LWT - Food Science and Technology 149 (2021) 111783

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Microbial stabilisation of white wine by filtration through silica microparticles functionalised with natural antimicrobials

María Ruiz-Rico [a], Estéfani García-Ríos [b,*], José Manuel Barat [a], José Manuel Guillamón [b]

[a] Departamento de Tecnología de Alimentos, Universitat Politècnica de València, Camino de Vera S/n, 46022 Valencia, Spain
[b] Departamento de Biotecnología de los alimentos, Instituto de Agroquímica y Tecnología de los Alimentos (CSIC), Avda. Agustín Escardino, 7, E-46980-Paterna, Valencia, Spain

ARTICLE INFO

Keywords:
Acetic acid bacteria
Yeast
Lactic acid bacteria
Wine
Filtering material

ABSTRACT

During wine production and storage, undesired effects can appear mainly due to the microbiological activity of the present microorganisms leading to economic losses. Wine stabilisation is needed for controlling the unwanted microbiological activity to improve wine safety and final quality. Nowadays, a plethora of viticultural and technological solutions is available but, in some cases, the stabilisation process changes the wine sensory profile. The aim of this study was the development of filter aids functionalised with phenolic compounds (PHE) with enhanced antimicrobial properties. The filters' removal capability was evaluated using white wine inoculated with *Acetobacter aceti*, *Lactobacillus plantarum*, *Dekkera bruxellensis*, *Zygosaccharomyces bailii* and *Saccharomyces cerevisiae*. Wine was filtered through PHE-coated filters and the microbial load was determined by plate count. The influence of filtration on the physicochemical wine parameters was also assessed. The removal capacity results showed the PHE-functionalised filters were capable of reducing 3 logarithmic values, being eugenol the most effective compound. The results evidenced different influence of filtration on wine properties according to the immobilised PHE showing that eugenol-functionalised supports had a very low impact on physicochemical parameters. Thus our results support the relevance of using antimicrobial-coated filters at different stages of the winemaking process for wine microbiological stabilisation.

1. Introduction

Winemaking is a microbiologically complex process which requires the control of microorganisms load at different stages to reduce the number of wild microorganisms to ensure the imposition of starter cultures for adequate alcoholic or malolactic fermentation (Lisanti, Blaiotta, Nioi, & Moio, 2019). Furthermore, wine can be spoiled due to the growth of some undesirable yeasts, lactic and acetic acid bacteria, producing an irreversible effect on wine quality and considerable economic losses (Avramova, Vallet-Courbin, Maupeu, Masneuf-Pomarède, & Albertin, 2018; Bartowsky, 2009; Oro, Canonico, Marinelli, Ciani, & Comitini, 2019). These spoilage microorganisms can survive in harsh environments such as low nutrient, low pH, high ethanol content, and presence of SO_2 (Renouf, Perello, de Revel, & Lonvaud-Funel, 2007). In fact, some spoilage microorganisms like *Dekkera bruxellensis* have been reported to decrease cell size and enter a viable but non-culturable (VBNC) state, thereby potentially hampering the techniques required for their removal (i.e. decreasing porosities of filtration) (Millet & Lonvaud-Funel, 2000; Umiker, Descenzo, Lee, & Edwards, 2013). For these reasons, wine should be microbiologically stabilised before bottling (Gialleli, Kallis, Bekatorou, Kanellaki, & Koutinas, 2015).

The microbial stabilisation of wine is usually performed by chemical and physical techniques. SO_2 is the most widely used chemical additive in wine industry given its antimicrobial and antioxidant properties, but current concerns about its potential adverse effects have forced its reduction in food and beverages promoting the development of alternative control methods that allow the reduction or even elimination of this preservative (Lisanti et al., 2019). In addition, the massive employ

Abbreviations: **ΔE***, colour difference; ***Aa***, *Acetobacter aceti*; **APTES**, (3-aminopropyl)triethoxysilane; ***Db***, *Dekkera bruxellensis*; **EU**, eugenol; **FE**, ferulic acid; **GC-MS**, gas chromatography-mass spectrometry; **HPLC**, High-performance liquid chromatography; ***Lp***, *Lactobacillus plantarum*; **LRVs**, logarithmic reduction values; **LSD**, least significant difference; **MRS**, Man, Rogosa and Sharpe; **PHE**, phenolic compounds; ***Sc***, *Saccharomyces cerevisiae*; **TPI**, total phenolic index; **VA**, vanillin; **VBNC**, viable but non-culturable; **YPD**, Yeast Extract Peptone Dextrose; ***Zb***, *Zygosaccharomyces bailii*.

* Corresponding author. National Center for Microbiology, Instituto de Salud Carlos III, Majadahonda, Madrid, Spain.
E-mail addresses: e.garcia.rios@iata.csic.es, egarcia@isciii.es (E. García-Ríos).

https://doi.org/10.1016/j.lwt.2021.111783
Received 31 March 2021; Received in revised form 10 May 2021; Accepted 24 May 2021
Available online 27 May 2021

of SO_2 is not always compatible with production of high quality wines, and it does not always avoid the risk of wine deviations, because of the emergence of tolerant/resistant spoilage microorganisms (Avramova et al., 2018). Among physical techniques, filtration is a separation technique used to remove suspended particles from a liquid by passing them through a filter medium (Mierczynska-Vasilev & Smith, 2015). Many forms of filtration are available in the wine industry, mainly classified as depth filtration and membrane filtration. Depth filtration is commonly applied in the early stages of winemaking, such as must settling, clarification, fining and stabilisation, whereas membrane filtration is used at the end of the process to sterilise wine (Gialleli et al., 2015). Membrane filters based on very low porosity are capable of effectively removing cells, but have some disadvantages, such as frequent fouling, regeneration problems, relatively high cost, and partial retention of desirable wine components (Gialleli et al., 2015; Girard, Fukumoto, & Sefa Koseoglu, 2000).

In addition to conventional stabilisation techniques, other non-thermal technologies are being explored including pulsed electric fields, high pressure, ultrasound or UV irradiation, and natural products, such as bacteriocin, lysozyme, chitosan or phenolic compounds (Bartowsky, 2009; Paulin et al., 2020; Scansani, Rauhut, Brezina, Semmler, & Benito, 2020). Grape phenolic compounds are important for the colour, flavour, astringency and bitterness of the wine (Ugliano & Henschke, 2009). Among them, some hydroxycinnamic acids such as ferulic acid (Monagas & Bartolomé, 2009), phenolic aldehydes like vanillin and other volatile phenols such as eugenol (Baumes, 2009; Pérez-Coello & Díaz-Maroto, 2009) are phenolic compounds (PHE) naturally present in wine that present well-known antimicrobial properties (Fitzgerald, Stratford, Gasson, & Narbad, 2005; García-Ríos, Ruiz-Rico, Guillamón, Pérez-Esteve, & Barat, 2018; Marchese et al., 2017; Shi et al., 2016). However, the use of these PHEs as natural preservatives is limited by their strong sensory properties that can have negative effects on wine colour, aroma and flavour (Burt, 2004).

Given the limitations of current techniques to manage stabilisation of wine, the co-application of different approaches can be a good alternative to ensure a safe final product. In this context, our research group has developed functionalised supports formed from biocompatible materials (silica gel as support and natural antimicrobials as biocidal agents) as antimicrobial devices, preventing the release of the grafted bioactive molecules and providing advanced properties to the supports, maintaining the features of the pristine materials. The potential use of these supports as filter aids has recently been evaluated against different spoilage microorganisms present in beverages such as water (Peña-Gómez, Ruiz-Rico, Pérez-Esteve, Fernández-Segovia, & Barat, 2019b), juice (Peña-Gómez, Ruiz-Rico, Fernández-Segovia, & Barat, 2019a) and beer (Peña-Gómez, Ruiz-Rico, Pérez-Esteve, Fernández-Segovia, & Barat, 2020).

