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Innovative "Soft" Maceration Techniques in Red Grape Fermentation

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Abstract: Two innovative soft maceration techniques of vinification based on red grape Cabernet Sauvignon were compared in 2020 and 2021 vintages with the most used system of maceration (pump-over and delestage) in today's wine sector in order to verify the efficiency in polyphenol extraction and fermentation rate. Fermentation kinetics and final wine characteristics were evaluated as the main parameters for comparing the systems. The AIR MIX (AIRMIXING M.I.™) technique is based on the use of a fixed sequential small injections of compressed air (3 jets) from the bottom of the tank, aimed at creating waves (resonance waves) able to prevent the cap formation. The ADCF (NECTAR-ADCF™) technique uses the overpressure produced by carbon dioxide in the wine tank during alcoholic fermentation to keep the cap submerged and to favor its disruption by the CO_2 outside release through a valve. As a reference, the control vinification consisted of the use of "delestage" and pump-over to facilitate the extraction and good management of the cap. ADCF, at the end, extracted a greater quantity of polyphenols and anthocyanins. AIR MIX speeded up the fermentation, which ended 4–7 days before the control and, initially, provoked a greater extraction of phenols and anthocyanins as ADCF. By the end, the concentration of polyphenols and anthocyanins was the highest in ADCF, followed by control and AIR MIX approximately at the same amount. The control wine had a slightly higher volatile acidity. AIR MIX consumed more than 60% less energy because the nonuse of pump-over and delestage, and also, no personnel was requested.

Keywords: maceration; extraction; ADCF™; AIR MIXING™; alcoholic fermentation; phenolic compounds

Citation: Pettinelli, S.; Pardini, L.; De Angeli, G.; Bianchi, A.; Najar, B.; Cerreta, R.; Bellincontro, A.; Floridia, G.; Mencarelli, F. Innovative "Soft" Maceration Techniques in Red Grape Fermentation. *Beverages* **2022**, *8*, 62. https://doi.org/10.3390/beverages8040062

Academic Editor: António Manuel Jordão

Received: 7 August 2022
Accepted: 2 October 2022
Published: 6 October 2022

1. Introduction

In a highly competitive wine market, wineries need to invest in technology in order to increase productivity and to improve the wine quality [1].

Polyphenols are secondary plant metabolites that are implicated in a number of varied roles, including UV protection, pigmentation, disease resistance, and nodule production [2,3]. Polyphenols characterize body, color, and some of the main organoleptic attributes in red wines [4–6]. Wine polyphenols can be extracted from grapes and wood, or they can be metabolized by yeasts [7]. Grape juice fermentation is a critical stage in wine production, and maceration coupled to fermentation is the most crucial step of red wine vinification [8]. Transfer of polyphenols from berry skin to liquid during maceration/fermentation depends on various factors: chemical and physical ones [9,10]. The phenomena are complex and are not limited to a regular increase in extracted substances [11,12]. During a traditional fermentation on the grape skins, the alcohol content, carbon dioxide, and sulfur dioxide, together with the heat of fermentation, increase the permeability of cell membrane. Other mechanical or physical treatments that destroy the cell membranes and walls may also increase the release of these pigments. Two factors limiting the extraction

are the solubility and the instability as for anthocyanins and tannins [13]. The early peak and subsequent decline in anthocyanins during fermentation is inconsistent with solubility being the limiting factor for these compounds and instead reflects their instability [14]. Anthocyanins are very important for the visual red wine properties, but they also play a role through their interactions, reactions, and combinations with the other wine phenols, such as tannins and flavonols [15]. Other components such as proanthocyanins or tannins relate to the mouthfeel attributes, bitterness, and astringency, and they influence mouthfeel perception [16,17]. In the first step of cell degradation after crushing operation, cell wall enzymatic activity plays an important role [18] because the cell membrane and wall must be fractioned and not only collapsed since, in the latter case, membrane structure residues could retain phenolic components [19]. Enzyme activity provokes the deconstruction of cell walls polysaccharide networks, permitting the other factors to proceed with the extraction during maceration and fermentation [20–22], but this activity is strongly dependent on the internal temperature of the tank. Alternating temperature in the postharvest cooling treatment of Fiano and Falanghina wine grapes affects the cell wall enzyme rate, berry softening, and polyphenol [23]. A great deal of research has been carried out on these aspects to facilitate and optimize substances translocation from solid to liquid medium and on the environmental factors that determine its best outcome [24].

