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The Use of Overripe Grapes and Their Skins for Naturally Sweet Wines Production in a Warm Climate Zone

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Abstract: Due to global warming and the effects associated with it, the wine industry is facing important challenges during the winemaking process and the production of high-quality wines. In this study, mistelas and naturally sweet wines were produced with the 'Pedro Ximénez' grapevine cultivar, overripened by sun drying and fermented with and without the presence of grape skins. Some oenological parameters related to alcoholic fermentation and low-molecular-weight polyphenols and furans were considered. Naturally sweet wines with skins presence showed a higher value of viable biomass than those with grape skins absence. However, in terms of density and ethanol production, sweet wines with grape skins absence presented lower and higher values, respectively, than the other elaborations. No significant differences in the organic acids and low-molecular-weight polyphenols and furans contents, with respect to the presence or absence of grape skins, were observed. In this sense, this research proves that the production of sweet wines from sun-dried grapes with the presence/absence of grape skins during alcoholic fermentation could be a possible choice in areas where agro-climatic conditions make it possible.

Keywords: sweet wine; grape skin; alcoholic fermentation; Pedro Ximénez; overripe

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1. Introduction

Grapevine (*Vitis vinifera* L.) is one of the world's most valuable horticultural crops [1] and, for this reason, the global wine sector plays a significant role in several national economies [2]. Internationally acclaimed wine-producing areas are typically located within regions recognized with an Appellation of Origin (AO; OIV/ECO Resolution 2/92) or a Designation of Origin (DO; European Community 479/2008 Art. 34 1a). These designations guarantee the distinctive characteristics of the wine [3]. These areas are known for their predominant environmental attributes, including their climate, soil, and the types of grapevines cultivated. However, numerous studies have questioned the viability of major viticulture regions under future climatic forecasts [4–7]. Grapevine is a phenotypically flexible plant in order to respond to environmental stimuli and biotic/abiotic conditions, and, in this sense, this fact impacts its metabolic outputs and the composition of the resulting berries and wine [8]. Among the different effects associated with climate change, early cultivar ripening, a generalized advance of harvest dates [9,10], and an excess of grape ripening can be highlighted. This excessive ripening results in the production of must with a higher potential alcohol content, elevated pH levels, reduced acidity, and significant nutritional deficiencies [2,11,12]. The challenges posed by global warming on grapes have notable implications for the winemaking process and the creation of high-quality wines. These influences impact the presentation of varietal scents and the chemical and

microbiological stability, as well as the sensory equilibrium [11]. To address these challenges, Sancho-Galán et al. [13] recommended exploring new winemaking techniques as a strategy for adapting to climate change, particularly in warm regions. This approach aims to align with current trends and the expectations of wine consumers, seeking to provide new sensory experiences (experiential marketing) [14]. Consequently, emerging market trends emphasise the search for a typical character in wines, which directly contributes to the recovery of ancient techniques such as grape overripening [15].

DO Jerez-Xérès-Sherry is the southernmost wine region in Europe. This region is considered to be an area with a warm climate and is characterized by dry and sweet fortified wines from mainly three grapevine varieties: Palomino Fino, Pedro Ximénez, and Muscat of Alexandria [16]. For sweet wine production, the Pedro Ximénez and Muscat of Alexandria cultivars are used and their grape musts are fortified with alcohol to stop alcoholic fermentation, which allows for the production of mistelas (a liqueur made with a mixture of grape juice and alcohol) or natural sweet wines [17]. In other DO wine regions, another type of sweet wines, known as naturally sweet wines, are also produced [18,19], where no fortification is carried out and, in this case, all alcohol content is produced exclusively from the alcoholic fermentation. However, this type of wine is hardly produced at present in the different wine-growing regions of southern Spain.

In warm regions with dry summers, the conditions established by climate change (high radiation and temperatures) could be considered as an advantage for grape overripening procedures and making wines without alcohol addition (fortification). In this sense, there are previous works published on dry white wines made with overripening grapes from the Palomino Fino cultivar (the main grapevine cultivar in SW Spain) [20], also fermented with or without the presence of their skins [13,20], also with the aim to develop new types of wine and cover the demands of consumers with new products with differential organoleptic characteristics [21].

In view of the above precedents, the aim of this research was to study the viability of new production processes for naturally sweet wines that allow for obtaining new wines and expanding and diversifying the production of wines in warm climate zones like SW Spain. This work presents a study, for the first time, on the elaboration of naturally sweet white wines from the 'Pedro Ximénez' grapevine cultivar fermented with or without the presence of grape skins.