Taking these results into account, filtration based on antimicrobial-coated granulated particles could be applied to various stages of the winemaking process to control a variety of spoilage microorganisms without compromising the wine features. These filters could be an alternative technique to conventional separation processes in different steps of the fermentation process because the combination of different immobilised bioactive compounds and different particle size would allow the filtration technique to be adapted to the target step. Therefore, the aim of the study was to develop filter aids with enhanced properties in comparison with conventional depth filtration materials in order to apply this technology at different stages of the winemaking process for wine microbiological stabilisation. To this purpose, the capacity of silica supports functionalised with eugenol, ferulic acid and vanillin (PHE-functionalised filtering aids) to remove representative spoilage microorganisms (*Acetobacter aceti*, *Lactobacillus plantarum*, *Dekkera bruxellensis*, *Zygosaccharomyces bailii* and *Saccharomyces cerevisiae)* from wine was evaluated. Wine samples were filtered through a bed of filtering aids and the microbial load was determined by plate count. In addition, the influence of filtration on the physicochemical wine parameters was assessed by determining total colour difference (ΔE*), total phenolic index (TPI), wine main components and potential PHE leaching.

2. Materials and methods

2.1. Estimation of filtering parameters for white wine

As a first step, the wine flow rate through the bed of different supports was determined as an important factor to technologically apply the developed supports (Peña-Gómez et al., 2019b). To this end, 50 mL of commercial white wine (pH 3.5; ethanol 100 g/L) were filtered through a bed of different commercial materials cellulose membrane 0.45 μm (Merck Millipore, Darmstadt, Germany), silica particles of mean size of 5, 10, 25, 50, 75, 200 and 375 μm (Sigma-Aldrich, Madrid, Spain), cellulose particles of mean size of 50 μm (Sigma-Aldrich, Madrid, Spain) and diatomaceous earth pure food grade (Lumino Wellness, Vancouver, USA) and different bed thicknesses of each support in a filtration funnel (volume 250 mL, Ø 47 mm). After filtration, the colour, total phenolic index and wine main components of the samples was determined (see Section 2.4 for details). These assays allowed to establish the amount of particles needed to obtain the target bed thickness, the filtration time, the volume of permeate and the influence of the process on one of the most relevant physicochemical wine parameter. The supports that least affected the wine parameters with adequate filtration time and volume of permeate were selected as supports for the development of the functionalised filtering materials.

2.2. Synthesis and characterisation of the PHE-functionalised silica supports

Silica microparticles of mean particle size of 50 and 200 μm (Sigma-Aldrich, Madrid, Spain) were used as inorganic support for the immobilisation of the natural antimicrobials eugenol (Sigma-Aldrich, Madrid, Spain), vanillin (Ernesto Ventós S.A., Barcelona, Spain) and *trans*-ferulic acid (Sigma-Aldrich, Madrid, Spain) resulting in the PHE-functionalised materials. Surface silanisation was the approach used for the covalent immobilisation of the different phenolic compounds but the grafting procedure was specific for each compound (see Supplementary material and Fig. 1 for details) (García-Ríos et al., 2018).

The bare and PHE-functionalised silica microparticles were characterised by standard techniques to determine their particle size, surface charge and degree of functionalisation. Particle size distribution was analysed in deionised water with a laser diffractometer (Mastersizer 2000; Malvern Instruments, Worcestershire, UK) applying the Mie theory (refractive index of 1.45 and an absorption index of 0.01). Surface charge was determined by zeta potential analysis using a Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK). Samples were suspended with deionised water (1 mg/mL) and sonicated before being measured to prevent the agglomeration of microparticles. The electrophoretic mobility measurements were converted into zeta potential values by the Smoluchowsky mathematical model. Degree of functionalisation was established by elemental analysis for C, H and N in a CHNOS Vario EL III model (Elemental Analyses System GMHB, Germany). All the analyses were conducted in triplicate.

2.3. Assessment of the PHE-functionalised supports as filtering materials for white wine microbial stabilisation

The capability of the PHE-functionalised filtering materials to remove spoilage wine microorganisms was assessed by using commercial white wine (pH 3.5; ethanol 100 g/L) inoculated with *Acetobacter aceti* (CECT 298) (*Aa*), *Lactobacillus plantarum* (CECT 748) (*Lp*), *Dekkera bruxellensis* (CECT 11045) (*Db*), *Zygosaccharomyces bailii* (CECT 11042) (*Zb*) and *Saccharomyces cerevisiae* (ICV GRE, Lallemand France) (*Sc*). These microorganisms were chosen according to their relevance in the

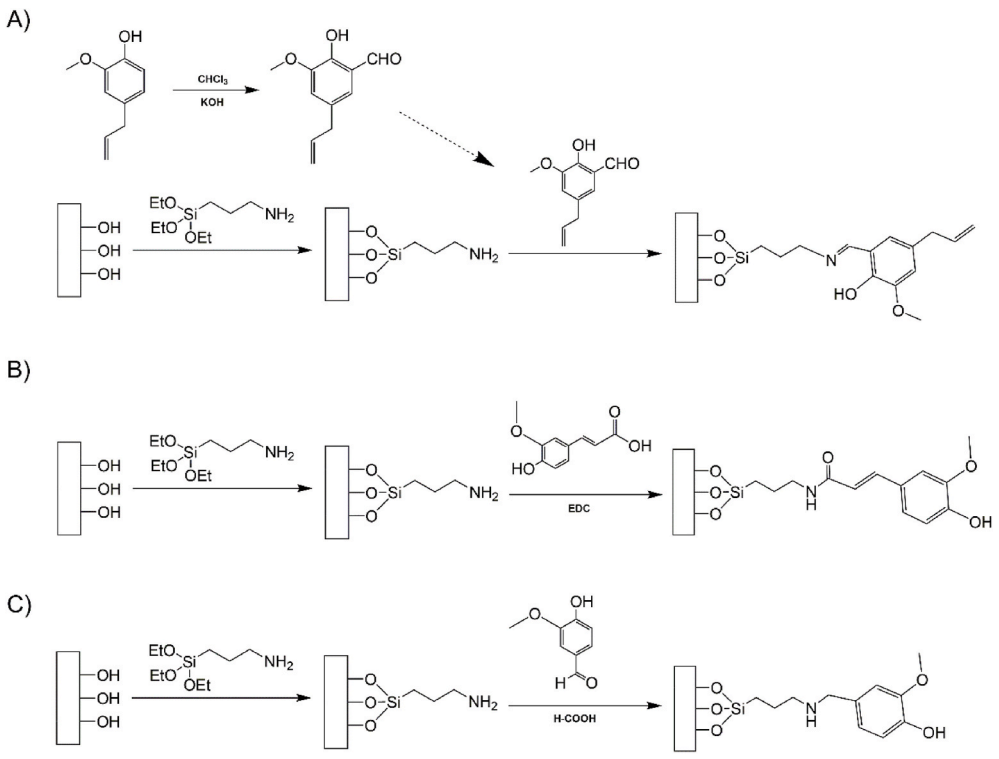

Fig. 1. Synthetic procedure of the silica supports functionalised with eugenol (a), ferulic acid (b) and vanillin (c).

spoilage of several beverages and food including wine (Bartowsky, 2009; Oro et al., 2019). De Man, Rogosa and Sharpe (MRS) broth and agar for bacteria or Yeast Extract Peptone Dextrose (YPD) broth and agar for the yeasts, were used to grow the microorganisms. All media were purchased from Scharlab (Barcelona, Spain). The strains were reconstituted following the provider instructions and microbial stocks were stored at 4 °C in MRS or YPD agar before being used. Inocula were prepared by introducing one single colony from each strain into 10 mL of MRS or YPD broth. The mixture was incubated at 28 °C for 24 h to obtain an inoculum with a density of approximately 10^8 cells/mL of broth. After incubation, the cell concentration of the inoculum was checked by determining optical density at 600 nm in a Helios Zeta UV-VIS instrument (Thermo Scientific, Hampton, New Hampshire, USA). Then appropriate dilution of the five tested microorganisms were prepared to individually inoculate commercial white wine with a cell density of 10^3 cells/50 mL.

