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ORIGINAL ARTICLE

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Modeling the antimicrobial effects of olive mill waste extract, rich in hydroxytyrosol, on the growth of lactic acid bacteria using response surface methodology

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Abstract: The objective of this study is to assess the inhibitory effects of an aqueous extract from olive oil mill waste (alperujo) on the growth of a lactic acid bacteria (LAB) cocktail consisting of various strains of *Lactiplantibacillus pentosus* and *Lactiplantibacillus plantarum* species. For this purpose, response surface methodology was employed using two independent variables (pH levels 3.5–5.55; hydroxytyrosol concentration ranging from 0.93–2990 ppm). The response variable was the average inhibition per treatment on the LAB cocktail (expressed as a percentage). The developed model identified significant terms, including the linear effect of hydroxytyrosol and pH, their interaction, and the quadratic effect of pH. Maximum inhibition of the LAB cocktail was observed at progressively higher concentrations of hydroxytyrosol and lower pH values. Therefore, complete inhibition of LAB in the synthetic culture medium could only be achieved for concentrations of 2984 ppm hydroxytyrosol at a pH of 3.95. These findings suggest that extracts derived from "alperujo" could be utilized as a natural preservative in acidified foods with a bitter flavor and antioxidant requirements.

KEYWORDS
alperujo, hydroxytyrosol, *Lactiplantibacillus*, pH, predictive models

1 | INTRODUCTION

The term "alperujo" refers to a semi-solid paste composed of vegetation water and olive pomace, which is obtained during the olive oil extraction process when the two-phase system is applied (Klen & Vodopivec, 2012). Low water activity and a pH ranging from 4.0 to 6.0 typically characterize it. Furthermore, it contains numerous organic compounds, including diverse types of bio-phenols (Alburquerque et al., 2004; Dermeche et al., 2013; El-Abbassi et al., 2012). Consequently, this byproduct of olive oil production is an excellent source of naturally occurring

polyphenols, such as hydroxytyrosol (Hy), which can be easily recoverable and possess high-added value (Rubio-Senent et al., 2015; Suárez et al., 2010). Due to its tendency to partition into the aqueous phase, these polyphenols have limited biodegradability within the "alperujo" matrix (Artajo et al., 2007; Morillo et al., 2009).

"Alperujo" possess potent inhibitory activity against fungi and pathogenic microorganisms, which is attributed to their phenolic fraction (Brenes et al., 2011; Caballero-Guerrero et al., 2022; Capasso et al., 1995; Leouifoudi et al., 2015; Yakhlef et al., 2018). These compounds have also demonstrated antimicrobial activity against the growth of lactic acid bacteria (LAB) (Hurtado et al., 2012). However, certain species/strains are capable of bioconverting oleuropein to Hy (Santos et al., 2012). In the specific case of Hy, its antimicrobial activity has been related to producing reactive oxygen species as a mechanism to kill bacteria (Kohanski et al., 2007).

Even though LAB are widely recognized for their use in the biopreservation of foods (Ibrahim et al., 2021), various studies have reported that LAB species can also contribute to food spoilage. Romero-Gil, Rodríguez-Gómez, et al. (2016) identified a specific genotype of *Lactiplantibacillus pentosus* associated with the spoilage of packaged *Aloreña de Málaga* table olives. Another study by Montaño et al. (2013) revealed an olive *L. pentosus* strain capable of metabolizing sorbate from the medium, compromising the packed product's stability. Various LAB species, including *Lactiplantibacillus*, have been reported to metabolize preservatives and organic acids, leading to the formation of unpleasant compounds, such as diacetyl, CO_2, ethanol, and formic or acetic acid, and causing alterations in food packaging (Basak et al., 2002; Jay & Anderson, 2001; Johanningsmeier & McFeeters, 2013; Pothakos et al., 2015). Lastly, De Castro et al. (2015) attributed the production of unpleasant odors, specifically 4-ethylphenol, during the "alperujo" storage to *L. pentosus* species.

The antimicrobial activity of phenolic compounds from "alperujo" makes them potential candidates for controlling microbial growth in the food industry. Response surface methodology (RSM), also known as polynomial models, has proven to be a suitable approach for assessing the influence of environmental variables on the microbial response, such as growth or inhibition (Pérez-Rodríguez & Valero, 2013). D-optimal designs are appropriate for further application in secondary Respond Surface (RS) models (Khuri & Mukhopadhyay, 2010; Myers & Montgomery, 2002). In a recent study, Dauber et al. (2022) utilized RSM to optimize the recovery of phenols from "alperujo" using supercritical fluid extraction.

In this survey, we employed RSM to evaluate the inhibitory effects of a treated aqueous extract from "alperujo" on a cocktail of LAB native-to-table olive processing. We modified the synthetic culture medium by adding different doses of this olive oil extraction byproduct to achieve different levels of Hy and pH capable of inhibiting the growth of LAB.

2 | MATERIALS AND METHODS

2.1 | Growth medium

The concentrated extract (AE-2) used in this survey was obtained from a Picual olive mill waste (SCA San Isidro Labrador, Los Villares, Spain). The original "alperujo" was subjected to a thermo-malaxation process using the Pieralisisi apparatus (Pieralisis, Jesi, Italy) with slow stirring at 60°C for 90 min. Then, the liquid phase was purified using a chromatographic system developed within the European Project Phenoliva (EIT-FOOD, Leuven, Belgium). Our group also previously used this same extract to assess the antimicrobial effect of AE-2 on the growth of diverse foodborne pathogen species, being an olive extract rich in Hy with almost 80% of the total phenolic fraction (see Caballero-Guerrero et al., 2022).

