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Innovative red winemaking strategy: biosurfactant-assisted extraction and stabilization of phenolic compounds

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Abstract. In the present study, a biosurfactant extract obtained from a fermented residual stream of agri-food waste from corn industry, named corn steep liquor (CSL), has been used during winemaking to improve the colour features of red wines. Therefore, the first aim of this study was to evaluate the effectiveness of CSL biosurfactant to increase the release and preservation of anthocyanins during skin simulated macerations, and to compare its effect with four treatments based on exogenous tannin additions (grape seeds, grape skins, quebracho, and acacia tannins), considering two red winegrape varieties ('Cabernet sauvignon' and 'Aglianico'). Then, the pre-fermentative addition of CSL biosurfactant was evaluated through nano-vinifications of 'Merlot' winegrapes, during spontaneous or inoculated fermentations with commercial *Saccharomyces cerevisiae* yeasts. Technological parameters, colour characteristics, and phenolic composition were determined, as well as the fermentation dynamics. For the simulated maceration trial, the results obtained showed that the addition of the CSL biosurfactant increased colour intensity from the beginning of maceration with respect to untreated control, particularly for 'Cabernet sauvignon'. During 'Merlot' grapes nano-vinifications, the inoculated samples confirmed higher values of colour intensity with the addition of CSL biosurfactant and, at the end of alcoholic fermentation, a higher percentage of large polymeric pigments was also observed. After malolactic fermentation, the colour of CSL biosurfactant-added wines was also darker.

1. Introduction

The colour is the first attribute perceived by consumers and a major factor determining the quality of red wines. This depends mainly on the content of grape anthocyanins and their extraction into the juice/wine during winemaking. Furthermore, these compounds can undergo reactions that influence the chemical and sensory characteristics of the wine. Monomeric forms are prone to oxidation and adsorption on solid parts. Nevertheless, polymerization and copigmentation reactions with other metabolites are highly valuable for colour stability and preservation.

Several studies have evaluated the effect of adding different oenological tannins or copigments on anthocyanin preservation and colour stabilization during maceration. A novel alternative strategy has promoted the anthocyanin solubilisation within micelles by using surface-active compounds, such as polysorbate-based chemical surfactants [1]. However, the chemical surfactants are not suitable for food use. Nowadays, the production of biological surfactants, namely biosurfactants, by microorganisms opens new opportunities in food industry due to their low toxicity, biodegradability, and biocompatibility**.** The biosurfactant extracted from corn steep liquor (CSL), being a spontaneously fermented agri-food residue, is cost-competitive and its content of phenolic compounds gives antioxidant activity [2]. It can solubilize a great diversity of compounds as a consequence of its amphiphilic nature, with a hydrophobic tail composed of fatty acids and a hydrophilic head containing nitrogen from lipopeptides [3]. Hydrogen bonds and hydrophobic interactions may

promote the solubilisation of hydrophobic compounds in water-based solutions.

To our knowledge, prior to this study a biosurfactant extract has never been tested during red winemaking with grape skin maceration to improve the colour traits of red wines and related phenolic compounds through the combined effect of its surface-active, antioxidant and solubilizing properties.

2. Materials and methods

2.1. Grape samples

Vitis vinifera L. cv. 'Aglianico' and 'Cabernet sauvignon' red winegrapes, harvested at ripeness (about 24 Brix) from the ampelographic collection of Grinzane Cavour (Cuneo province, north-west Italy), were used for skin simulated macerations. For each grape variety, the berries were density-sorted by flotation in saline solutions and then washed with water; those belonging to the most representative density class were selected (1106 kg/m^3 for 'Aglianico' and 1100 kg/m^3 for 'Cabernet sauvignon').

On the other hand, destemmed and crushed *Vitis vinifera* L. cv. 'Merlot' red winegrapes, harvested at ripeness (about 24 Brix) from an experimental vineyard (Cuneo province, north-west Italy), were used for nano-vinification trials.

2.2. Total extraction of skin phenolic compounds

Three replicates of ten berries were manually peeled. The skins were manually separated from the pulp and quickly immersed into 25 mL of a buffer solution at pH 3.40 containing 14% v/v of ethanol, 5 g/L of tartaric acid, and 2 g/L of sodium metabisulphite [4]. After homogenization with an Ultra-Turrax T25 high-speed homogenizer (IKA Labortechnik) for 1 min at 8000 rpm and subsequent centrifugation at 3000 x g for 15 min at 20 ºC, the supernatant was obtained.