During the traditional red wine production, grape skins form a floating cap supported by the carbon dioxide released during fermentation, which inhibits an efficient yeast fermentation and skin maceration. Therefore, this cap should be broken down to submerge berry skins into the fermenting juice typically a few times a day [25]. In this context, various alternative systems and techniques, either soft (use of gas movement) or hard (mechanical movement), have been developed in order to reduce the time and labor cost, providing better phenolic extraction [16,26].

On this basis, the aim of this study was to compare the traditional and very invasive technique, using pump-over and delestage (named here as hard), with two innovative techniques (soft) to manage the fermentation: AIR MIX and ADCF^{TM}. The first one is an air-modulated injection using the resonance wave physical law to prevent the cap formation, while the second one keeps a slight overpressure of CO_2 released by alcoholic fermentation through an accurate pressure sensor and employs this overpressure together with a sudden pressure dropping by a valve opening to disrupt the cap. Our hypothesis is that the soft techniques provide better polyphenol extraction at lower energy and personnel cost because they maintain a more uniform temperature and a continuous leaching activity in the tank by circulating the must (AIR MIX) or by keeping the cap immersed and by a soft but quick movement due to the sudden change in pressure (ADCF).

2. Materials and Methods

2.1. Materials, Fermentation, and Maceration Protocol

Experimental tests were conducted in 2020 and 2021 vintages with grapes coming from the same vineyard and adopting the same protocols for both years.

Bunches of red grape variety cv Cabernet Sauvignon (*Vitis vinifera* L.) were hand harvested upon Famiglia Cotarella (Montecchio, TR, Italy). After harvest, grapes were sorted for absence of visual defects and, on the basis of uniform color, immediately shipped to the department to carry out all the analytical determinations (Tables 1 and 2).

Table 1. Eno-chemical characteristics of Cabernet Sauvignon grape at harvest and the three wines at the racking for the 2020 year. The data are expressed as mean (±SD) of three set of berries at harvest and three 500 mL bottles, each one taken immediately after wine mixing in the tank. Different letters in a row for the wine comparison indicate statistically different data (Tukey, $p \leq 0.05$). n.d., not detected; n.m., not measured.

Parameters	Unit	Grape at Harvest 2020	Control	ADCF	AIR MIX
Alcohol	% V/V	n.d.	14.77 ± 0.23 a	14.64 ± 0.14 a	14.62 ± 0.17 a
Sugars	g/L hexoses	244.5 ± 4.1	0.35 ± 0.13 b	1.03 ± 0.11 a	0.78 ± 0.19 a
pH		3.47 ± 0.09	3.84 ± 0.05 a	3.79 ± 0.03 a	3.81 ± 0.05 a
Titratable acidity	g/L tartaric acid	6.14 ± 0.17	5.72 ± 0.07 a	5.36 ± 0.05 b	5.31 ± 0.07 b
Volatile acidity	g/L acetic acid	n.d.	0.51 ± 0.02 a	0.27 ± 0.01 c	0.33 ± 0.02 b
Malic acid	g/L	1.34 ± 0.11	0.92 ± 0.06 b	1.20 ± 0.09 a	0.90 ± 0.06 b
Lactic acid	g/L	n.d.	0.25 ± 0.09 a	0.18 ± 0.05 a	0.24 ± 0.07 a
Tartaric acid	g/L	4.71 ± 0.19	3.63 ± 0.13 a	3.47 ± 0.21 a	3.51 ± 0.18 a
Citric acid	g/L	0.17 ± 0.06	0.14 ± 0.03 a	0.13 ± 0.02 a	0.16 ± 0.02 a
Total extract	g/L	271.3 ± 5.1	35.9 ± 1.9 a	33.3 ± 2.4 ab	31.0 ± 1.7 b
Ash	g/L	2.02 ± 0.11	1.71 ± 0.05 a	1.82 ± 0.08 a	1.82 ± 0.04 a
YAN	mg/L	202 ± 9	63 ± 2 c	91 ± 3 a	85 ± 2 b
Total anthocyanins	mg/L malvidin	745 ± 23	475 ± 16 b	520 ± 15 a	480 ± 19 b
Total polyphenols	mg/L gallic acid	3140 ± 123	2388 ± 103 b	2773 ± 92 a	2300 ± 128 b
Color intensity		n.m.	0.77 ± 0.09 b	0.99 ± 0.05 a	0.81 ± 0.07 b
Tonality		n.m.	0.82 ± 0.07 b	0.98 ± 0.06 a	0.88 ± 0.04 b