The basal growth medium used in this survey was De Man Rogosa Sharpe (MRS) broth (Oxoid, Basingstoke, UK), which was modified before inoculation by adding different doses of AE-2 to reach the Hy and pH levels shown in Table 1. Before use, the AE-2 extract was treated by centrifugation (10.000 rpm for 10 min) and filtered (0.45 μm) to remove impurities and sterilize.

2.2 | cocktail

Two strains of the species *L. pentosus* (TOMC LAB2 and TOMC LAB10) and two strains of *Lactiplantibacillus plantarum* (TOMC LAB8 and TOMC LAB9) were obtained from the Table Olive Microbial Collection of Instituto de la Grasa (CSIC, Sevilla, Spain). All these strains have been previously isolated from table olive processing and identified by molecular methods using multiplex PCR of the *recA* gene (Torriani et al., 2001). They were stored at −80°C in the MRS broth culture medium with 20 g/L glycerol until use. Before experiments, each strain was refreshed and cultured independently in MRS broth medium at 30°C to reach 0.25 optical density (OD_{600}). Then, the media were centrifuged, and the pellet was washed and suspended in a sterile saline solution (0.9% NaCl). A unique LAB cocktail was obtained by mixing the same quantities of the different *L. pentosus* and *L. plantarum* strains. The mix received approximately 8 $\log_{10}$ CFU/mL of each strain, which was confirmed by spreading by duplicate in MRS agar. We prefer to work with an LAB cocktail instead of individual species/strains because this represents better the real food conditions.

TABLE 1 D-optimal experimental design used in the present study, using pH and hydroxytyrosol (Hy) (ppm) as independent variables and %inhibition (%I) as the dependent (response) variable. The design was replicated to fill four 100-well Honeycomb microplates with lids.

Run	pH	Hy	Area**	%I
1	5.55	1500	6725.78	34.58
2	5.25	440	7960.83	22.57
3	5.25	2160	5819.99	43.39
4	4.53	0.93	7785.69	24.27
5	4.53	1500	5559.83	45.92
6	4.53	1500	5434.73	47.14
7	4.53	1500	5687.87	44.68
8	4.53	1500	5315.63	48.30
9	4.53	1500	5129.49	50.11
10	4.53	2999	2692.68	73.81
11	3.88	440	5596.13	45.57
12	3.8	2160	150.96	98.53
13	3.5	1500	35.54	99.65
14*	6.5	0	10,281.10	0.00

Note: Area values for each experimental run were obtained by integrating the respective OD/growth curve after subtracting the OD of the uninoculated well.
*Optimal growth conditions, not included in the experimental design but necessary to obtain %I.
**Average area per run.

2.3 | In vitro antimicrobial assay

The microbial response was monitored measuring OD changes in an automated spectrophotometer (Bioscreen C, Lab system, Turku, Finland) using the wideband filter (420–580 nm). After pre-shaking for 5 s, readings were recorded every 30 min for 4 days (96 h). The experiments were performed at 30°C. The microplate wells were filled with 340 μL of MRS broth medium (modified to the different doses of pH and Hy after AE-2 addition) and 10 μL of the LAB inoculum, reaching an initial OD value above 0.1 (inoculum level 6 $\log_{10}$ CFU/well). For each experimental condition, negative controls (uninoculated wells) were also introduced to subtract the noise signal due to absorbance changes exclusively due to color modifications of the medium. The complete set of experiments (design + control, Table 1) was replicated 14 times.

The inhibitory effect of "alperujo" extract was determined by calculating the area under the OD/time growth curve of the LAB cocktail, according to Hy and pH levels (Table 1), in comparison with the area of the positive control (optimal conditions, MRS broth medium without Hy and pH = 6.5). As the amount of inhibitory compound in the well increases, microbial growth is reduced and causes a diminution in the area under the growth curve compared to the control (Bonatsou et al., 2015; Caballero-Guerrero et al., 2022; Romero-Gil, García-García, et al., 2016). OriginPro 7.5 software (OriginLab Corporation, Northampton, MA, USA) was used to obtain the areas under the corresponding OD/time growth curves for each treatment.

Ultimately, the average inhibition (expressed as a percentage) caused by each run was estimated as follows:

$$\%I = 100 - ((\text{Testarea}/\text{Controlarea}) \times 100). \quad (1)$$

2.4 | Secondary model

Table 1 shows the different combinations of pH (3.50–5.55) and Hy (0.93–2999 ppm) included in the D-optimal experimental design applied in this work. The data analysis was performed using Design-Expert v.12 (StatEase software, Minneapolis, MN, USA). Analysis of variance (ANOVA) was executed to select significant coefficients ($p \leq 0.05$) of the model, their standard error, and confidence limits. The adequacy of the model's fit was checked by lack of fit estimation, adjusted R-squared, and normal probability versus the internally studentized residuals graphs. For graphical representation, the equations in actual values were deduced, and the two- or three-dimensions plots were obtained.