2.3. Skin simulated maceration tests

For 'Aglianico' and 'Cabernet sauvignon' varieties, eighteen sets (6 tests × 3 independent replicates) of 20 sorted berries were selected. The six skin simulated maceration tests evaluated the effect of the CSL biosurfactant and four single oenological tannin formulations (grape seeds, grape skins, quebracho, or acacia) with respect to unadded control. The berry skins, carefully separated from the pulp, were quickly immersed into 100 mL of a buffer solution at pH 3.40 containing 5 g/L of tartaric acid (control), in which an established dose of tannin formulation (4/5 of the maximum recommended dose: 20, 25, 40, and 32 g/hL for grape seeds, grape skins, quebracho, and acacia, respectively) or CSL biosurfactant extract (100 g/hL to overcome the critical micellar concentration of 200 mg/L ensuring the micelle formation [5]) was added. Wine fermentative maceration process was simulated by macerating the berry skins for 7 days at 25 ºC with progressive addition of 96% v/v ethanol at 6, 24, 48, 72, and 96 h of maceration, achieving respectively 2.50, 4.80, 7.10, 10.6, and 14.0% v/v ethanol. Just before each addition, an equal aliquot of skin extract was taken, maintaining constant the volume of the macerating solution. After 168 h of maceration, the liquid skin extract was taken for a more complete analytical determination.

2.4. Nano-vinifications

Previously destemmed and crushed 'Merlot' grapes were distributed in 12 sterile Erlenmeyer flasks (4 tests × 3 independent replicates) with the same grape must/solids ratio. For each one of 12 flasks (CSL biosurfactant addition of 100 g/hL and without addition for both spontaneous and inoculated fermentation), 150 g of grape must and 100 g of grape marc were used. To ensure a complete fermentation process, organic nutrition was added (20 g/hL of Fermaid O, Lallemand). For inoculated trials, commercial LSA *Saccharomyces cerevisiae* yeasts (Lalvin EC1118®, Lallemand) were used at 1.0×10^6 cells/mL.

All flasks were closed with a fermentation air-lock containing sterile liquid paraffin and kept at 25 °C. Skin maceration lasted 15 days, then the racking was carried out. At the end of alcoholic fermentation, commercial *Oenococcus oeni* lactic acid bacteria (Lalvin VP41®, Lallemand) were inoculated at 1.0×10^6 cells/mL to drive the malolactic fermentation.

Samples were taken during maceration (1, 2, 3, 4, 7, 11, and 14 days), at the end of alcoholic fermentation, and at the end of malolactic fermentation.

2.5. Analytical determinations

Standard physico-chemical parameters of grape must were determined after manual crushing of 100 grape berries and centrifugation at 3000 × g for 15 min at 20 °C. Two replicates were performed. Total acidity (expressed as g/L of tartaric acid) and pH determinations were conducted using OIV methods [6]. Reducing sugars, ethanol, and glycerol were quantified using a HPLC system equipped with refractive index detector [7]. Phenolic ripeness indices, cell maturity index (EA%) and seed maturity index (Mp%), were assessed on other two replicates of 200 berries each [8].

Yeast population evolution during the fermentation process was followed by culture-dependent approach, using WLN culture media [9].

The phenolic composition was determined through spectrophotometric methods [10] using a UV-1800 spectrophotometer (Shimazdu). Total anthocyanins, total flavonoids, total polyphenols index (TPI), monomeric and oligomeric flavanols (as flavanols reactive to vanillin), and proanthocyanidins (Bate–Smith reaction) were determined. Polymeric pigments (Adams-Harbertson assay) and copigmentation (Boulton method) were assessed for their contribution to wine colour [11, 12].

The anthocyanin profile was also determined by HPLC-DAD with an Agilent 1260 system (Agilent Technologies) [10]. Each skin extract was diluted 1:1 with an HCl solution at pH 0.5, filtered through a 0.45 µm PTFE membrane filter, and then injected (50 µL). Individual anthocyanins were quantified and expressed as percentage, whereas the sum of all individual forms was expressed as mg of malvidin-3-glucoside chloride/kg of grape berries.

During skin maceration, colour intensity and hue values were obtained according to the OIV-MA-AS2-07B method [6]. CIELab parameters, namely lightness (L*), red/green colour coordinate (a*), and yellow/blue colour coordinate (b*), were determined following the OIV-MA-AS2-11 method [6]. The colour difference between control and treated samples (ΔE*) was calculated as follows: $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ [6].