The removal capability assays were carried out by filtering 50 mL of inoculated white wine through a bed of the PHE-functionalised particles used as filtering materials. The filtration assays were performed using a filtration funnel (volume 250 mL, Ø 47 mm) coupled to a stainless steel manifold (Microfil® filtration system, Merck Millipore, Darmstadt, Germany) and connected to an Erlenmeyer flask to collect the wine samples. The filtering bed placed in the funnel consisted of three layers: (i) a cellulose membrane filter (0.45 μm) that retained the microbial cells on the manifold base; (ii) a cellulosic paper; and (iii) a bed of PHE-functionalised supports (layer of 0.5–3 cm thickness) (Umiker, Descenzo, Lee, & Edwards, 2021). After filtration, the cellulose membrane filter was transferred to MRS or YPD plates and incubated at 28 °C for 24–48 h. The cultivable cell numbers were counted, and the results were expressed as relative viability of $\log_{10}$ CFU/50 mL according to the viability of control samples. Then the collected white wine was placed in a conical tube and stored at −18 °C prior to physicochemical and lixiviate analyses. The non-filtered sample (the control sample filtered only with the 0.45 μm cellulose membrane filter, but not with filtering supports) and the wine samples filtered through a bed of bare silica microparticles were included in the assays as the control samples to quantify the microbial count in the absence of treatment and after filtration through bare supports. The assays were run in triplicate.

2.4. *Influence of filtration on the physicochemical properties of white wine*

After establishing the capability of the PHE-functionalised supports as filtering materials to microbiologically stabilise commercial inoculated wine, the influence of the filtration process on different physicochemical parameters of white wine was evaluated. Wine colour was measured by a Minolta spectrophotometer CM 3600-d (Osaka, Japan). The intrinsic colour of samples was measured in the transmittance mode using standard illuminant D65 and 10° observer. Data were converted in the CIE-L*a*b* colour space to calculate the colour parameters: L* (brightness), a* (red–green) and b* (yellow–blue). With these values the total colour difference (ΔE*) between the unfiltered sample and the treated samples was calculated (Peña-Gómez et al., 2019a). The total phenolic index (TPI) was established by a spectrophotometric method using a Helios Zeta UV-VIS instrument (Thermo Scientific, Hampton, New Hampshire, USA). Wine was diluted with water in a 1:50 ratio and the absorbance was directly measured at 280 nm. The TPI value was calculated by multiplying the absorbance × 50 (Guise et al., 2014).

Extracellular metabolites were analysed in all the filtered samples. Analytical high-performance liquid chromatography (HPLC) was carried out in a Surveyor Plus Chromatograph (Thermo Fisher Scientific, Waltham, MA) equipped with a refraction index detector, an autosampler and a UV–Visible detector (Pérez-Través, Lopes, González, Barrio, & Querol, 2015). Prior to injection, samples were centrifuged at 13,300 rpm for 5 min, and samples were diluted 10-fold and filtered through 0.22 μm pore size nylon filters (Micron Analitica, Spain). A total volume of 25 μL was injected into a HyperREZ XP Carbohydrate H + 8 mm column (Thermo Fisher Scientific) assembled to its correspondent guard. The mobile phase used was 1.5 mmol/L H_2SO_4 with a flux of 0.6 mL/min and a column temperature of 50 °C. The concentration of each compound was calculated using external standards. Each sample was analysed in duplicate. The assays were run in triplicate.

2.5. Leaching of the immobilised antimicrobials

The possible leaching of the immobilised bioactive compounds was determined by gas chromatography-mass spectrometry (GC-MS) with previous extraction of the released compounds after wine filtration (see Supplementary material for details). The results were expressed as PHE leaching (mg/mL) and the relative percentage of the leached compounds by taking into account the total attached PHE to the supports (elemental analysis results, Section 2.2) and the amount of particles needed to prepare the bed of particles (Peña-Gómez et al., 2019b).

2.6. Statistical analysis

Data were statistically analysed using Statgraphics Centurion XVI (Statpoint Technologies, Inc., Warrenton, VA, USA). The results obtained in the characterisation of the filtering supports, the evaluation of their inhibitory capacity and the effect of filtration on the physicochemical wine parameters was analysed by an analysis of variance (ANOVA). The LSD (least significant difference) procedure was used to test the differences between averages at the 5% significance level.

3. Results and discussion

3.1. Determination of support type and size

As a preliminary study, different support materials with different granulometry were tested as filtering aids to establish the most suitable supports in terms of key parameters for the technological application of filters on a laboratory scale (flow rate and permeate volume) and according to the influence of filtration on the physicochemical properties of wine. The material supports evaluated in this study were selected due to their well-known properties as filtering materials in the winemaking process. Wine clarification is traditionally done by using depth filtration through sand, diatomaceous earth or polymeric fibres (cellulose) or by pad filtration (based on cellulose and diatomaceous earth) with perpendicular-flow systems (Mierczynska-Vasilev & Smith, 2015). In addition, bentonite and colloidal silica are used as fining agents in the wine industry for clarification and protein stability given their charged surface resulting in protein binding (Mierczynska-Vasilev & Smith, 2015).

Table 1 shows the filtering parameters of the different pristine supports determined after a single filtration of 50 mL of a commercial white wine through a bed thickness of 1.5 cm. For the mass of particles needed to achieve the same layer thickness, a higher quantity of particles is required for silica supports with a larger mean particle size, given their lower bulk density. In terms of flow rate and permeate volume, filtering wine through 5 μm particles lasted several minutes and somewhat retained the wine, which implies the influence of particle size on the flow of the liquid through the bed. The use of supports with large mean particle size (50–375 μm) allowed to broadly reduce the filtration time with partial retention of the sample.

Table 1
Mass of particles (g), filtration time (min) and permeate (mL) after filtering 50 mL of white wine through a bed thickness of 1.5 cm of supports according to particle type.

Support	m (g)	t (min)	Permeate (mL)
Silica 5 μm	2	7	37.5
Silica 10 μm	7	2	40
Silica 25 μm	7.5	3	37.5
Silica 50 μm	10	0.5	37.5
Silica 75 μm	10	0.5	40
Silica 200 μm	13	2	37.5
Silica 375 μm	17	0.5	40
Cellulose	10	0.5	37.5
Diatomaceous earth	7	5	37.5

The impact of wine filtration through the different supports on its physicochemical properties is presented in Table 2. The ΔE^* between the unfiltered wine and the wine samples filtered through the bare supports revealed the minor influence of filtration on the colour of the samples, being these differences not distinguished by the human eye ($\Delta E^* < 3$) (Gómez-Míguez, González-Miret, & Heredia, 2007). The TPI determination showed a significant influence of filtration in this parameter with slight increase and decrease of the index for different supports. In addition, the analysis of the main metabolites present in the wine showed very low influence of almost all the component except for the biggest size of silica particle (S375) which had great influence on these wine parameters.

Taking into account the results of this preliminary study, silica particles of mean size of 50 and 200 μm were chosen as supports due to their slight effect on the physicochemical wine properties with adequate filtering parameters.