2. Materials and Methods

2.1. Raw Material

The raw material was 'Pedro Ximénez' grape berries, handpicked from a vineyard situated at 36°52′46″ N, 6°11′47″ W in the municipality of Trebujena (Cádiz, Spain). Fertilization and irrigation were not employed in the vineyard, and conventional phytosanitary products were used to ensure proper grape development. As an overripening method, the technique called asoleo or sun drying was employed, in which the grapes were spread out under the sun in a single layer. The grapes were dried for 11 days. After reaching overripeness, the grapes were manually destemmed and pressed using a vertical press (MECAMAQ M030, Mollerussa, Spain) at a pressure of 50 bars. Once grape must was obtained, and it underwent acidification using tartaric acid (Agrovin, Ciudad Real, Spain) and an additional 80 mg/L of potassium metabisulphite (Agrovin, Ciudad Real, Spain). The grape must showed the following characteristics after correction: pH = 3.45 ± 0.01, 21 °Bé (sugar concentration), total acidity of 5.43 ± 0.18 g L^{-1} of tartaric acid, Free Amino Nitrogen (FAN) = 195 ± 0 mg/L, malic acid concentration of 0.63 ± 0.00 g L^{-1}, and 0.21 ± 0.00 g L^{-1} of gluconic acid. After all the pre-fermentative corrections were performed, the grape must was divided and distributed in stainless steel 5 L tanks. Mistelas (M) and Naturally Sweet Wines (NSW) were elaborated with grape skins presence (SP) and without them (SA). Thus, four types of elaborations were made: M_SA, M_SP, NSW_SA, and NSW_SP. To make the mistelas, wine alcohol at 96.2% (*v*/*v*) was added to reach a final alcohol content of 18% *v*/*v*, as is conventionally performed for this type of liqueur. An optimal dose of 20% (*w*/*v*) grape

skins was added to the musts fermented with skins and Mistelas (M_SP), according to a previous work [20]. For the Naturally Sweet Wines' alcoholic fermentation, an Actiflore BO213 (Laffort Inc., Bordeaux, France) *Saccharomyces cerevisiae* yeast was employed at 15 g hL^{-1}. The fermentations were carried out under controlled conditions at 22 °C for 21 days and were stopped in a natural way, without adding alcohol. Once the alcoholic fermentation finished, grape skins and lees were removed, and potassium metabisulphite was added up to 200 mg L^{-1}.

2.2. Analytical Methodology

A physicochemical assessment of the grape must, including the pH, total acidity, and °Bé, followed the protocols outlined by the International Organization of Vine and Wine (OIV) [22]. The quantification of Free Amino Nitrogen (FAN) was conducted using the method proposed by Abernathy et al. [23].

Alcoholic fermentation monitoring involved measurements of the viable biomass, density, and FAN levels. Density measurements were performed using a DMA 5000 M densimeter from Anton Paar (Graz, Austria). Viable biomass counts were carried out using an optical Leica CME Microscope (Houston, TX, USA) and the methylene blue staining technique within a Neubauer chamber (Brightline, Germany). Wine analytical assessments, including total acidity and alcohol content, followed the established procedures outlined by the OIV [22]. The residual sugar content was assessed using the dinitrosalicylic acid (DNS) method, as outlined in the procedure by Gonçalves et al. [24]. To determine the organic acid levels, ionic chromatography was employed, using a Metrohm 930 compact IC Flex ionic chromatograph with a conductimetric detector and a Metrosep organic acids column (250 × 7.8 mm; Herisau, Switzerland). The separation of organic acids was accomplished using a 0.4 mM H_2SO_4 solution in a 12% acetone mixture as the eluent, at a at a constant flow rate of 0.4 mL/min. The different organic acids (tartaric acid, acetic acid, lactic acid, succinic acid, and malic acid) were identified by comparing their retention times and analytical signals against those of the commercially available standards (Sigma Aldrich, Barcelona, Spain). Each one of them was quantified using a calibration curve constructed at five concentration points from its commercial standard.