2.6. Statistical analysis

Statistical analysis was carried out using R statistic software, version 3.6.2, or SPSS version 29. For each studied variable distributed normally and with homogeneity in variance, one-way analysis of variance (ANOVA) using the Tukey HSD post-hoc test was used to evaluate significant differences. When populations were heterogeneous in variance or were not distributed normally, non-parametric tests were performed (Welch-one-way ANOVA test with Games–Howell post-hoc and Kruskal–Wallis test with Conover post-hoc, respectively).

3. Results and discussion

3.1. Skin-simulated maceration tests

3.1.1. Analytical characterization of grape berries

The average values of standard analytical parameters determined at harvest in sorted samples were the following: reducing sugars 262 g/L, pH 3.30, and 7.1 g/L as tartaric acid for total acidity in 'Aglianico'; reducing sugars 234 g/L, pH 3.49, and 5.7 g/L as tartaric acid for total acidity in 'Cabernet sauvignon'. Phenolic ripeness indices were quite similar for the two varieties (43.7 and 40.0 for EA%, 75.7 and 69.8 for Mp% in 'Aglianico' and 'Cabernet sauvignon', respectively).

Regarding the skin phenolic composition, both red winegrape varieties were quite different (data not shown). 'Cabernet sauvignon' grapes were richer in total skin phenolic compounds, flavanols, and anthocyanins. Although 'Aglianico' and 'Cabernet sauvignon' were prevalent in trisubstituted anthocyanins (70.48% and 61.72%, respectively), with a clear prevalence of malvidin-3-glucoside, the anthocyanin profile showed some differences. 'Cabernet sauvignon' had a significantly lower percentage of malvidin-3-glucoside but higher one of delphinidin-3-glucoside, as well it was richer in acetylated derivatives (22.21% compared to 3.76%). On the other hand, 'Aglianico' grapes were richer in cinnamoylated forms (22.92% compared to 9.10%). These results agree with those previously published for these varieties [13].

3.1.2. Colour evolution during maceration

The evolution of colour intensity and hue throughout skin simulated maceration was similar for 'Aglianico' and 'Cabernet sauvignon' winegrapes, whereas significant differences were observed at different sampling times for the different oenological tannins and CSL biosurfactant tested (Figure 1). Colour intensity increased progressively until reaching a maximum value (72 h for 'Aglianico' and 48 h for 'Cabernet sauvignon') and then decreased in the latter stages of maceration. For 'Aglianico' winegrapes, the skin extracts showed the highest values of colour intensity when quebracho tannin was used, even though the increase observed was not always significant with respect to control. Regarding 'Cabernet sauvignon', CSL biosurfactant was most efficient in increasing the colour intensity of skin extracts, followed by quebracho tannin. Nevertheless, at the end of maceration (168 h), significant differences were not observed for colour intensity among the treatments tested for 'Aglianico' whereas the macerating solutions showed higher values for treated samples with CSL biosurfactant on 'Cabernet sauvignon', particularly when compared to control as well as to grape-derived tannins.

Hue value evolved similarly during maceration for the two varieties, independently on the treatment. After an initial decrease, it increased after 72 h of maceration probably due to a loss of red colour component. The CSL biosurfactant addition, differently from oenological tannins, did not increase significantly hue values at any sampling time when compared to the control. At the end of maceration, the differences were not significant among treatments and control for the two varieties.

The treatments tested affected the visually perceived colour of skin extracts during the simulated maceration process. For each variety and at each maceration time, ΔE* values were calculated to quantify the colour differences for each treatment in relation to the control. In most cases, ΔE* values were greater than 3.0 units (threshold to correctly detect wine colour differences by the human eye [14]), except for 'Aglianico' variety at 24, 48, 72, and 96 h of maceration when acacia tannin was used, as well as CSL biosurfactant at 48 and 72 h. The perceived colour of 'Cabernet sauvignon' skin extracts was visually different in all samples added with the single tannin formulations tested compared to the control (ΔE* values above 3.49), but particularly for the CSL biosurfactant, leading to a progressive increase of ΔE* values during the skin simulated maceration process (ΔE* values from 6.35 at 6 h to 11.53 at the end of maceration). It is interesting to highlight that the colour differences are visible for the use of the CSL biosurfactant for the two varieties, not only with respect to the control but also with respect to all the tannin formulations evaluated (ΔE* values from 5.02 to 11.53). On the contrary, the perceived colour differences

decreased in most of cases when maceration progressed for the other treatments.