3.2. PHE-functionalised supports design and characterisation

Six supports functionalised with phenolic compounds (two supports and three antimicrobials) were prepared to assess their potential application as filtering aids for wine stabilisation. The phenolic compounds immobilised on the surface of the supports were selected based on their presence in wine matrices naturally and their reported antimicrobial activity (Bartowsky, 2009). Eugenol presented excellent antimicrobial activity in prior studies, being active against fungi and a wide range of gram-negative and gram-positive bacteria (Marchese et al., 2017). This volatile phenolic compound can be formed during wine aging and is considered an odorant with a positive sensory impact (Baumes, 2009). Ferulic acid is a hydroxycinnamic acid commonly present in wine and is associated with the colour of wine (Monagas & Bartolomé, 2009). The *in vitro* antimicrobial activity of ferulic acid has been previously evaluated corroborating its moderate inhibitory potential against different pathogens and spoilage microorganisms (Borges, Ferreira, Saavedra, & Simões, 2013; Pastorkova et al., 2013; Shi et al., 2016). Lastly, vanillin is other non-flavonoid phenolic contained in wine that contributes flavour (Ugliano & Henschke, 2009) which presents significant antimicrobial activity in accordance with former studies (Fitzgerald et al., 2004, 2005).

As detailed in the experimental section, the phenolic compounds were covalently grafted on the surface of the silica supports by surface silanisation obtaining the PHE-functionalised filtering materials. Fig. S1 shows the particle size distribution of the bare and PHE-functionalised silica supports. The 50 μm-supports (S50) exhibited a size distribution in the range of 20–120 μm with $d_{0.5}$ values between 38 and 43 μm, whereas the 200 μm-supports (S200) revealed a size distribution between 90 and 480 μm and an average $d_{0.5}$ value of 176 μm. As can be observed in the figure, the supports presented a similar size distribution regardless the immobilised phenolic compound, confirming that the functionalisation procedure did not affect the morphological properties of the supports.

The zeta potential values of the pristine and PHE-functionalised supports are shown in Fig. 2. The bare supports (S50 and S200) showed negative zeta potential due to the presence of silanol moieties on the surface of the materials. In contrast, the PHE-functionalised materials exhibited positive zeta potential due to the phenolic compounds-alkoxysilane derivatives grafted on their surface. This change on zeta potential values confirmed the efficiency of the functionalisation method.

The yield of the functionalisation procedure was estimated by elemental analysis. Table 3 shows the content of organic matter of the PHE-functionalised supports. The 200 μm-supports exhibited a slightly higher content of phenolic-alkoxysilane derivatives immobilised on their surface than the 50 μm-supports, which could imply a higher yield of the immobilisation process on this support. The degree of functionalisation results are in accordance with previous studies (Peña-Gómez

Table 2
Physicochemical parameters of samples after filtering 50 mL of white wine through a bed thickness of 1.5 cm of filtering materials according to particle type. Mean values ± SD (n = 3).

Support	*ΔE**	*TPI*	Glucose (g/L)	Fructose (g/L)	Erythritol (g/L)	Glycerol (g/L)	Ethanol (g/L)
Control	0.019 ± 0.016^{a}	8.13 ± 0.39^{c}	0.31 ± 0.01^{bc}	0.70 ± 0.00^{b}	0.28 ± 0.01^{c}	5.17 ± 0.17^{bc}	10.34 ± 0.32^{abcd}
Cont 0.45	0.367 ± 0.039^{bc}	8.15 ± 0.42^{c}	0.31 ± 0.01^{bc}	0.65 ± 0.02^{b}	0.26 ± 0.01^{c}	4.88 ± 0.19^{ab}	9.69 ± 0.50^{ab}
S5	0.342 ± 0.230^{bc}	8.55 ± 0.07^{cde}	0.32 ± 0.01^{bc}	0.71 ± 0.01^{b}	0.29 ± 0.00^{c}	5.34 ± 0.01^{d}	10.52 ± 0.01^{de}
S10	0.128 ± 0.008^{ab}	8.55 ± 0.07^{cde}	0.33 ± 0.01^{bc}	0.72 ± 0.01^{b}	0.29 ± 0.01^{c}	5.33 ± 0.05^{cd}	10.30 ± 0.16^{bcd}
S25	0.137 ± 0.002^{ab}	9.03 ± 0.39^{de}	0.32 ± 0.01^{bc}	0.71 ± 0.01^{b}	0.28 ± 0.00^{c}	5.28 ± 0.02^{cd}	10.31 ± 0.06^{bcd}
S50	0.156 ± 0.014^{ab}	8.85 ± 0.35^{de}	0.32 ± 0.00^{bc}	0.70 ± 0.01^{b}	0.27 ± 0.02^{c}	5.23 ± 0.01^{cd}	10.15 ± 0.07^{abc}
S75	1.452 ± 0.070^{e}	7.50 ± 0.00^{b}	0.29 ± 0.01^{b}	0.65 ± 0.01^{b}	0.19 ± 0.01^{a}	5.35 ± 0.05^{d}	10.44 ± 0.09^{cde}
S200	0.503 ± 0.320^{c}	6.73 ± 0.04^{a}	0.29 ± 0.00^{b}	0.65 ± 0.00^{b}	0.17 ± 0.00^{a}	5.34 ± 0.02^{d}	9.98 ± 0.05^{a}
S375	1.764 ± 0.108^{f}	6.80 ± 0.14^{a}	0.10 ± 0.12^{a}	0.28 ± 0.32^{a}	0.25 ± 0.04^{b}	4.90 ± 0.30^{a}	10.19 ± 0.18^{abc}
Cellul	0.807 ± 0.089^{d}	8.73 ± 0.04^{de}	0.35 ± 0.01^{c}	0.71 ± 0.02^{b}	0.29 ± 0.01^{c}	5.29 ± 0.13^{cd}	10.62 ± 0.22^{e}
Diatom	0.275 ± 0.006^{abc}	8.45 ± 0.00^{cd}	0.32 ± 0.01^{bc}	0.70 ± 0.01^{b}	0.28 ± 0.00^{c}	5.24 ± 0.08^{cd}	10.39 ± 0.09^{bcde}

Same letters in a column indicate homogeneous group membership ($p < 0.05$).

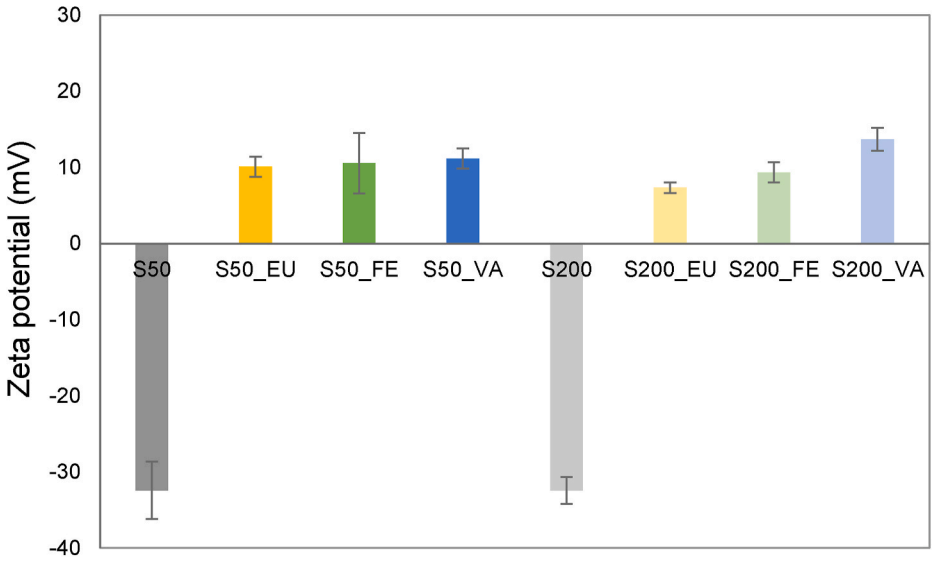

Fig. 2. Zeta potential values (mV) of the different bare and PHE-functionalised supports of mean size of 50 μm (S50) and 200 μm (S200) dispersed in distilled water. EU: eugenol-functionalised particle; FE: ferulic acid-functionalised particle; VA: vanillin-functionalised particle. Mean values ± SD (n = 3).