A duplicate analysis of low-molecular-weight polyphenols and furanic compounds individually was conducted using an Acquity UPLC system from Waters Corporation (Milford, MA, USA), which was equipped with a diode array detector. The methodology that was previously optimized by Schwarz et al. [25] was followed. Individual analyses of low-molecular-weight polyphenols and furanic compounds were carried out, in duplicate, by the means of an Acquity UPLC system (Waters Corporation, Milford, MA, USA), equipped with a diode array detector, following the methodology previously optimized by Schwarz et al. [25]. Different commercial standards from Fluka (Buchs, Switzerland) and Sigma (St. Louis, MO, USA) were employed for identification and quantification purposes. The quantification of the identified compounds was performed using external calibration, using five levels of concentration for each compound and covering the expected range for each one. Benzoic acids were measured at a wavelength of 280 nm, while cinnamic acids were quantified at 320 nm. For *p*-Hydroxybenzoic acid, the quantification was performed at 255 nm.

2.3. Statistical Analysis

Significant differences among the samples were assessed using a two-way ANOVA, following Bonferroni's multiple range (BSD) test at a significance level of $p < 0.05$. The statistical analysis was conducted using the GraphPad Prism version 8.0.1 (GraphPad Software, San Diego, CA, USA) statistical package for Windows.

3. Results and Discussion

3.1. Effect of Grape Skin Presence on Alcoholic Fermentation

Along the alcoholic fermentation, some basic oenological parameters were considered and measured: viable yeast population, relative density, and Free Amino Nitrogen (FAN) content. Figure 1 shows the evolution of the viable yeast population during the alcoholic fermentation process with (SP) and without (SA) the presence of grape skins.

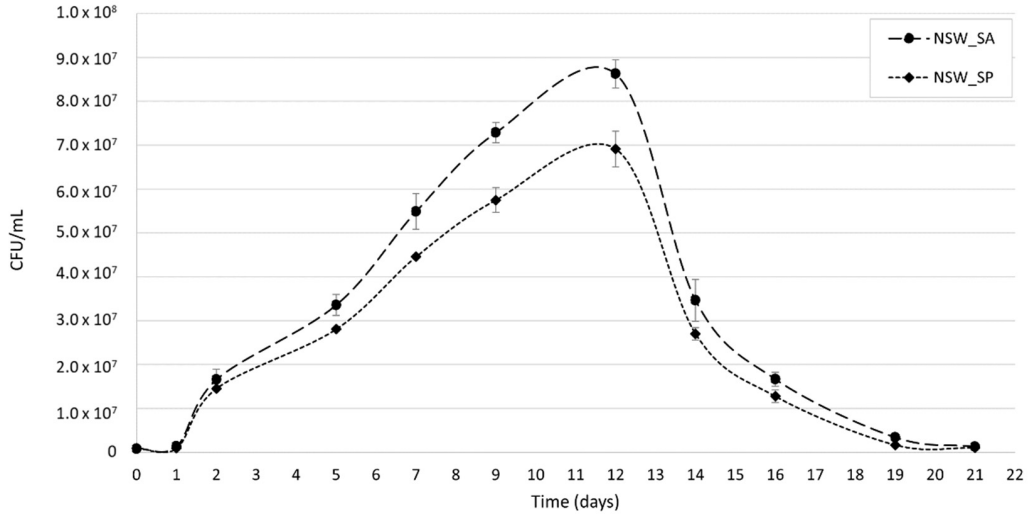

Figure 1. Viable biomass during 'Pedro Ximénez' grape must alcoholic fermentation with and without the presence of grape skins. NSW_SP: Naturally Sweet Wine with Skins Presence. NSW_SA: Naturally Sweet Wine with Skins Absence. CFU: Colony-Forming Units.

During the first days after the inoculation, no significant growth was observed in the yeast populations of each fermentation tank. This fact, as expected, can be explained considering the characteristic lag phase in which yeasts adapt to the conditions of a medium before multiplying to a large extent [26].