3 | RESULTS AND DISCUSSION

A lot of "alperujo" is obtained during olive oil extraction, representing up to 80% of the processed raw material (Morillo et al., 2009). Up to 98% of the majority of phenolic compounds present in fruits can remain in this olive oil byproduct (Fernández-Bolaños et al., 2002). Therefore, many studies on the treatment of "alperujo" have been conducted to reduce its environmental impact and improve its exploitation, mainly by extracting biophenols (Fermoso et al., 2018). 3,4-Dihydroxyphenylethanol, or Hy, is one of the most important hydroxy aromatic components of secoiridoids, and it is a highly bioactive alcoholic *ortho*-diphenol. Hy is predominant in "alperujo", but its purification usually requires the use of several physical, hydrothermal, or supercritical fluid treatments (Cubero-Cardoso et al., 2020; Dauber et al., 2022; Fernández-Prior et al., 2020; Lama-Muñoz et al., 2019). Recently, Romeo et al. (2021) also treated olive mill wastewater with a strain of *L. plantarum* that can produce esterase and β-glucosidase, thereby increasing Hy content through enzymatic activity.

The original AE-2 aqueous concentrated extracts used in the present study for the antimicrobial assays had an acidic pH (3.5 units), low sugar content (14.63 g/L), and high Hy (7536 ppm) content, representing 80% of the

TABLE 2 The most appropriate model suggested by the sequential model sum of squares (type I).

Source	Sum of squares	*df*	Mean square	*F* value	*p*-Value Prob > F
Mean vs. total	35,414.57	1	35,414.57		
Linear vs. mean	6172.15	2	3086.07	33.40	<0.0001
Two-factor Interaction vs. Linear	236.59	1	236.59	3.10	0.1123
*Quadratic vs. two-factor interaction	637.27	2	318.63	44.44	0.0001
Cubic vs. quadratic	32.52	3	10.84	2.45	0.2030
Residual	17.67	4	4.42		
Total	42,510.76	13	3270.06		

*Suggested model.

total phenolic fraction (Caballero-Guerrero et al., 2022). It was used to modify the LAB culture broth medium according to the experimental design. This specific AE-2 "alperujo" extract had previously exhibited antimicrobial activity against the main foodborne pathogens associated with foods (Caballero-Guerrero et al., 2022). In this study, we aimed to assess the inhibitory activity of the AE-2 extract on the growth of the major LAB species found in table olive products and other fermented vegetables. LAB have also been occasionally isolated from "alperujo" samples and are associated with producing undesirable odors (De Castro et al., 2015; Ruiz-Méndez et al., 2013).

The estimation of the area under the OD/time growth curve between optimal and inhibitory conditions is a commonly used method in predictive microbiology to determine the inhibitory effect of antimicrobial compounds (Bonatsou et al., 2015; Lambert, 2001; Romero-Gil, García-García, et al., 2016). Negative values (<0.0) %I indicate that the bacteria grow better under the environmental conditions, evaluated for its optimal culture medium. Conversely, values between 0.0 and 99.9 %I indicate that the microorganism can grow but with reduced performance compared to optimal conditions. Finally, values of 100% indicate that the bacteria cannot grow at the factor levels analyzed (Caballero-Guerrero et al., 2022).

This survey used 5376 OD raw data (obtained from the 14 LAB growth curves) (design runs+ non-inoculated controls). First, the area under OD/growth curves was calculated and then the average inhibition per treatment (%I) and model building were determined for each condition. Table 1 presents the average area and %I values obtained for the LAB cocktail under the different treatment assayed, ranging from 22.57% (Run 2, pH 5.25 and Hy 440 ppm) to 99.65% (Run 13, pH 3.5 pH and Hy 1500 ppm).

Based on the sequential model sum of squares analysis, the quadratic model was found to be appropriate as it significantly contributed to explaining the sum of squares. In contrast, the cubic model was aliased (Table 2). The

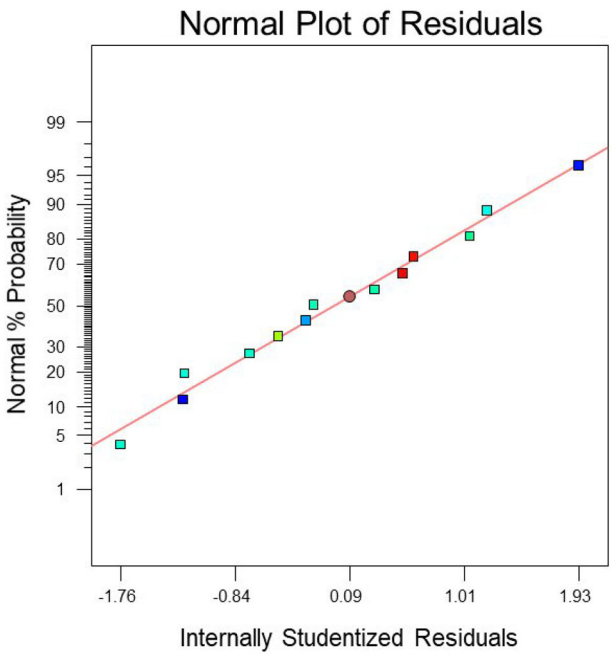

FIGURE 1 Lactic acid bacteria (LAB) cocktail inhibition (%I) by "alperujo" extracts. Normal probability plot versus internally studentized residuals concerning LAB cocktail inhibition and based on the deduced quadratic model.