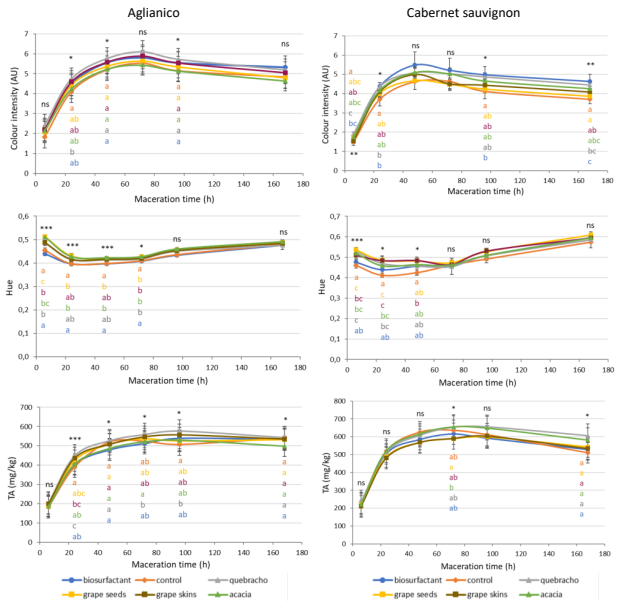

Figure 1. Colour intensity, hue, and total anthocyanins of skin extracts during simulated maceration with enological tannins from different origin and corn steep liquor (CSL) biosurfactant for 'Aglianico' and 'Cabernet sauvignon' winegrapes. All data are expressed as average value (n=3). Sign: *, **, ***, and ns indicate significance at $p < 0.05$, 0.01, 0.001, and not significant, respectively, for the differences among treatments for each maceration time according to ANOVA. Different Latin letters indicate significant differences according to Tukey HSD ($p < 0.05$). TA: total anthocyanins expressed as mg malvidin-3-glucoside chloride/kg grapes.

Figure 1 shows that the extracted content of total anthocyanins cannot explain the evolution of colour intensity during skin maceration. For 'Aglianico' 'Cabernet sauvignon' winegrapes, the highest contents of total anthocyanins corresponded to quebracho tannin, which is not in agreement with the highest values found of colour intensity for the CSL biosurfactant on 'Cabernet sauvignon'. For this last variety, the smaller decrease observed in total content of anthocyanins after 72 h of skin maceration when quebracho or acacia tannins were used is interesting. At the end of maceration (168 h), these two treatments increased the anthocyanin extraction yield between +9% and 7% with respect to control.

3.1.3. Phenolic composition at the end of maceration

Table 1 shows the phenolic composition of the skin extracts at the end of the maceration process (168 h). For 'Cabernet sauvignon' variety, the percentage of copigmented anthocyanins increased significantly with the addition of the CSL biosurfactant when compared to control samples, in detriment of free forms. The bathochromic shift and hyperchromic effect on absorbance at 520 nm related to copigmentation explains the more coloured skin extracts obtained for this treatment [15].

Regarding 'Aglianico', there is no significant modification of the contribution of the copigmentation phenomenon to the colour of skin extracts resulting from the treatment with the CSL biosurfactant, contrary to the decrease observed for other treatments, but a significant effect on polymeric pigments was not observed either probably due to the initial phase of winemaking. However, the acacia tannin and CSL biosurfactant were the treatments that most increased the percentage of polymeric pigments for 'Aglianico' (+1.91% and +1.09%, respectively, compared to control).

The variety effect on copigmentation could be attributable to the higher richness in coumaroylated anthocyanins of 'Aglianico' skins, that could reduce the intermolecular copigmentation with exogenous tannins or CSL biosurfactant [16]. Therefore, a combined effect of total content of anthocyanins, copigmentation, and polymerization reactions could have better preserved the colour for CSL biosurfactant-added skin macerated solutions on 'Aglianico'.

Table 1. Phenolic composition of skin extracts at the end of maceration with tannins from different origin and CSL biosurfactant for 'Aglianico' and 'Cabernet sauvignon' winegrapes.