A short latency phase was observed in all cases (two days). This fact could have been due to the dehydration process during the "asoleo" stage, leading to a high increase in the sugar concentration and FAN initial values in yhr grape must. These high values of nitrogen compounds could be responsible for shortening the lag phase, as has been observed by other authors in trials for producing sweet wines [18]. Moreover, this effect could have been accentuated due to the low volume used at the laboratory scale (5 L). From day 5 on, there was a slight drop in the fermentation kinetics slopes for musts with the presence of skins (SP) compared to those fermented with the absence of skins (SA; significant differences at $p < 0.05$). Comparing the two fermentations, significant differences were observed in the fermentation speed. On the one hand, NSW_SA showed an FAL rate of 1.062 ± 0.030 g $\times$ mL^{-1}/day, while NSW_SP showed 0.978 ± 0.028 g $\times$ mL^{-1}/day. The presence of grape skins reduced the biomass growth rate by 28% compared to the musts without skins. It is likely that the polyphenols polymerized during the "asoleo" stage, present in greater amounts in those musts fermented with skins, were extracted during alcoholic fermentation and contributed with the inhibitions that slowed down the rate of biomass development. The polyphenolic compounds could affect the yeast growth kinetics due to the interactions between polyphenols and yeast plasma membrane and, in this sense, have effect on yeast metabolism [27]. Thus, an inhibitory effect of these compounds on the activity of the membrane enzyme H^+-ATPase during the initial phase of the fermentation could explain this phenomenon [28] The maximum viable biomass populations were reached on day 12 in both vinifications, reaching 25% more viable yeasts in grape musts without the presence of grape skins. This fact would support the hypothesis suggested above. From this maximum population, a similar downward slope was observed in both cases, but with significantly higher viable yeast populations in wines without the

presence of skins (ANOVA, $p < 0.05$). These differences became less pronounced as the end of alcoholic fermentation was reached.

Another parameter considered was the relative density. Figure 2 shows the evolution of the relative density during alcoholic fermentation with and without grape skins.

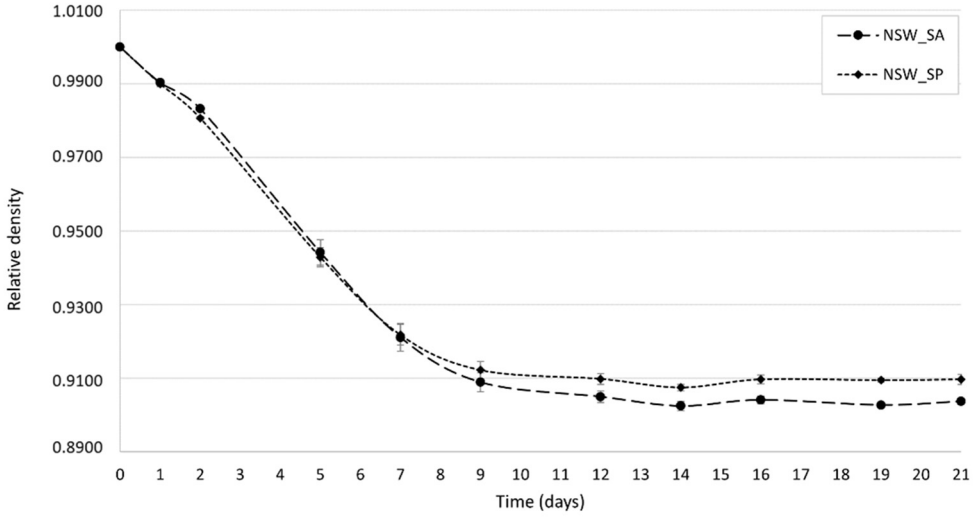

Figure 2. Evolution of the relative density throughout the alcoholic fermentation of "Pedro Ximénez" grape must with and without the presence of skins. NSW_SP: Naturally Sweet Wine with Skins Presence. NSW_SA: Naturally Sweet Wine with Skins Absence.

According to the evolution of the viable yeast biomass, the grape musts with the lowest density values were those in which the fermentation was carried out in the absence of grape skins, consistent with the values observed for the viable yeast population (Figure 1). However, in the case of this parameter, during the first days of the alcoholic fermentation process, no statistically significant differences were observed with respect to those fermented with grape skins. This fact could have been due to the low tolerance of the yeasts to a high osmotic pressure considering the high sugar content of musts [29]. High osmotic pressures [29,30], together with the presence of high levels of polyphenolic compounds in the must, could have been responsible for the fermentation slowing down at the beginning for musts fermented with grape skins (Figure 1) [31]. Subsequently, in grape skins with the absence of musts (NSW_SA), there was a significantly greater decrease in relative density on those days (from day 9) when the yeasts reproduced faster ($p < 0.05$; Figure 1), coinciding with the tumultuous phase of the fermentation process, given that, in this phase, the transformation of sugars into ethanol occurs at maximum speed [32]. As expected, this behaviour agreed with that observed in the evolution of the viable biomass, where wines fermented without skins (NSW_SA) showed the highest fermentative biomass evolution, reaching a 9% decrease in density values compared to those for wines fermented with skins (NSW_SP).