ANOVA results for the suggested model (Table 3) showed its significance at $p < 0.0001$, and the adjusted R-square indicated that it accounted for 98.92% of the variance. Additionally, the lack of fit was not significant ($p = 0.2759$). Furthermore, the precision, which evaluates the signal noise/ratio, was determined to be 49.99, well above the minimum limit (4.00) considered an adequate level. The plot of residuals (Figure 1) followed a normal distribution. Therefore, all these parameters indicate that the model is suitable for predictions within the experimental region.

The estimated coefficients for the suggested quadratic model were calculated, as shown in Table 4. None of the coefficients included zero within their confidence

TABLE 3 Analysis of variance (ANOVA) analysis for the RS-reduced quadratic model.

Source	Sum of squares	*df*	Mean square	*F* value	*p*-Value Prob > F
Model	7045.0902	4	1761.27,255	275.723,846	<0.0001
pH	3911.61,465	1	3911.61,465	612.355,786	<0.0001
Hy	2486.64,644	1	2486.64,644	389.279,741	<0.0001
pH*Hy	198.031,078	1	198.031,078	31.0,013,862	0.0005
pH^2	636.352,546	1	636.352,546	99.619,773	<0.0001
Residual	51.1,025,092	8	6.38,781,365		
Lack of fit	33.4,365,092	4	8.3,591,273	1.89,270,402	0.2759
Pure error	17.666	4	4.4165		
Cor total	7096.19,271	12			

TABLE 4 Estimated significant coefficients for the RS quadratic model deduced for the lactic acid bacteria (LAB) cocktail inhibition.

Factor	Coefficient	*df*	Standard error	95% CI low	95% CI high	VIF
Intercept	47.68	1	1.10	45.15	50.20	
pH	−31.43	1	1.54	−34.97	−27.89	1.03
Hy	27.88	1	1.65	24.07	31.70	1.00
pH*Hy	−20.29	1	3.69	−28.79	−11.79	1.03
pH^2	18.66	1	2.28	13.40	23.92	1.00

Abbreviations: CI, confidence interval; VIF, variance inflation factor.

limits, indicating that they all were significant, except the quadratic effect of Hy. Therefore, the final model consisted of the linear effects of pH and Hy, their interaction, and the quadratic effect of pH (Table 4). Furthermore, the coefficients had variance inflation factor values close to 1.0, indicating a balanced influence of the diverse terms and the absence of a collinearity problem. In terms of the actual values, the equation is as follows:

$$\%\mathrm{I\,(LAB)} = 432.51 - 171.57\mathrm{pH} + 0.08\,\mathrm{Hy} - 0.01\mathrm{pH} \times \mathrm{Hy} + 17.75\mathrm{pH}^2 \quad (2)$$

The equation can be represented in a three-dimensional plot (Figure 2), where a plane rises sharply as the pH level decreases and the concentrations of Hy increase. The interaction effect is best illustrated in a two-dimensional plot (Figure 3), indicating that the inhibitory effect of Hy is weak at high pH levels but slightly increases as the pH decreases. From both figures, it can be deduced that the inhibition (%I) increases with higher concentrations of Hy and lower pH values. Therefore, the inhibitory effect of Hy decreases as pH increases. Complete inhibition of the LAB cocktail is achieved only at very high concentrations of Hy and a pH value below 4.0 (Figure 3). Figure 4 also represents the interaction between pH and Hy, showcasing the quadratic effect of pH, especially for high concentrations of

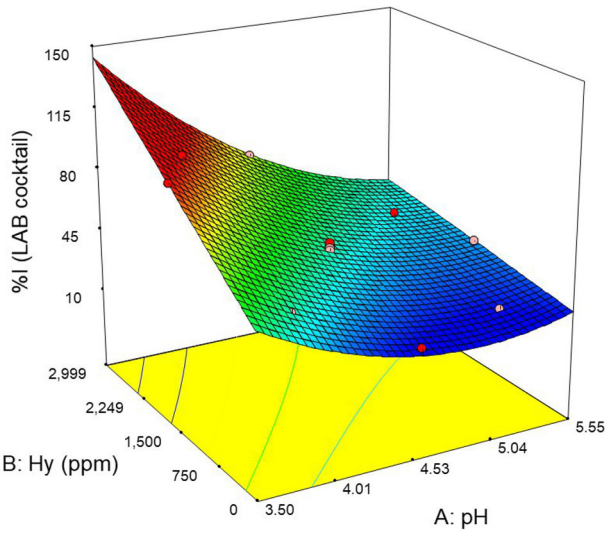

FIGURE 2 Lactic acid bacteria (LAB) cocktail inhibition (%I) by "alperujo" extracts. Response surface and contour plot of the equation depicting the inhibition (%I) of the LAB cocktail as a function of pH (units) and hydroxytyrosol (Hy) (ppm).

Hy. It is important to note that obtaining a complete inhibition of the LAB cocktail in the absence of Hy is impossible, even with a low pH of 3.50.