Treatment	Copigmented anthocyanins (%)	Free anthocyanins (%)	Polymeric pigments (%)	TPI (mg/kg grapes)	TA (mg/kg grapes)	FNA (mg/kg grapes)
			Aglianico			
control	26.13 ± 1.14 b	63.55 ± 0.25 a	19.90 ± 0.63	2014 ± 148 a	542 ± 57	639 ± 30 a
grape seeds	21.62 ± 0.72 a	67.57 ± 0.31 b	20.71 ± 0.95	2285 ± 68 ab	532 ± 19	828 ± 21 b
grape skins	24.50 ± 0.64 ab	64.37 ± 1.16 ab	20.19 ± 0.35	2320 ± 89 b	535 ± 5	814 ± 22 b
acacia	25.24 ± 1.40 ab	64.11 ± 1.14 ab	21.81 ± 0.29	2283 ± 37 ab	498 ± 13	831 ± 25 b
quebracho	24.34 ± 3.42 ab	65.32 ± 3.54 ab	20.62 ± 0.34	2641 ± 18 c	544 ± 6	1045 ± 28 c
biosurfactant	25.70 ± 1.20 b	64.12 ± 1.66 ab	20.99 ± 1.45	2141 ± 184 ab	533 ± 46	632 ± 70 a
Sign	*	***	ns	***	ns	***
			Cabernet sauvignon			
control	18.42 ± 2.15 a	64.88 ± 0.97 b	36.60 ± 10.66	2192 ± 222 a	510 ± 58 a	482 ± 38 a
grape seeds	19.98 ± 2.30 ab	64.23 ± 2.75 b	29.20 ± 1.31	2733 ± 139 bc	543 ± 13 ab	713 ± 6 b
grape skins	22.62 ± 3.82 ab	61.96 ± 3.03 ab	28.83 ± 0.67	2671 ± 51 ab	534 ± 28 ab	751 ± 21 b
acacia	20.14 ± 1.20 ab	64.93 ± 1.32 b	29.62 ± 0.31	2856 ± 166 bc	582 ± 11 ab	860 ± 19 c
quebracho	23.30 ± 0.65 ab	63.02 ± 0.66 ab	30.53 ± 1.19	3191 ± 208 c	606 ± 41 b	1145 ± 57 d
biosurfactant	24.95 ± 1.55 b	59.62 ± 1.92 a	32.41 ± 3.37	2659 ± 238 ab	529 ± 16 ab	406 ± 19 a
Sign	*	***	ns	***	*	***

All data are expressed as average value ± standard deviation (n=3). Sign: *, ***, and ns indicate significance at $p < 0.05$, 0.001, and not significant, respectively, for the differences among treatments according to ANOVA or Welch's ANOVA tests. Different Latin letters within the same column indicate significant differences according to Tukey HSD and Games-Howell tests ($p < 0.05$) for ANOVA and Welch's ANOVA, respectively. TPI: total polyphenols index expressed as mg (-)-epicatechin/kg grapes, TA: total anthocyanins expressed as mg malvidin-3-glucoside chloride/kg grapes, FNA: non-anthocyanin flavonoids expressed as mg (+)-catechin/kg grapes.

Higher total polyphenols index, as well total contents of anthocyanins and non-anthocyanin flavonoids, are not directly related to the contribution of these copigmentation and polymerization reactions.

Regarding individual anthocyanin forms, the CSL biosurfactant played a protective role on delphinidin-3-glucoside and petunidin-3-glucoside in 'Aglianico' winegrape variety whereas on acylated derivatives in 'Cabernet sauvignon', similarly to other tannins. The CSL biosurfactant has antioxidant activity derived from its phenolic composition [2]. Nevertheless, its efficacy was less than that corresponding to some enological tannins such as quebracho.

3.2. Nano-vinification tests

3.2.1. Analytical characterization of grape berries

With the aim of evaluating if the colour increase obtained during simulated skin maceration/fermentation using a buffer solution and the addition of CSL biosurfactant extract can be also attained during real winemaking, nano-vinifications of 'Merlot' red winegrapes were carried out. The grape berries used for this study were analysed at harvest and showed the following average values of standard physico-chemical parameters: reducing sugars 249 g/L, pH 3.56, and 5.1 g/L as tartaric acid for total acidity. Phenolic ripeness indices, EA% and Mp%, were 33.5 and 68.0, respectively. Regarding the phenolic composition, 'Merlot' grapes were also prevalent in malvidin-3-glucoside [17].