In relation to FAN evolution, Figure 3 shows its evolution for all alcoholic fermentations, both for those with and without skins in the fermentation medium.

Generally, in all cases, the FAN values' evolution was in accordance with the viable biomass and relative density behaviour observed (Figures 1 and 2). At the beginning of fermentation (from day 1 to day 5), there were significant differences between the samples depending on the presence or absence of grape skins (ANOVA, $p < 0.05$), showing higher values for those musts fermented in the presence of skins, which could be explained by their contribution [32].

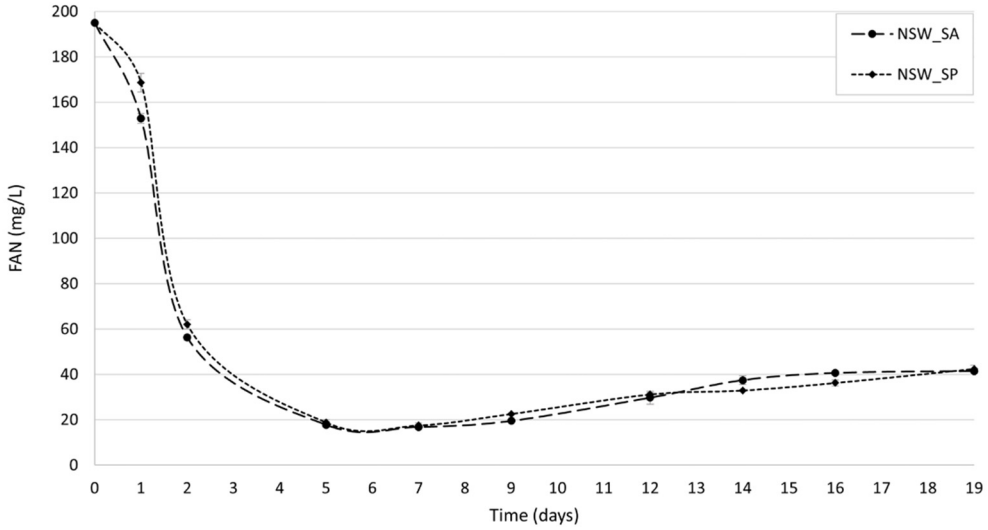

Figure 3. Evolution of free amino nitrogen (FAN) concentration during alcoholic fermentation of grape musts without and with the presence of skins. NSW_SP: Naturally Sweet Wine with Skins Presence. NSW_SA: Naturally Sweet Wine with Skins Absence.

The FAN content decreased from the first days after the yeast inoculation in the fermentation media in all cases, reaching a 91% decrease, corresponding to the exponential phase of biomass growth (Figure 1). The minimum FAN values (maximum consumption) were reached between days 5 and 7 (Figure 3), slightly before reaching the maximum population values (Figure 1). Possibly, this behaviour was influenced by the conditions of the high sugar content in grape juice. When the minimum FAN concentration was reached, there was a slight general increase in both fermentation styles, regardless of the presence or absence of grape skins, with significantly higher levels found in those fermentations with skins absence at the end of the process (days 14–18; ANOVA, $p < 0.05$). This phenomenon could be explained, on the one hand, by the effect of yeast autolysis in both cases that would release nitrogen-enriched compounds such as proteins and some free amino acids into the medium [33], and, on the other hand, due to the increase in biomass experienced in those musts fermented without skins (Figure 1) in comparison to those fermented with skins. An average increase in FAN levels of 13.0% was observed in grape musts fermented without skins in comparison to those fermented with grape skin presence. The final FAN values in the wines ensured that these were stable from a microbiological point of view, considering that these values were less than 60 mg L^{-1}, values for which the development lactic and acetic bacteria can be a risk factor.

3.2. Effect of Grape Skins Presence on the Physicochemical Composition of Mistelas and Final Wines

Table 1 shows the mistelas and final wines physicochemical characterization made from overripe grapes in grape skins presence (SP) or absence (SA).