Table 3
Functionalisation's degree of the PHE-functionalised supports according to the organic matter content (α).

Support size (μm)	Immobilised compound	α (g organic matter/g solid)
50	Eugenol	0.086
	Ferulic acid	0.084
	Vanillin	0.057
200	Eugenol	0.101
	Ferulic acid	0.092
	Vanillin	0.156

et al., 2019b; Verdú et al., 2020). These values were also used to calculate the relative percentage of leached PHE (*vide infra*).

3.3. Effect on cell viability of the PHE-functionalised supports against spoilage wine microorganisms

After characterising the PHE-functionalised supports developed, the removal capacity of them as filtering aids was evaluated by deep filtration of white wine inoculated with common spoilage wine microorganisms. Fig. 3 shows the percentage of viable cells as a function of its control (S50 and S200) after filtering 50 mL of wine through a bed thickness of 3 cm of the bare or PHE-functionalised supports. The control survival value is shown as a dotted line in the graphs (100%). Filtering with bare particles (Fig. S2) slightly reduced the filtered wine's microbial load (below 20%) for all the evaluated microorganisms. These results prove the weak impact of using bare silica supports of large particle size as filtering materials for the microbial removal. In contrast, filtering wine samples through the PHE-functionalised supports resulted in significant reduction of the microbial load.

Eugenol-functionalised support (Fig. 3A) (S50 and S200) proved to be the most effective (viability below 10%) against all the tested microorganism with two exceptions (*Sc* S200 and *Db* S50) in which the viability reached higher values. In general, bacteria (*Aa* and *Lp*) seem to be more affected by eugenol-functionalised supports compared to yeasts. Ferulic acid (Fig. 3B) supports presented the lowest removal capacity, especially for S200 in the bacterium *Aa* and the yeast *Zb*. Even though the viability of the microorganisms was reduced, the effectiveness of these supports was not as good as in the case of eugenol. Vanillin (Fig. 3C) exhibited an intermediate removal capacity between eugenol and ferulic acid. Although, the complete set of tested microorganisms were affected by vanillin, in general terms the viability percentages were higher comparing with those obtained in the eugenol-functionalised supports.

Taking these results into account, it can be summarized that *Aa* filtered through the three S50 PHE-functionalised supports allowed reduced the microorganism to undetectable levels (log reduction value (LRV) *ca.* 3), being the eugenol-functionalised support (S200_EU) the filter that exhibited the highest removal capacity followed by vanillin-functionalised support (S200_VA). On the other hand, *Lp* was the most

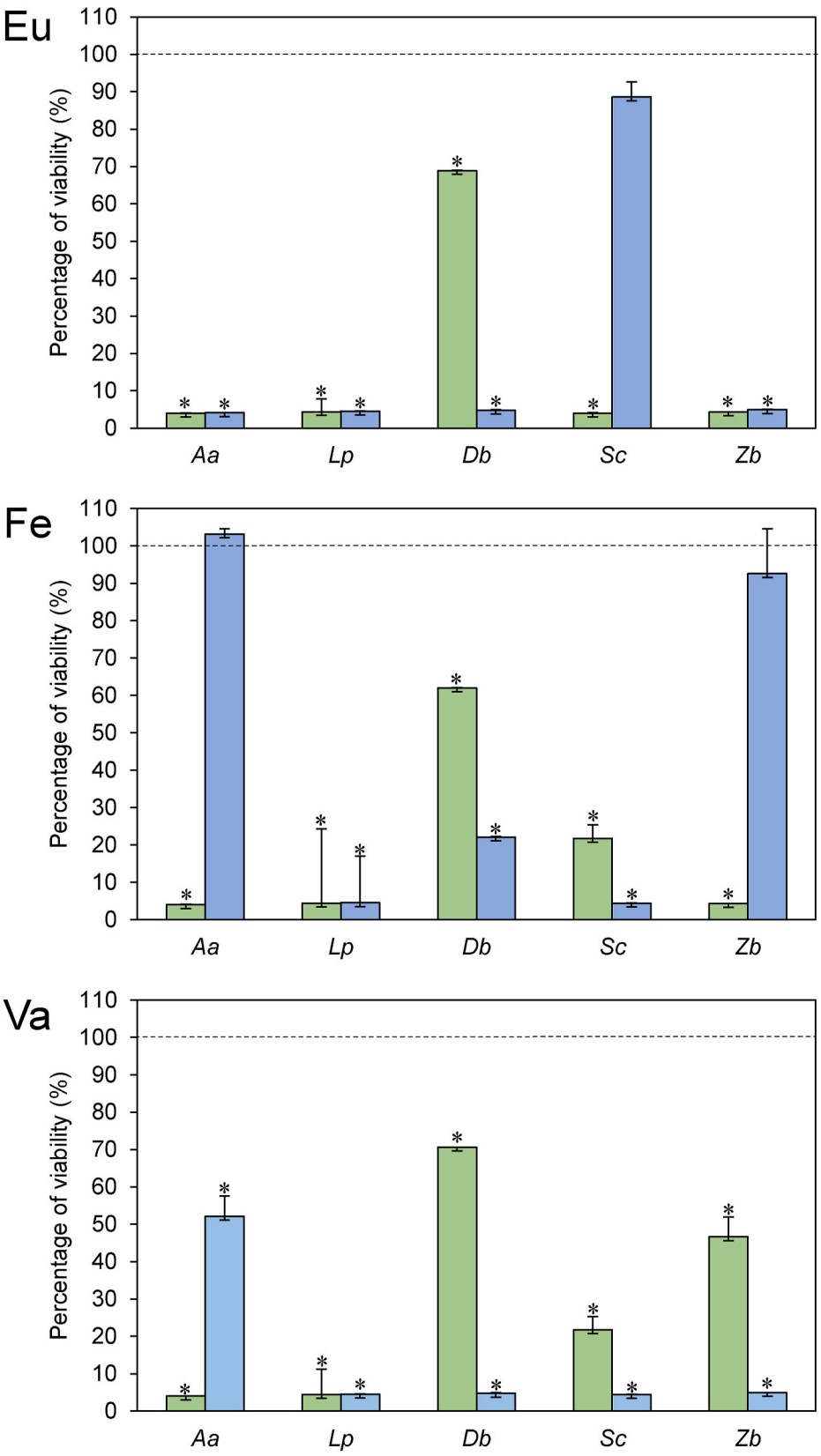

Fig. 3. Microbial relative viability (%) comparing with the bare particle S50 (green) and S200 (blue) of *A. aceti* (Aa), *L. plantarum* (Lp), *D. bruxellensis* (Db), *S. cerevisiae* (Sc) and *Z. bailii* (Zb) from white wine filtered through a bed thickness of 3 cm of the PHE-functionalised supports. EU: eugenol-functionalised particle; FE: ferulic acid-functionalised particle; VA: vanillin-functionalised particle. The dotted line indicated the 100% of viability corresponding to the control viability. *Significant differences compared with the control. Mean values ± SD (n = 3). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

susceptible microorganism against all the PHE-functionalised supports with viability values below 5% in all the tested conditions. In this sense, total removal of *Lp* was achieved by using all PHE-functionalised supports irrespective of grafted compound or particle size. In the case of the yeast, for *Db*, the use of the filtering aids gave different results, in this case the 200 μm-supports showing better microbial removal capacity. The results obtained with *Sc* in the removal capability assays showed an effective removal of the microorganisms for almost all the coated

supports. With *Zb*, the S200_FE and S50_VA supports were the filters with the lowest removal capacity, whereas the rest of the supports were capable of completely eliminating the microorganism from wine.