Once the equation linking pH level and Hy concentrations with inhibitory activity is known, it becomes possible

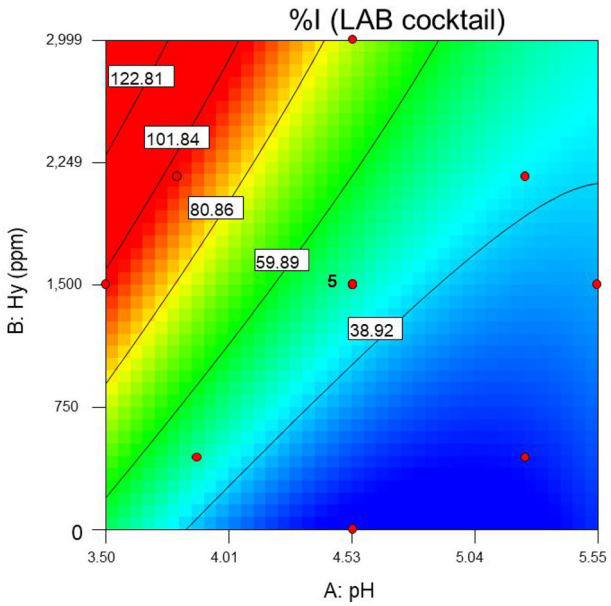

FIGURE 3 Lactic acid bacteria (LAB) cocktail inhibition (%I) by "alperujo" extracts. Two-dimensional contour plot of the equation, illustrating the inhibition (%I) of the LAB cocktail as a function of pH (units) and hydroxytyrosol (Hy) (ppm).

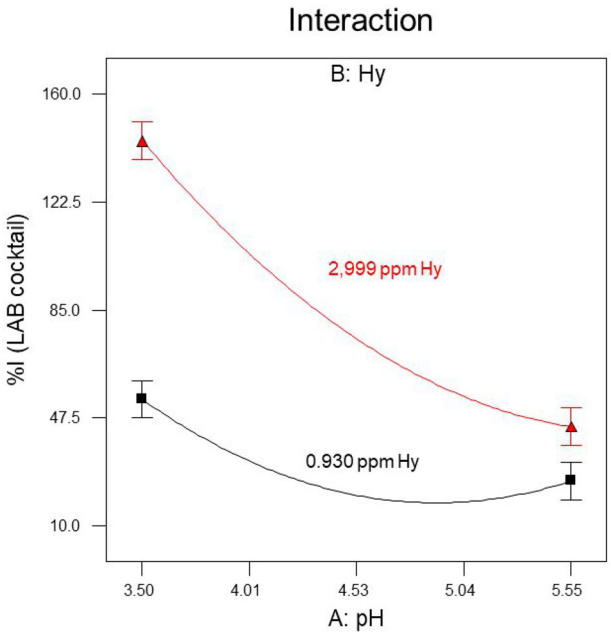

FIGURE 4 Lactic acid bacteria (LAB) cocktail inhibition (%I) by "alperujo" extracts. Interaction graph depicting the percentage of inhibition (I%) of the LAB cocktail according to pH (units) and two levels of Hy (0.93 ppm, black line; 2999 ppm, red line).

to determine a set of optimum conditions (Table 5) to prevent LAB growth (Desirability 1). Complete inhibition of the LAB cocktail can be achieved under all conditions indicated in Table 5. The lowest Hy concentration required for inhibition was 1674 ppm (solution number 16) at a pH of 3.53. It is worth noting the marked increase in the required Hy concentration as pH increases in the interval 3.50–4.00 (Figure 5). For instance, at pH 3.95, the concentration required for inhibition reaches 2984 ppm. The optimum conditions can also be represented graphically (Figure 5), indicating the point of complete inhibition at pH 3.53 and the region where no growth is expected. However, it should be noted that achieving total inhibition above a pH of 4.00 is challenging, even at the highest tested Hy levels.

Therefore, the LAB cocktail used in this study demonstrated greater resistance to the AE-2 extract compared to foodborne pathogens such as *Listeria monocytogenes, Staphylococcus aureus, Escherichia coli*, and *Salmonella* enterica (Caballero-Guerrero et al., 2022). Mathematical models developed by our group predicted complete

TABLE 5 Optimum inhibitory conditions, predicted by the RS equation, for achieving maximum inhibition (%I = 100%) of the lactic acid bacteria (LAB) cocktail tested in this study.

Number	pH	Hy (ppm)	%I	Desirability
1	3.58	1830.13	103.326	1.000
2	3.70	2076.18	101.624	1.000
3	3.78	2978.26	120.481	1.000
4	3.72	2111.06	101.719	1.000
5	3.60	1848.62	102.296	1.000
6	3.88	2480.33	100.823	1.000
7	3.60	2167.64	111.573	1.000
8	3.97	2708.49	100.643	1.000
9	3.72	2358.46	108.099	1.000
10	3.93	2796.41	105.280	1.000
11	3.56	2010.41	109.832	1.000
12	3.83	2804.73	112.476	1.000
13	3.57	1753.61	101.156	1.000
14	3.55	2316.94	119.661	1.000
15	3.75	2225.05	102.565	1.000
16	3.53	1673.69	102.103	1.000
17	3.91	2727.19	105.072	1.000
18	3.52	2146.05	116.888	1.000
19	3.69	2065.18	102.227	1.000
20	3.63	2876.48	129.123	1.000
21	3.70	2274.97	107.428	1.000
22	3.59	2563.68	123.867	1.000
23	3.58	1770.39	101.323	1.000
24	3.64	1877.42	100.587	1.000
25	3.69	2770.70	121.871	1.000
26	3.69	2232.84	106.726	1.000
27	3.95	2923.81	106.964	1.000
28	3.52	2841.30	137.002	1.000
29	3.72	2590.06	114.239	1.000
30	3.59	2775.64	130.073	1.000