3.2.2. Yeast dynamics and fermentation kinetics

The CSL biosurfactant addition did not affect significantly yeast dynamics and fermentation kinetics for inoculated and spontaneous trials. As it can be observed in Figure 2, growth kinetics of *S. cerevisiae* and non-*Saccharomyces* yeasts species, as well as the contents of reducing sugars, ethanol, and glycerol, were similar for inoculated and spontaneous fermentations between biosurfactant-added and control samples.

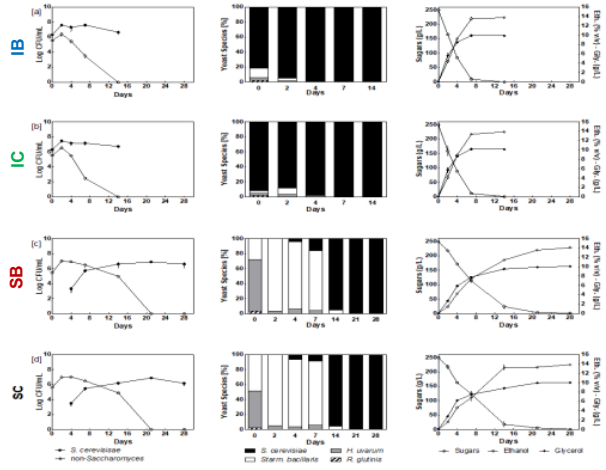

Figure 2. Viable cells of suspected *S. cerevisiae* and non-*Saccharomyces*, yeast species identification of *S. cerevisiae* colonies, and reducing sugars, ethanol, and glycerol contents during inoculated (I) and spontaneous (S) alcoholic fermentation with CSL biosurfactant addition (B) or without addition (C) (n=3). CFU/mL: number of colony forming units per millilitre.

3.2.3. Colour evolution during maceration

Figure 3 shows the evolution of colour intensity, hue, total anthocyanins, and total polyphenols index during maceration. The biosurfactant-added samples showed higher values of colour intensity with respect to control for both inoculated and spontaneous fermentations whereas significant differences were not observed for hue values at each sampling point. After an initial increased total content of anthocyanins for the samples added with the CSL biosurfactant in the inoculated fermentation test, the differences with respect to control were smaller when fermentation progressed. However, in the last days of maceration, a significantly higher content of total polyphenols was observed, only for inoculated trials, probably as consequence of a higher extraction of grape tannins when using the CSL biosurfactant. It agreed with the lower percentage of monomeric anthocyanins found in the wine just after racking (in biosurfactant-added and control samples, respectively, 57.9% and 58.6% for inoculated fermentation, 59.0% and 59.5% for spontaneous fermentation). The surface-active properties and amphiphilic nature of CSL biosurfactant may have contributed to an increased release and solubilisation of these less water-soluble compounds than anthocyanins. These compounds could have promoted the colour stabilization, increasing the values of colour intensity at the end of maceration for both inoculated and spontaneous fermentations.

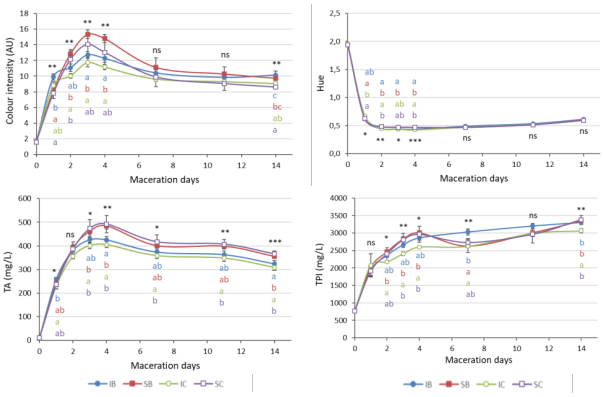

Figure 3. Colour intensity, hue, total anthocyanins, and total polyphenols index of must/wine samples during maceration with CSL biosurfactant for Merlot winegrapes and control with addition. All data are expressed as average value (n=3). Sign: *, **, ***, and ns indicate significance at $p < 0.05$, 0.01, 0.001, and not significant, respectively, for the differences among treatments for each maceration time according to ANOVA. Different Latin letters indicate significant differences according to Tukey HSD ($p < 0.05$). TA: total anthocyanins expressed as mg malvidin-3-glucoside chloride/L, TPI: total polyphenols index expressed as mg (–)-epicatechin/L.