The reductions of wine spoilage microorganisms obtained in this study are similar to the inactivation efficacy of other non-thermal preservation technologies such as pulse electric fields (Marsellés-Fontanet, Puig, Olmos, Mínguez-Sanz, & Martín-Belloso, 2009) or high hydrostatic pressure (Morata et al., 2015) with LRVs between 1 and 4 according to the technique and the treated microorganism.

Besides the removal of the largest bed thickness of the supports, Fig. S3 presents the percentage of viable cells of the inoculated wine after filtration with a bed thicknesses of 0.5 and 1.5 cm. These results show the significant influence of the bed thickness on the removal properties of the PHE-functionalised supports. The larger the bed of particles, the greater the reduction in microbial load (Gialleli et al., 2015).

Overall, the most effective bioactive phenolic compound was eugenol. These results are consistent with our previous study in which the *in vitro* antimicrobial activity of silica microparticles of mean size of 5 μm functionalised with thymol, vanillin, eugenol and carvacrol was evaluated against wine spoilage microorganisms, being the eugenol-functionalised silica particles the antimicrobial agent with the greatest enhancement in its effectiveness compared to free eugenol (García-Ríos et al., 2018).

Some winemakers implement filtration systems with porosities as large as possible to sustain adequate flow rates with low retention of desirable colour/aroma/flavour while removing microorganisms of concern (Umiker et al., 2013). According to the results of the removal capability tests, these filtering materials would allow depth filtration with large porosity that lets the passage of the components of the liquid matrix but capable of render a wine microbiologically stable against yeast and bacteria due to the fact that they have a inhibitory effect on filtered microorganisms. The stabilisation of wine results from a combination of physical absorption on the bed and the inhibitory effect of the phenolic compounds immobilised on the surface of the filtering aids, as has been previously stated by our research group (Peña-Gómez et al., 2019b; Ruiz-Rico, Moreno, & Barat, 2020). This fact prevents the presence of microorganisms in a VBNC state in which could become smaller during wine storage (after exposure to SO_2, O_2 deprivation, etc.), decreasing the porosities of the filtration membranes required for their removal (Millet & Lonvaud-Funel, 2000; Umiker et al., 2013).

3.4. Influence of filtration on white wine parameters

Table 4 shows the colour difference, the phenolic content and the concentration of the major metabolites present in white wine before filtration and after filtering through the bare and PHE-functionalised supports with a bed thickness of 3 cm. In addition, Fig. S4 presents the ΔE* and TPI values of the inoculated wine after filtration with a bed thicknesses of 0.5 and 1.5 cm.

ΔE* results indicated a significant influence of filtration on the wine colour. The use of pristine supports resulted in ΔE*<3, which is in accordance with the results obtained in the preliminary assays using a smaller bed of supports (see Section 3.1). The colour differences observed between the unfiltered wine and the wine filtered through the eugenol-functionalised supports (S50_EU and S200_EU) were in the range between 2.4 and 3.5 that can be considered appropriate colour differences. In contrast, the samples filtered through the supports coated with ferulic acid and vanillin presented ΔE* values within 6.6–8.2, being changes easily detected by observers (ΔE*>3) (Gómez-Míguez et al., 2007). The colour differences found with these filtering aids are in the same range than other fining agents (Cosme, Capão, Filipe-Ribeiro, Bennett, & Mendes-Faia, 2012; Guise et al., 2014). Other novel stabilisation techniques evaluated in wine matrices have shown significant influence on the colour parameter with even higher ΔE* values, as it has been previously reported for the pulsed electric field treatment (ΔE*>9) (López, Puértolas, Hernández-Orte, Álvarez, & Raso, 2009).

The TPI results showed a significant influence of filtration in this parameter. The use of the bare supports or the filters functionalised with eugenol and vanillin slightly increased the content of total phenol, but the differences with the control wine were non-significant. In contrast, the wine samples filtered through the ferulic acid-functionalised supports (S50_FE and S200_FE) presented very high TPI values. These results suggest the partial leaching of the grafted ferulic acid from the supports' surface to the liquid matrix during the filtration process (*vide infra*).

Regarding the metabolites determined by HPLC, there was slightly differences comparing with the control without any particle, mainly in the quantity of the residual sugars (glucose and fructose) and in a lesser extent in some conditions of the other metabolites. In any case, these differences although significant may not imply organoleptic changes in the final product. Similar results have been obtained in other works describing new filtering materials, such as continuous cold pasteurisation of wine by filtration with tubular cellulose (Gialleli et al., 2015). After first day of the filtration and after filter regeneration, the concentration of the wine components and the colour index were reduced, in a similar range than our results.

3.5. Leaching of the immobilised antimicrobials

Table 5 and Table S1 present the amount of lixiviated phenolic compounds in the permeate after filtration through the PHE-functionalised supports. Besides the amount of grafted compounds released from the supports, the percentage of leached compounds was calculated by considering the total phenolic compounds attached to the supports (Section 3.2) and the amount of particles needed to prepare the bed of particles. For immobilised eugenol, a very low content of this compound was quantified after filtering wine which had a virtually no effect on the wine properties and wine stability. In fact, the released eugenol concentration fell far below the minimum inhibitory concentration determined for free eugenol against these target wine spoilage microorganisms in a previous study (0.5–13.2 mg/mL) (García-Ríos

Table 4
Physicochemical parameters of white wine filtered through a bed thickness of 3 cm of the non-functionalised particles and PHE-functionalised supports. Mean values ± SD (n = 3).

Support	*ΔE**	*TPI*	Glucose (g/L)	Fructose (g/L)	Erythritol (g/L)	Glycerol (g/L)	Ethanol (g/L)
Control	0.67 ± 0.87^{a}	9.49 ± 2.33^{a}	0.38 ± 0.11^{bc}	0.30 ± 0.09^{cd}	0.22 ± 0.06	4.84 ± 0.59	9.69 ± 1.07^{ab}
S50	1.46 ± 0.76^{ab}	8.82 ± 0.69^{a}	0.35 ± 0.05^{ab}	0.19 ± 0.15^{ab}	0.20 ± 0.07	4.97 ± 0.36	9.69 ± 0.65^{abc}
S50 EU	3.46 ± 1.39^{b}	10.52 ± 2.13^{a}	0.38 ± 0.05^{bc}	0.20 ± 0.11^{abc}	0.21 ± 0.06	4.95 ± 0.23	10.00 ± 0.41^{bc}
S50 FE	6.64 ± 2.93^{c}	104.05 ± 40.14^{b}	0.36 ± 0.07^{abc}	0.12 ± 0.11^{a}	0.20 ± 0.04	5.03 ± 0.11	10.17 ± 0.20^{c}
S50 VA	7.51 ± 4.42^{c}	14.91 ± 6.12^{a}	0.45 ± 0.18^{c}	0.12 ± 0.09^{a}	0.21 ± 0.04	4.93 ± 0.11	9.90 ± 0.27^{bc}
S200	2.99 ± 2.22^{b}	12.65 ± 5.72^{a}	0.36 ± 0.03^{abc}	0.25 ± 0.12^{bcd}	0.20 ± 0.03	4.88 ± 0.24	9.27 ± 0.59^{a}
S200 EU	2.37 ± 0.64^{ab}	12.04 ± 1.90^{a}	0.39 ± 0.04^{bc}	0.23 ± 0.12^{bcd}	0.21 ± 0.04	5.01 ± 0.15	9.86 ± 0.20^{bc}
S200 FE	6.57 ± 3.19^{c}	114.68 ± 35.23^{b}	0.26 ± 0.14^{a}	0.20 ± 0.06^{abc}	0.20 ± 0.03	4.99 ± 0.14	10.02 ± 0.41^{bc}
S200 VA	8.15 ± 3.66^{c}	25.62 ± 21.01^{a}	0.31 ± 0.19^{ab}	0.31 ± 0.15^{d}	0.19 ± 0.05	4.80 ± 0.31	9.93 ± 0.45^{bc}

Same letters in a column indicate homogeneous group membership ($p < 0.05$).