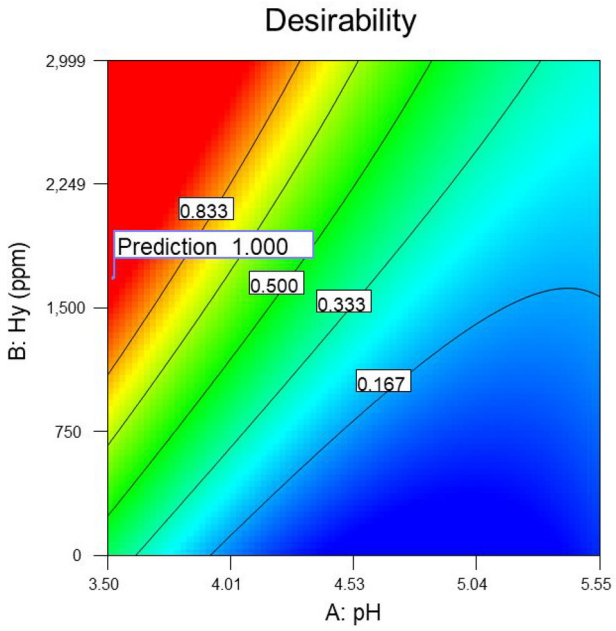

FIGURE 5 Lactic acid bacteria (LAB) cocktail inhibition (%I) by "alperujo" extracts. Graphical optimization of the maximum %I on the LAB cocktail. The plot displays the desirability contours based on the RS equation within the experimental conditions of pH, and hydroxytyrosol (Hy) tested in this work.

inhibition for these four foodborne pathogen species at pH 3.9 and an Hy concentration of 1314 ppm (Caballero-Guerrero et al., 2022). In another study, Romeo et al. (2021) found that a strain of *L. plantarum* exhibited enzymatic activity in olive mill wastewater at a pH of 4.8 with an Hy content of around 300 ppm. Arroyo-López et al. (2005) observed LAB growth during the storage of *Aloreña de Málaga* table olives in packaging brines with a pH of 4.1, 567 ppm of Hy, and 0.0175% sodium sorbate. De Leonardis et al. (2007) reported that 3.200 ppm of Hy obtained from olive leaves was insufficient to inhibit *Lactobacillus delbruechii* completely. However, due to the LAB's susceptibility to very low pH, the acidification of "alperujo" to pH 2.0 prevented *L. pentosus* growth and the subsequent occurrence of odor emissions during storage (De Castro et al., 2015).

4 | CONCLUSION

RSM proved highly valuable in investigating the inhibitory effects of concentrated aqueous extracts derived from "alperujo" on a native olive LAB cocktail. The inhibitory effect caused by this olive oil mill byproduct was influenced by the interaction between Hy and pH, necessitating high levels of the phenolic compound to achieve complete inhibition. Further studies should be conducted to validate these initial findings in actual food systems. Nevertheless, these extracts could be employed as preservatives with antimicrobial properties to enhance the nutritional value and quality of acidic food matrices with bitter flavor and antioxidant requirements (such as table olives, capers, and beer).

AUTHOR CONTRIBUTIONS

Belén Caballero-Guerrero: Methodology; Formal analysis; Supervision. **Antonio Garrido-Fernández**: Conceptualization; Formal analysis; Writing-original draft; Writing-review & editing. **Fernando G. Fermoso**: Conceptualization; Funding acquisition; Project administration. **María África Fernández-Prior**: Methodology. **Juan Cubero-Cardoso**: Methodology. **Claudio Reinhard**: Conceptualization; Writing-review & editing; Funding acquisition; Project administration. **Laura Nyström**: Methodology. **Antonio Benítez-Cabello**: Writing-original draft; Formal analysis. **Elio López-García**: Formal analysis; Writing-original draft; Writing-review & editing. **Francisco Noé Arroyo-López**: Formal analysis; Writing-original draft; Writing-review & editing; Conceptualization.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

REFERENCES

Alburquerque, J. A., Gonzálvez, J., Garcıa, D., & Cegarra, J. (2004). Agrochemical characterization of "alperujo", a solid by-product of the two-phase centrifugation method for olive oil extraction. *Bioresource Technology*, *91*(2), 195–200.

Artajo, L.-S., Romero, M.-P., Suárez, M., & Motilva, M.-J. (2007). Partition of phenolic compounds during the virgin olive oil industrial extraction process. *European Food Research and Technology*, *225*(5), 617–625.

Basak, S., Ramaswamy, H. S., & Piette, J. P. G. (2002). High-pressure destruction kinetics of *Leuconostoc mesenteroides* and *Saccharomyces cerevisiae* in single strength and concentrated orange juice. *Innovative Food Science and Emerging Technologies*, *3*, 223–231.