Throughout the alcoholic fermentation metabolic pathway, the sugar in grape must is transformed into ethanol and other by-products by yeasts. However, in this metabolic process, these microorganisms are exposed to a wide range of stressors, including high sugar concentrations and ethanol accumulation when the fermentation progress starts [34]. Wines elaborated without grape skins (SA) presented a higher alcohol content and lower residual sugar concentration compared to wines made with grape skins presence (SP). These differences could be related to the polyphenolic compounds release contained in the grape skins during alcoholic fermentation, which could have induced significant modifications in yeast metabolism, resulting in a significant impact on fermentation kinetics [28]. Mekoue-Nguela et al. [28] found that the polyphenol presence in the fermentation medium resulted in a significant decrease in yeast growth, a lower CO_2 production rate, and a

lower nitrogen consumption, which led to longer fermentation times, both in stressed and unstressed conditions.

Table 1. Physicochemical and polyphenolic characterization of mistelas and final naturally sweet wines.

	M_SA	M_SP	NSW_SA	NSW_SP
Parameter	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
% Alc.	18.04 ± 0.00 ^{a}	18.01 ± 0.00 ^{a}	13.14 ± 0.19 ^{b}	11.43 ± 0.7 ^{b}
RS (gL^{-1})	353.44 ± 12.01 ^{a}	336.27 ± 3.11 ^{a}	156.78 ± 0.49 ^{b}	172.25 ± 1.8 ^{b}
Tartaric acid (gL^{-1})	1.010 ± 0.00 ^{b}	0.886 ± 0.00 ^{c}	1.69 ± 0.05 ^{a}	1.58 ± 0.04 ^{a}
Acetic acid (gL^{-1})	0.53 ± 0.00 ^{b}	0.53 ± 0.00 ^{b}	1.94 ± 0.05 ^{a}	1.95 ± 0.01 ^{a}
Malic Acid (gL^{-1})	0.65 ± 0.00 ^{b}	0.61 ± 0.00 ^{b}	0.91 ± 0.01 ^{a}	0.90 ± 0.01 ^{a}
Lactic Acid (gL^{-1})	0.02 ± 0.00 ^{b}	0.02 ± 0.00 ^{b}	0.07 ± 0.01 ^{a}	0.08 ± 0.01 ^{a}
Succinic acid (gL^{-1})	0.03 ± 0.00 ^{b}	0.03 ± 0.00 ^{b}	0.28 ± 0.01 ^{a}	0.31 ± 0.01 ^{a}
Glycerine (gL^{-1})	4.24 ± 0.09 ^{c}	5.86 ± 0.05 ^{c}	18.65 ± 1.28 ^{a}	16.55 ± 0.20 ^{b}
Gallic acid (mgL^{-1})	17.70 ± 0.10 ^{a}	16.20 ± 0.44 ^{a}	14.87 ± 0.19 ^{a}	17.26 ± 2.17 ^{a}
HMF (mgL^{-1})	1.89 ± 0.00 ^{c}	2.35 ± 0.04 ^{a}	2.05 ± 0.01 ^{b}	2.06 ± 0.04 ^{b}
Protocatechuic acid (mgL^{-1})	6.30 ± 0.04 ^{c}	4.27 ± 0.04 ^{d}	6.98 ± 0.10 ^{b}	8.40 ± 0.70 ^{a}
Furoic acid (mgL^{-1})	1.41 ± 0.07 ^{a}	1.32 ± 0.02 ^{a}	1.44 ± 0.01 ^{a}	1.48 ± 0.05 ^{a}
Furfural (mgL^{-1})	0.32 ± 0.03 ^{b}	0.37 ± 0.03 ^{b}	0.41 ± 0.00 ^{a}	0.42 ± 0.02 ^{a}
p-Hydroxybenzoic acid (mgL^{-1})	1.91 ± 0.00 ^{b}	0.03 ± 0.00 ^{c}	2.23 ± 0.03 ^{a}	2.52 ± 0.16 ^{a}
Tyrosol (mgL^{-1})	1.76 ± 0.32 ^{b}	1.66 ± 0.43 ^{b}	4.46 ± 0.05 ^{a}	5.99 ± 3.39 ^{a}
Catechin (mgL^{-1})	2.75 ± 1.51 ^{c}	4.73 ± 0.59 ^{b}	4.22 ± 0.60 ^{b}	5.48 ± 0.43 ^{a}
Syringic acid (mgL^{-1})	2.51 ± 0.12 ^{c}	2.00 ± 0.12 ^{d}	3.42 ± 0.08 ^{b}	9.22 ± 0.15 ^{a}
p-Coumaric acid (mgL^{-1})	4.15 ± 0.34 ^{ab}	4.39 ± 0.21 ^{a}	2.73 ± 0.10 ^{c}	2.92 ± 0.53 ^{bc}
cis-*p*-Coutaric acid (mgL^{-1})	4.43 ± 0.19 ^{b}	5.69 ± 0.10 ^{a}	4.25 ± 0.03 ^{b}	2.73 ± 0.23 ^{c}
GRP (mgL^{-1})	7.69 ± 0.23 ^{a}	7.08 ± 0.32 ^{a}	7.63 ± 0.50 ^{a}	6.34 ± 0.71 ^{a}
2,4-Dihydroxybenzoic acid (mgL^{-1})	1.63 ± 0.17 ^{b}	2.24 ± 0.08 ^{a}	2.29 ± 0.42 ^{a}	2.80 ± 0.96 ^{a}
Caffeic acid (mgL^{-1})	3.90 ± 0.19 ^{a}	3.58 ± 0.12 ^{a}	3.22 ± 0.23 ^{ab}	2.69 ± 0.32 ^{b}
Ethyl caffeate (mgL^{-1})	1.27 ± 0.02 ^{a}	0.82 ± 0.01 ^{b}	1.02 ± 0.02 ^{a}	1.24 ± 0.30 ^{a}
Ethyl *p*-coumarate (mgL^{-1})	0.80 ± 0.04 ^{a}	0.56 ± 0.01 ^{b}	0.61 ± 0.04 ^{b}	0.65 ± 0.08 ^{b}
Ethyl gallate (mgL^{-1})	0.29 ± 0.01 ^{c}	0.28 ± 0.05 ^{c}	0.49 ± 0.08 ^{b}	0.79 ± 0.07 ^{a}