The perceived colour of 'Merlot' must/wine was visually different in the samples added with CSL biosurfactant with respect to the control (ΔE* values above 3.0), from the fourth day of skin maceration, achieving values of 10.64 and 5.53 at the end of maceration (14 days) for inoculated and spontaneous fermentations, respectively.

3.2.4. Phenolic composition in the wines obtained

At the end of malolactic fermentation, the CSL biosurfactant influenced positively the colour stability of the resulting wines, achieving significantly higher values of colour intensity only for inoculated alcoholic fermentation (Table 2). The wines obtained using the CSL biosurfactant were richer in total anthocyanins and

polyphenols (particularly tannins) with respect to control wines. In this case, the effect of CSL biosurfactant was higher for flavanols than anthocyanins. Therefore, the presence of CSL biosurfactant may have increased the solubility of hydrophobic compounds such as tannins, preserving the colour intensity, even though not through copigmentation and polymerization reactions but antioxidant capability. However, no significant effect was observed on colour properties and related compounds when spontaneous fermentation was conducted.

Table 2. Phenolic composition of Merlot wines at the end of malolactic fermentation with addition CSL biosurfactant *versus* control.

	Inoculated			Spontaneous			
	IB	IC	Sign[a]	SB	SC	Sign[b]	Sign
Phenolic composition							
TA (mg/L)	169±6c	147±7b	*	65±3a	74±3a	*	***
TF (mg/L)	1074±6c	942±17b	***	680±12a	711±10a	*	***
PRO (mg/L)	7493±299c	6268±193b	**	715±33a	724±33a	ns	***
FRV (mg/L)	485±48c	379±51b	ns	156±11a	161±4a	ns	***
TPI (mg/L)	2840±52c	2524±84b	**	2216±82a	2255±31a	ns	***
Chromatic treats							
Colour intensity (AU, 10 mm)	8.89±0.36c	7.69±0.38bc	*	5.87±0.56a	6.41±0.71ab	ns	***
Hue	0.85±0.01a	0.86±0.01a	ns	1.12±0.03b	1.09±0.02b	ns	***
Polymeric pigments (%)	52.7±0.9a	52.4±0.3a	ns	66.5±0.0b	66.1±2.7b	ns	***
Copigmentation (%)	7.6±0.1b	7.3±0.8ab	ns	5.2±0.7a	5.8±1.4ab	ns	*
Free anthocyanins (%)	44.8±1.1b	45.1±0.8b	ns	26.7±0.3a	25.6±1.0a	ns	***

All data are expressed as average value ± standard deviation (n=3). Sign: *, **, ***, and ns indicate significance at $p < 0.05$, 0.01, 0.001, and not significant, respectively, for the differences among treatments according to ANOVA test. Different Latin letters within the same row indicate significant differences according to Tukey HSD test ($p < 0.05$). TA: total anthocyanins expressed as mg malvidin-3-glucoside chloride/L, TF: total flavonoids expressed as mg (+)-catechin/L, PRO: proanthocyanidins expressed as cyanidin chloride, FRV: flavanols reactive to vanillin expressed as mg (+)-catechin/L, TPI: total polyphenols index expressed as mg (-)-epicatechin/L. Inoculated (I) and spontaneous (S) alcoholic fermentation with CSL biosurfactant (B) o without addition (C).

4. Conclusions

Considering the simulated maceration study, the results obtained showed that the addition of the CSL biosurfactant extract increased colour intensity from the beginning of maceration, particularly for 'Cabernet sauvignon'. Although total anthocyanin content was not significantly affected by the CSL biosurfactant, the colour preservation seems to be mainly due to copigmentation for 'Cabernet sauvignon' whereas a combined effect of copigmentation and polymerization reactions could be hypothesized for 'Aglianico'. During 'Merlot' grapes nano-vinifications, the inoculated samples confirmed higher values of colour intensity with the addition of CSL biosurfactant and, at the end of alcoholic fermentation, a higher percentage of large polymeric pigments was also observed. Moreover, the release and solubilization of anthocyanins and proanthocyanidins was higher, leading to significantly higher concentrations of these compounds in the final wine, as well as for total polyphenols. After malolactic fermentation, the colour of CSL biosurfactant-added wines was also darker for the inoculated trial. The CSL biosurfactant addition did not negatively affect the fermentation dynamics. When spontaneous fermentation was carried out, no significant changes were observed on colour features and related compounds. These results highlight the effectiveness of the CSL biosurfactant to protect and stabilize the colour traits through winemaking.

5. Acknowledgements

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