Table 5
Phenolic compounds leaching (mg/mL) and the relative percentage of the leached compounds after filtering 50 mL of white wine through the bed of the PHE-functionalised supports. Mean values ± SD (n = 4).

Support size (μm)	Immobilised compound	Leached compound (mg/mL)	Relative leached (%)
50	Eugenol	0.002 ± 0.001	0.006 ± 0.003
	Ferulic acid	0.819 ± 0.317	2.436 ± 0.945
	Vanillin	0.069 ± 0.053	0.303 ± 0.232
200	Eugenol	0.012 ± 0.005	0.023 ± 0.010
	Ferulic acid	0.554 ± 0.060	1.205 ± 0.130
	Vanillin	0.274 ± 0.173	0.352 ± 0.221

et al., 2018).

In contrast, wine filtered through the supports functionalised with vanillin and ferulic acid showed a significant lixiviate of the phenolic compounds with 0.07–0.27 mg/mL (0.3% relative leached) of the initial vanillin grafted to filters' surface and 0.82–0.55 mg/mL (1.2–2.4% relative leached) of the attached ferulic acid. This partial leaching is almost certainly responsible for the impact of the treated wine on the physicochemical properties mainly related with the ΔE* and TPI results. This leaching mainly limits the application of the ferulic acid-functionalised supports and the immobilisation procedure should be optimised to ensure the zero release of anchored molecules to prevent any influence on the physicochemical and sensory properties of wine.

4. Conclusions

In this study, silica microparticles functionalised with phenolic compounds were applied as filtering aids for microbial stabilisation of wine achieving 3 LRVs. The results evidenced the significant influence of the PHE immobilised on the removal capability of the filters as well as the impact on the wine properties. The use of eugenol-functionalised supports had a very low impact on the wine physicochemical parameters, which confirmed the preservation of the major attributes of the final product. The immobilisation of eugenol resulted in filters capable of removing microorganisms from wine similarly to other non-thermal preservation technologies. Besides, it is worth remarking the significant influence of the evaluated spoilage microorganisms on the antimicrobial properties of the PHE-functionalised supports. This fact suggests the application of a multilayer system to treat real wine samples in which a complex mix of microorganisms could be present in the matrix.

The overall results encourage the scale-up of the process for its application to wine or other liquid foods. The new filtering aids possess some advantages over standard depth filtration materials, such as increased removal capacity with low energy consumption and minimal effect on wine sensory properties. These coated filters could be used for clarification, microbiological stabilisation, and even sterile filtration in one single continuous operation reducing wine losses and energy costs by substitution of several treatments of traditional filtrations in a single operation and improving hygiene and safety of the process. However, some filtering features such as stability of the grafting, strength to environmental agents, conditions for reuse or service life should be studied before being applied in a real scenario.

CRediT authorship contribution statement

María Ruiz-Rico: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Estéfani García-Ríos:** Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **José Manuel Barat:** Supervision, Funding acquisition, Writing – original draft. **José Manuel Guillamón:** Supervision, Funding acquisition, Writing – original draft.

Acknowledgements

The authors gratefully acknowledge the financial support from the Ministerio de Ciencia, Innovación y Universidades, the Agencia Estatal de Investigación and FEDER-EU (Project RTI 2018-101599-B-C21 and ProjectPID 2019-108722RB-C31). M.R-R. acknowledges the Generalitat Valenciana for her postdoctoral fellowship (APOSTD/2019/118) and E. G-R. is supported by the Sara Borrell Program (CD18CIII/00007), Instituto de Salud Carlos III, Ministerio de Ciencia, Innovación y Universidades. We also thank to Judit Pardo and Antonio Ruiz for their technical assistance with the HPLC analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2021.111783.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Avramova, M., Vallet-Courbin, A., Maupeu, J., Masneuf-Pomarède, I., & Albertin, W. (2018). Molecular diagnosis of *Brettanomyces bruxellensis*' sulfur dioxide sensitivity through genotype specific method. *Frontiers in Microbiology, 9*(JUN), 1–9. https://doi.org/10.3389/fmicb.2018.01260

Bartowsky, E. J. (2009). Bacterial spoilage of wine and approaches to minimize it. *Letters in Applied Microbiology, 48*(2), 149–156. https://doi.org/10.1111/j.1472-765X.2008.02505.x

Baumes, R. (2009). Wine aroma precursors. In *Wine chemistry and biochemistry* (pp. 251–274). New York: Springer. https://doi.org/10.1007/978-0-387-74118-5_14.

Borges, A., Ferreira, C., Saavedra, M. J., & Simões, M. (2013). Antibacterial activity and mode of action of ferulic and gallic acids against pathogenic bacteria. *Microbial Drug Resistance, 19*(4), 256–265. https://doi.org/10.1089/mdr.2012.0244

Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods—a review. *International Journal of Food Microbiology, 94*(3), 223–253. https://doi.org/10.1016/J.IJFOODMICRO.2004.03.022

Cosme, F., Capão, I., Filipe-Ribeiro, L., Bennett, R. N., & Mendes-Faia, A. (2012). Evaluating potential alternatives to potassium caseinate for white wine fining: Effects on physicochemical and sensory characteristics. *LWT - Food Science and Technology, 46*(2), 382–387. https://doi.org/10.1016/j.lwt.2011.12.016

Fitzgerald, D. J., Stratford, M., Gasson, M. J., & Narbad, A. (2005). Structure−function analysis of the vanillin molecule and its antifungal properties. *Journal of Agricultural and Food Chemistry, 53*(5), 1769–1775. https://doi.org/10.1021/JF048575T

Fitzgerald, D. J., Stratford, M., Gasson, M. J., Ueckert, J., Bos, A., & Narbad, A. (2004). Mode of antimicrobial action of vanillin against *Escherichia coli, Lactobacillus plantarum* and *Listeria innocua*. *Journal of Applied Microbiology, 97*(1), 104–113. https://doi.org/10.1111/j.1365-2672.2004.02275.x

García-Ríos, E., Ruiz-Rico, M., Guillamón, J. M., Pérez-Esteve, É., & Barat, J. M. (2018). Improved antimicrobial activity of immobilised essential oil components against representative spoilage wine microorganisms. *Food Control, 94*(May), 177–186. https://doi.org/10.1016/j.foodcont.2018.07.005

Gialleli, A. I., Kallis, M., Bekatorou, A., Kanellaki, M., & Koutinas, A. A. (2015). Continuous cold pasteurisation of contaminated wine using Nano- and micro-tubular cellulose. *Food and Bioprocess Technology, 8*(3), 539–547. https://doi.org/10.1007/s11947-014-1416-5

Girard, B., Fukumoto, L. R., & Sefa Koseoglu, S. (2000). Membrane processing of fruit juices and beverages: A review. *Critical Reviews in Biotechnology, 20*(2), 109–175. https://doi.org/10.1080/07388550008984168

Gómez-Míguez, M., González-Miret, M. L., & Heredia, F. J. (2007). Evolution of colour and anthocyanin composition of Syrah wines elaborated with pre-fermentative cold maceration. *Journal of Food Engineering, 79*(1), 271–278. https://doi.org/10.1016/j.jfoodeng.2006.01.054