% Alc.: Alcohol; RS: Residual Sugars; HMF: 5-Hydroxymethylfurfural; GRP: 2-S-glutathionyl caftaric acid. Different superscript letters for each row mean significant differences between the samples (ANOVA $p < 0.05$) determined by two-way ANOVA and applying Bonferroni multiple range (BSD) test. M_SA: Mistela with Skin Absence. M_SP: Mistela with Skin Presence. NSW_SA: Naturally Sweet Wine with Skins Absence. NSW_SP: Naturally Sweet Wine with Skins Presence.

In the present work, when low-molecular-weight polyphenols and furans were submitted to an ANOVA study considering fermentation and skins as possible significant factors, the main significant factor was "fermentation". Polyphenol extraction during alcoholic fermentation takes place as a consequence of the degradation of the cell wall pectin layer and depends on the grape ripeness degree [35]. In the present work, as could be expected, higher concentrations for *p*-hydroxybenzoic acid, protocatechuic acid, furfural, catechin, and syringic acid were found in the fermented wines with respect to mistelas (Table 1).

In relation to the presence of grape skins, in general, its effect was not significant, with only some low-molecular-weight polyphenols at higher concentrations in those wines fermented with grape skins (NSW_SP) (protocatechuic acid, catechin, syringic acid, and ethyl gallate; Table 1; $p < 0.05$). These low releases from the skins could be explained by the previous sun-drying stage, which could have induced significant changes/losses/polymerizations in the grapes' phenolic composition [36]. Another possible explanation could be that the phenolic compounds considered in this study, hydroxycinnamic and benzoic acids (the main phenolic compounds in white grapes), are located mainly in the pulp, so the contact with skins during alcoholic fermentation would not modify their presence in wines. Catechins and proanthocyanidins are the phenolic compounds

found mainly in grape skins. As can be seen in Table 1, the catechin concentrations were higher in those wines elaborated with grape skins.

Another parameter of interest in alcoholic fermentation is the content in organic acids. Most of them are produced during alcoholic fermentation and their final content may depend both on the specific conditions under which the yeasts transform sugar into alcohol and even on the yeast strain used to carry out this stage of winemaking [37].

