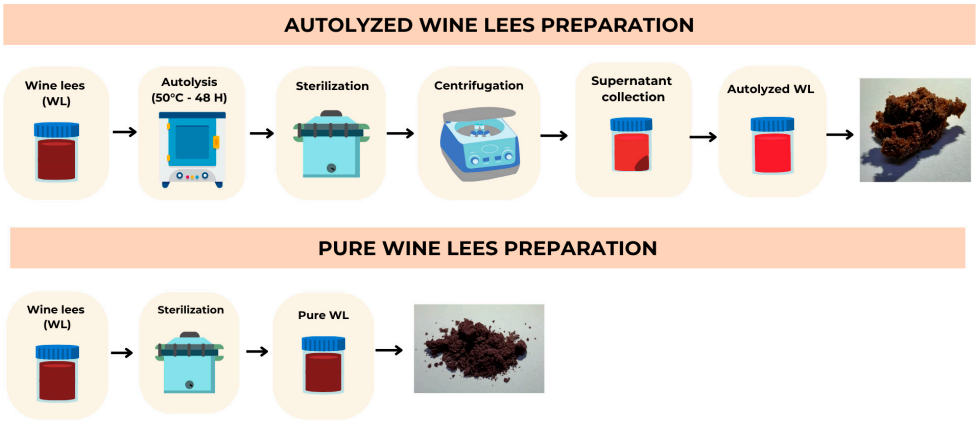


Figure S1. - Scheme for obtaining pure wine lees and used wine lees.

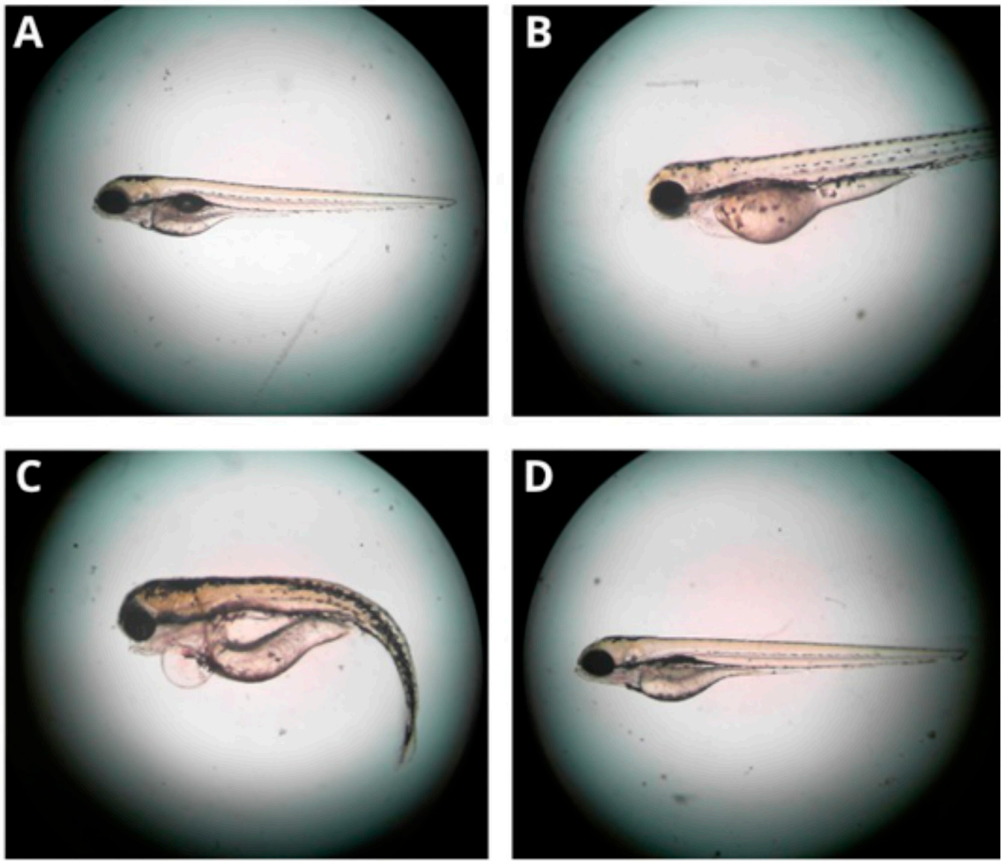


Figure S2. Representative morphological abnormalities observed in zebrafish embryos exposed to pure and autolyzed wine lees. Images are shown for qualitative illustration of the developmental effects described in the text and are not intended as quantitative analyses: (A) Control embryo with normal development; (B) intestinal inflammation, edema, and collapsed swim bladder; (C) edema, axial curvature, malformed swim bladder, and yolk sac deformity; (D) collapsed swim bladder.

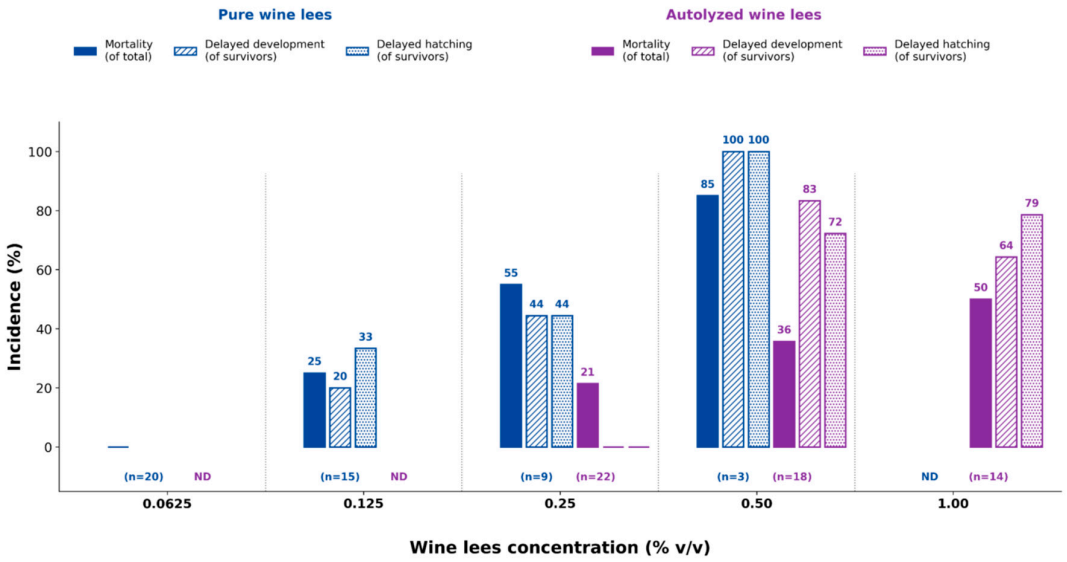


Figure S3. Developmental outcomes in zebrafish embryos exposed to pure and autolyzed wine lees. Mortality and the incidence of delayed development and delayed hatching were evaluated at different concentrations of pure wine lees and autolyzed wine lees. Mortality is expressed as the percentage of the total number of exposed embryos, whereas developmental abnormalities are expressed as the percentage of surviving embryos.The number of surviving embryos evaluated for developmental abnormalities (n) is indicated below each concentration. ND, not determined.

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Informed Consent Statement:.

Abbreviations

The following abbreviations are used in this manuscript:

LAB	Lactic acid bacteria
WL	Wine lees
YE	Yeast extract
DE	Disruption efficiency
WP	Whey permeate
Dpf	Days post-fertilization
Ppm	Parts per million

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