Guise, R., Filipe-Ribeiro, L., Nascimento, D., Bessa, O., Nunes, F. M., & Cosme, F. (2014). Comparison between different types of carboxylmethylcellulose and other oenological additives used for white wine tartaric stabilization. *Food Chemistry, 156*, 250–257. https://doi.org/10.1016/j.foodchem.2014.01.081

Lisanti, M. T., Blaiotta, G., Nioi, C., & Moio, L. (2019). Alternative methods to SO_2 for microbiological stabilization of wine. *Comprehensive Reviews in Food Science and Food Safety, 18*(2), 455–479. https://doi.org/10.1111/1541-4337.12422

López, N., Puértolas, E., Hernández-Orte, P., Álvarez, I., & Raso, J. (2009). Effect of a pulsed electric field treatment on the anthocyanins composition and other quality parameters of Cabernet Sauvignon freshly fermented model wines obtained after different maceration times. *LWT - Food Science and Technology, 42*(7), 1225–1231. https://doi.org/10.1016/j.lwt.2009.03.009

Marchese, A., Barbieri, R., Coppo, E., Orhan, I. E., Daglia, M., Nabavi, S. F., et al. (2017). Antimicrobial activity of eugenol and essential oils containing eugenol: A mechanistic viewpoint. *Critical Reviews in Microbiology, 43*(6), 668–689. https://doi.org/10.1080/1040841X.2017.1295225

Marsellés-Fontanet, À. R., Puig, A., Olmos, P., Mínguez-Sanz, S., & Martín-Belloso, O. (2009). Optimising the inactivation of grape juice spoilage organisms by pulse electric fields. *International Journal of Food Microbiology, 130*(3), 159–165. https://doi.org/10.1016/j.ijfoodmicro.2008.12.034

Mierczynska-Vasilev, A., & Smith, P. A. (2015). Current state of knowledge and challenges in wine clarification. *Australian Journal of Grape and Wine Research, 21*, 615–626. https://doi.org/10.1111/ajgw.12198

Millet, V., & Lonvaud-Funel, A. (2000). The viable but non-culturable state of wine micro-organisms during storage. *Letters in Applied Microbiology, 30*(2), 136–141. https://doi.org/10.1046/j.1472-765x.2000.00684.x

Monagas, M., & Bartolomé, B. (2009). Anthocyanins and anthocyanin-derived compounds. In *Wine chemistry and biochemistry* (pp. 439–462). New York: Springer. https://doi.org/10.1007/978-0-387-74118-5_21.

Morata, A., Loira, I., Vejarano, R., Bañuelos, M. A., Sanz, P. D., Otero, L., et al. (2015). Grape processing by high hydrostatic pressure: Effect on microbial populations, phenol extraction and wine quality. *Food and Bioprocess Technology, 8*(2), 277–286. https://doi.org/10.1007/s11947-014-1405-8

Oro, L., Canonico, L., Marinelli, V., Ciani, M., & Comitini, F. (2019). Occurrence of *Brettanomyces bruxellensis* on grape berries and in related winemaking cellar. *Frontiers in Microbiology, 10*(MAR), 1–10. https://doi.org/10.3389/fmicb.2019.00415

Pastorkova, E., Zakova, T., Landa, P., Novakova, J., Vadlejch, J., & Kokoska, L. (2013). Growth inhibitory effect of grape phenolics against wine spoilage yeasts and acetic acid bacteria. *International Journal of Food Microbiology, 161*(3), 209–213. https://doi.org/10.1016/j.ijfoodmicro.2012.12.018

Paulin, M., Miot-Sertier, C., Dutilh, L., Brasselet, C., Delattre, C., Pierre, G., et al. (2020). *Brettanomyces bruxellensis* displays variable susceptibility to chitosan treatment in wine. *Frontiers in Microbiology, 11*(September), 1–13. https://doi.org/10.3389/fmicb.2020.571067

Peña-Gómez, N., Ruiz-Rico, M., Fernández-Segovia, I., & Barat, J. M. (2019). Study of apple juice preservation by filtration through silica microparticles functionalised with essential oil components. *Food Control, 106*, Article 106749. https://doi.org/10.1016/j.foodcont.2019.106749

Peña-Gómez, N., Ruiz-Rico, M., Pérez-Esteve, É., Fernández-Segovia, I., & Barat, J. M. (2019). Novel antimicrobial filtering materials based on carvacrol, eugenol, thymol and vanillin immobilized on silica microparticles for water treatment. *Innovative Food Science & Emerging Technologies, 58*, Article 102228. https://doi.org/10.1016/j.ifset.2019.102228

Peña-Gómez, N., Ruiz-Rico, M., Pérez-Esteve, É., Fernández-Segovia, I., & Barat, J. M. (2020). Microbial stabilization of craft beer by filtration through silica supports functionalized with essential oil components. *Lebensmittel-Wissenschaft & Technologie, 117*, Article 108626. https://doi.org/10.1016/j.lwt.2019.108626

Pérez-Coello, M. S., & Díaz-Maroto, M. C. (2009). Volatile compounds and wine aging. In *Wine chemistry and biochemistry* (pp. 295–311). New York: Springer. https://doi.org/10.1007/978-0-387-74118-5_16.

Pérez-Través, L., Lopes, C. A., González, R., Barrio, E., & Querol, A. (2015). Physiological and genomic characterisation of *Saccharomyces cerevisiae* hybrids with improved fermentation performance and mannoprotein release capacity. *International Journal of Food Microbiology, 205*, 30–40. https://doi.org/10.1016/j.ijfoodmicro.2015.04.004

Renouf, V., Perello, M.-C., de Revel, G., & Lonvaud-Funel, A. (2007). Survival of wine microorganisms in the bottle during storage. *American Journal of Enology and Viticulture, 58*(3).

Ruiz-Rico, M., Moreno, Y., & Barat, J. M. (2020). In vitro antimicrobial activity of immobilised essential oil components against *Helicobacter pylori*. *World Journal of Microbiology and Biotechnology, 36*(1), 1–9. https://doi.org/10.1007/s11274-019-2782-y

Scansani, S., Rauhut, D., Brezina, S., Semmler, H., & Benito, S. (2020). The impact of chitosan on the chemical composition of wines fermented with *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae*. *Foods, 9*(10). https://doi.org/10.3390/foods9101423

Shi, C., Zhang, X., Sun, Y., Yang, M., Song, K., Zheng, Z., et al. (2016). Antimicrobial activity of ferulic acid against *Cronobacter sakazakii* and possible mechanism of action. *Foodborne Pathogens and Disease, 13*(4), 196–204. https://doi.org/10.1089/fpd.2015.1992

Ugliano, M., & Henschke, P. A. (2009). Yeasts and wine flavour. In *Wine chemistry and biochemistry* (pp. 313–392). New York: Springer. https://doi.org/10.1007/978-0-387-74118-5_17.

Umiker, N. L., Descenzo, R. A., Lee, J., & Edwards, C. G. (2013). Removal of *Brettanomyces bruxellensis* from red wine using membrane filtration. *Journal of Food Processing and Preservation, 37*(5), 799–805. https://doi.org/10.1111/j.1745-4549.2012.00702.x

Umiker, N. L., Descenzo, R. A., Lee, J., & Edwards, C. G. (2021). Efficient reduction in vegetative cells and spores of Bacillus subtilis by essential oil components-coated silica filtering materials. *Journal of Food Science*, 1–14. https://doi.org/10.1111/1750-3841.15748

Verdú, S., Ruiz-Rico, M., Perez, A. J., Barat, J. M., Talens, P., & Grau, R. (2020). Toxicological implications of amplifying the antibacterial activity of gallic acid by immobilisation on silica particles: A study on *C. elegans*. *Environmental Toxicology and Pharmacology, 80*, Article 103492. https://doi.org/10.1016/j.etap.2020.103492