Bonatsou, S., Benítez, A., Rodríguez-Gómez, F., Panagou, E. Z., & Arroyo-López, F. N. (2015). Selection of yeasts with multifunctional features for application as starters in natural black table olive processing. *Food Microbiology*, *46*, 66–73.

Brenes, M., García, A., De Los Santos, B., Medina, E., Romero, C., De Castro, A., & Romero, F. (2011). Olive glutaraldehyde-like compounds against plant pathogenic bacteria and fungi. *Food Chemistry*, *125*(4), 1262–1266.

Caballero-Guerrero, B., Garrido-Fernández, A., Fermoso, F. G., Rodríguez-Gutierrez, G., Fernández-Prior, M. Á., Reinhard, C., Nyström, L., Benítez-Cabello, A., & Arroyo-López, F. N. (2022). Antimicrobial effects of treated olive mill waste on foodborne pathogens. *LWT Food Science and Technology*, *164*, 113628.

Capasso, R., Evidente, A., Schivo, L., Orru, G., Marcialis, M. A., & Cristinzio, G. (1995). Antibacterial polyphenols from olive oil mill wastewaters. *Journal of Applied Bacteriology*, *79*(4), 393–398.

Cubero-Cardoso, J., Trujillo-Reyes, Á., Serrano, A., Rodríguez-Gutiérrez, G., Borja, R., & Fermoso, F. G. (2020). High-value-added compound recovery with high-temperature hydrothermal treatment, steam explosion, and subsequent biomethanization of residual strawberry extrudate. *Foods*, *9*(8), 1082.

Dauber, C., Carreras, T., Fernández Fernández, A., Irigaray, B., Albores, S., Gámbaro, A., Ibáñez, E., & Vieitez, I. (2022). Response surface methodology for the optimization of biophenols recovery from "alperujo" using supercritical fluid extraction. Comparison between Arbequina and Coratina cultivars. *The Journal of Supercritical Fluids*, *180*, 105460.

De Castro, A., Asencio, E., Ruiz-Méndez, M. V., Romero, C., Brenes, M., & Brenes, M. (2015). Production of 4-ethylphenol in alperujo by *Lactobacillus pentosus*. *Journal of the Science of Food and Agriculture*, *95*(11), 2222–2227.

De Leonardis, A., Aretini, A., Alfano, G., Macciola, V., & Ranalli, G. (2007). Isolation of a hydroxytyrosol-rich extract from olive leaves (*Olea Europaea*) and evaluation of its antioxidant properties and bioactivity. *European Food Research and Technology*, *226*, 653–659.

Dermeche, S., Nadour, M., Larroche, C., Moulti-Mati, F., & Michaud, P. (2013). Olive mill wastes: Biochemical characterizations and valorization strategies. *Process Biochemistry*, *48*(10), 1532–1552.

El-Abbassi, A., Kiai, H., & Hafidi, A. (2012). Phenolic profile and antioxidant activities of olive mill wastewater. *Food Chemistry*, *132*(1), 406–412.

Fermoso, F. G., Serrano, A., Alonso-Fariñas, B., Fernández-Bolaños, J., Borja, R., & Rodríguez-Gutiérrez, G. (2018). Valuable compound extraction, anaerobic digestion, and composting: A leading biorefinery approach for agricultural wastes. *Journal of Agricultural and Food Chemistry*, *66*(32), 8451–8468.

Fernández-Bolaños, J., Rodríguez, G., Rodríguez, R., Heredia, A., Guillén, R., & Jiménez, A. (2002). Production in large quantities of highly purified hydroxytyrosol from liquid-solid waste of two-phase olive oil processing or "alperujo". *Journal of Agricultural and Food Chemistry*, *50*, 6804–6811.

Fernández-Prior, M. Á., Fatuarte, J. C. P., Oria, A. B., Viera-Alcaide, I., Fernández-Bolaños, J., & Rodríguez-Gutiérrez, G. (2020). New liquid source of antioxidant phenolic compounds in the olive oil industry: Alperujo water. *Foods*, *9*(7), 962.

Hurtado, A., Reguant, C., Bordons, A., & Rozès, N. (2012). Lactic acid bacteria from fermented table olives. *Food Microbiology*, *31*, 1–8.

Ibrahim, S. A., Ayivi, R. D., Zimmerman, T., Siddiqui, S. A., Altemimi, A. B., Fidan, H., Esatbeyoglu, T., & Bakhshayesh, R. V. (2021). Lactic acid bacteria as antimicrobial agents: Food safet and microbial food spoilage prevention. *Foods*, *10*(12), 3131.

Jay, S., & Anderson, J. (2001). Fruit juice and related products. In C. J. Moir, C. Andrew-Kabilafkas, G. Arnold, B. M. Cox, A. D. Hocking, & I. Jenson (Eds.), *Spoilage of processed foods: Causes and diagnosis* (pp. 187–198). Southwood Press.

Jerman Klen, T., & Mozetič Vodopivec, B. (2012). The fate of olive fruit phenols during commercial olive oil processing: Traditional press versus continuous two-and three-phase centrifuge. *LWT—Food Science and Technology*, *49*(2), 267–274.