In both cases (NSW_SA and NSW_SP), and from a general point of view, the tartaric acid concentration was slightly higher, but not significantly, in wines made with grape skins absence (NSW_SA) compared to those with their presence (NSW_SP). These slight differences could be related to the release of the potassium contained in grape skins, which would lead to a higher precipitation of this acid as potassium bitartrate [38]. Similarly, in some previous studies, slightly lower values for tartaric acid have been observed in white wines fermented with grape skins presence [37], and the same results were also observed by Sancho-Galán et al. [13] in a study on Pedro Ximénez sweet wines where dynamic prefermentative maceration was employed. Comparing the mistelas, M_SP presented significantly higher values than M_SA ($p < 0.05$). Since no alcoholic fermentation was carried out in these cases, this could only have been due to the release of this acid by the skins during maceration. Regarding acetic acid, as expected, both mistelas showed very similar and low acetic acid values. For the wines in both cases, the content was higher, something similar to that observed by Roca-Domènech et al. [39] and Yang et al. [40] in sweet white wines production. This acid is the main component of volatile acidity, so it has a strong influence on wine quality [41]. High concentrations of acetic acid in sweet wines are not considered to be a sensory and microbiologically risk [42], considering that this type of wine needs a high level of total acidity to balance its sweetness. In overripe grape winemaking procedures, this acid can be produced in considerable amounts during alcoholic fermentation, depending on the yeast species and strains employed [43]. The *Saccharomyces cerevisiae* yeast strain produces this acid as a by-product of alcoholic fermentation in response to high-osmolarity conditions due to the medium–high sugar concentration [44]. In relation to the malic acid, lactic acid, and succinic acid concentrations, as was previously observed for acetic acid, no significant differences were found between both mistelas and both wines fermented with and without skins (ANOVA, $p < 0.05$). Malic acid is normally consumed by the metabolic activity of certain wine microorganisms through malolactic [45] and malo-alcoholic [46] fermentation. It is worth mentioning that a significantly higher content in the wines than in the initial grape must was observed. This could have been due to the production of malic acid during alcoholic fermentation, via two possible metabolic pathways [47]: via fumarate catalysed by cytosolic or mitochondrial fumarase or via oxaloacetic acid catalysed by malate dehydrogenase (MDH) [48]. In relation to lactic acid, it is mainly a product of a biochemical process called malolactic fermentation, which is usually carried out in red and some white wines. This biochemical pathway consists of the enzymatic decarboxylation of malic acid by lactic acid bacteria [49]. From a general point of view, these wines presented a rather low lactic acid concentration, with no significant differences between the different winemaking processes (ANOVA, $p < 0.05$). Therefore, this fact suggests that the wines did not show any evidence of malolactic fermentation development [50], and, consequently, the amount of this acid present in the wines was derived from the alcoholic fermentation. Regarding succinic acid, the osmotic stress produced by high sugar media during alcoholic fermentation induces the transcription of the genes involved in the production of succinic acid from glutamate [51]. The succinic acid concentrations were in concordance with those obtained by Roca-Domènech et al. [39] in a study on sweet wines fermented with two osmotolerant *Saccharomyces cerevisiae* strains.

With respect to the glycerine concentrations, it could be observed that, in NSW_SA and NSW_SP, high concentrations were obtained with respect to mistelas. Ruiz et al. [52] also observed high glycerine levels as a consequence of osmotic stress, where yeasts were subjected in a partially fermented Pedro Ximénez wine. Additionally, NSW_SA

wines exhibited a higher glicerine concentration than the NSW_SP ones, with a significant difference (ANOVA, $p < 0.05$). This difference was not observed by Nadai et al. [53] in a study on the alcoholic fermentation of dried grapes of the 'Raboso Piave' white grapevine cultivar using two different yeast strains (*Starmerella bacilaris* and *Saccharomyces cerevisiae*) and grape skins maceration. This compound seems to be a metabolite synthesized by yeast to equilibrate the osmotic pressure in cells during wine fermentation with a high sugar content [52].

4. Conclusions

In terms of fermentation kinetics, this process started slowly due to the high osmotic conditions in the medium. However, grape overripening did not influence the yeast lag phase. From a physico-chemical point of view, the alcoholic fermentation process increased the concentration of important organic acids and polyphenols with respect to mistelas. It should be noted that neither sun drying nor the presence of grape skins were factors responsible for the significant deviations in the parameters considered with respect to the usual oenological criteria for sweet wines. Grape skins presence reduced the biomass growth rate and gave rise to lower final FAN and alcohol concentrations, without significant modifications in the organic acids and low-molecular-weight polyphenols contents.

In view of the results obtained, the production of naturally sweet wines from overripe grapes, with either grape skins presence or absence during alcoholic fermentation, should be considered as a viable option for producing new sweet wines in areas where agro-climatic conditions make it possible.

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