Johanningsmeier, S. D., & Mcfeeters, R. F. (2013). Metabolism of lactic acid in fermented cucumbers by *Lactobacillus buchneri* and related species, potential spoilage organisms in reduced salt fermentations. *Food Microbiology*, *35*, 129.

Khuri, A. I., & Mukhopadhyay, S. (2010). Response surface methodology. *Wiley Interdisciplinary Reviews: Computational Statistics*, *2*(2), 128–149.

Kohanski, M. A., Dwyer, D. J., Hayete, B., Lawrence, C. A., & Collins, J. J. (2007). A common mechanism of cellular death induced by bactericidal antibiotics. *Cell*, *130*, 797–810.

Lama-Muñoz, A., Rubio-Senent, F., Bermúdez-Oria, A., Fernández-Bolaños, J., Prior, Á. F., & Rodríguez-Gutiérrez, G. (2019). The use of industrial thermal techniques to improve the bioactive compounds extraction and the olive oil solid waste utilization. *Innovative Food Science & Emerging Technologies*, *55*, 11–17.

Lambert, R. J. W. (2001). Advances in disinfection testing and modelling. *Journal of Applied Microbiology*, *91*(2), 351–363.

Leouifoudi, I., Harnafi, H., & Zyad, A. (2015). Olive mill waste extracts: Polyphenols content, antioxidant, and antimicrobial activities. *Advances in Pharmacological Sciences*, *2015*, 1–11.

Montaño, A., Sánchez, A. H., Casado, F. J., Beato, V. M., & De Castro, A. (2013). Degradation of ascorbic acid and potassium sorbate by different *Lactobacillus* species isolated from packed green olives. *Food Microbiology*, *34*, 7–11.

Morillo, J. A., Antizar-Ladislao, B., Monteoliva-Sánchez, M., Ramos-Cormenzana, A., & Russell, N. J. (2009). Bioremediation and biovalorisation of olive-mill wastes. *Applied Microbiology and Biotechnology*, *82*(1), 25–39.

Myers, R. H., & Montgomery, D. C. (2002). *Response surface methodology* (2nd ed.). John Wiley & Sons, Inc.

Noé Arroyo López, F., Romero, C., Durán Quintana, M. D. C., López López, A., García García, P., & Garrido Fernández, A. (2005). Kinetic study of the physicochemical and microbiological changes in "seasoned" olives during the shelf-life period. *Journal of Agricultural and Food Chemistry*, *53*, 5285–5292.

Pérez-Rodríguez, F., & Valero, A. (2013). *Predictive microbiology in foods*. Springer.

Pothakos, V., Devlieghere, F., Villani, F., Björkroth, J., & Ercolini, D. (2015). Lactic acid bacteria and their controversial role in fresh meat spoilage. *Meat Sciences*, *109*, 66–74.

Romeo, F. V., Granuzzo, G., Foti, P., Ballistreri, G., Caggia, C., Rapisarda, P., & Rapisarda, P. (2021). Microbial application to improve olive mil wastewater phenolic extracts. *Molecules*, *26*(7), 1944.

Romero-Gil, V., García-García, P., Garrido-Fernández, A., & Arroyo-López, F. N. (2016). Susceptibility and resistance of lactic acid bacteria and yeasts against preservatives with potential application in table olives. *Food Microbiology*, *54*, 72–79.

Romero-Gil, V., Rodríguez-Gómez, F., Garrido-Fernández, A., García-García, P., & Arroyo-López, F. N. (2016). *Lactobacillus pentosus* is the dominant species in spoilt packaged *Aloreña de Málaga* table olives. *LWT Food Science and Technology*, *70*, 252–260.

Rubio-Senent, F., Martos, S., Lama-Muñoz, A., Fernández-Bolaños, J. G., Rodríguez-Gutiérrez, G., & Fernández-Bolaños, J. (2015). Isolation and identification of minor secoiridoids and phenolic components from thermally treated olive oil by-products. *Food Chemistry*, *187*, 166–173.

Ruiz-Méndez, M. V., Romero, C., Medina, E., García, A., De Castro, A., & Brenes, M. (2013). Acidification of alperujo paste prevents off-odours during their storage in the open air. *Journal of the American Oil Chemists' Society*, *90*, 401–406.

Santos, M. M., Piccirillo, C., Castro, P. M. L., Kalogerakis, N., Pintado, M. E., & Pintado, M. E. (2012). Bioconversion of oleuropein to hydroxytyrosol by lactic acid bacteria. *World Journal of Microbiology and Biotechnology*, *28*(6), 2435–2440.

Suárez, M., Romero, M.-P., & Motilva, M.-J. (2010). Development of phenol-enriched olive oil with phenolic compounds from olive cake. *Journal of Agricultural and Food Chemistry*, *58*(19), 10396–10403.

Torriani, S., Felis, G. E., & Dellaglio, F. (2001). Differentiation of *Lactobacillus plantarum*, *L. pentosus*, and *L. paraplantarum* by recA gene sequence analysis and multiplex PCR assay with recA gene-derived primers. *Applied Environmental Microbiology*, *67*(8), 3450–3454.

Yakhlef, W., Arhab, R., Romero, C., Brenes, M., De Castro, A., & Medina, E. (2018). Phenolic composition and antimicrobial activity of Algerian olive products and by-products. *LWT Food Science Technology*, *93*, 323–328.

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