Table 2. Eno-chemical parameters of Cabernet Sauvignon grape at harvest and of the three wines for the 2021 year. Data are expressed as mean (±SD) of three set of berries at harvest and three 500 mL bottles, each one taken immediately after wine mixing in the tank. Different letters in a row for the wine comparison indicate statistically different data (Tukey, $p \leq 0.05$). n.d., not detected; n.m., not measured.

Parameters	Unit	Grape at Harvest 2021	Control	ADCF	AIR MIX
Alcohol	% V/V	n.d.	14.76 ± 0.21 a	14.90 ± 0.24 a	14.78 ± 0.26 a
Sugars	g/L hexoses	247.5 ± 3.2	3.73 ± 0.14 a	2.05 ± 0.11 b	1.80 ± 0.19 b
pH		3.38 ± 0.07	3.55 ± 0.07 b	3.68 ± 0.03 a	3.64 ± 0.05 a
Titratable acidity	g/L tartaric acid	7.10 ± 0.23	7.02 ± 0.09 a	6.42 ± 0.05 b	6.49 ± 0.07 b
Volatile acidity	g/L acetic acid	n.d.	0.31 ± 0.03 a	0.19 ± 0.01 c	0.24 ± 0.02 b
Malic acid	g/L	1.87 ± 0.13	0.95 ± 0.02 b	1.14 ± 0.09 a	1.20 ± 0.06 a
Lactic acid	g/L	n.d.	0.21 ± 0.05 a	0.19 ± 0.05 a	0.12 ± 0.07 a
Tartaric acid	g/L	5.82 ± 0.23	5.63 ± 0.06 a	5.47 ± 0.02 b	5.51 ± 0.02 b
Citric acid	g/L	0.22 ± 0.08	0.20 ± 0.06 a	0.23 ± 0.02 a	0.21 ± 0.02 a
Total extract	g/L	279.4 ± 3.2	35.3 ± 2.8 a	35.2 ± 1.7 a	33.2 ± 2.9 a
Ash	g/L	2.25 ± 0.08	2.11 ± 0.04 a	2.02 ± 0.08 ab	1.97 ± 0.04 b
YAN	mg/L	253 ± 5	73 ± 5 b	87 ± 3 a	80 ± 6 ab
Total anthocyanins	mg/L malvidin	1083 ± 15	851 ± 31 a	894 ± 22 a	770 ± 27 b
Total polyphenols	mg/L gallic acid	3540 ± 42	2977 ± 106 b	3291 ± 132 a	2918 ± 90 b
Color intensity		n.m.	1.02 ± 0.03 b	1.24 ± 0.04 a	0.91 ± 0.05 c
Tonality		n.m.	0.93 ± 0.03 b	1.07 ± 0.07 a	0.90 ± 0.08 b

The winemaking processes were carried out in the winery cellar. The three tanks (Control, AIR MIX, and ADCF) used in the experimentation have the same characteristics: a cylinder with height of 5.3 m and a diameter of about 2 m, for a total volume of 208 hL. All the tanks were equipped with a cooling jacket for temperature control, a hydraulic system consisting of a pump and pipes to automate the practice of must movement (pump-over), a rotary extraction blade placed at the base of the tank, an automatic macro-oxygenation system, and a computerized system SAEn5000 (Parsec s.r.l., Sesto Fiorentino (FI), Italy) used for the automation and control of oenological practices. Fifteen tons of grapes, destemmed and crushed and harvested in the same vineyard, were used to fill each tank. During

the winemaking process, the same addition of substances to all tanks was carried out. On the first day, 8 g/hL of potassium metabisulfite ($K_2S_2O_5$) and 15 g/hL of Selectys® Italica CR1 yeast (OENOFRANCE Montebello Vicentino (VI), Italy) were added, following the commercial guidelines for the rehydration and use. On the second day, 20 g/hL of fermentation activator (Nutriferm® Vit Flo (Enartis, San Martino (NO), Italy)) were added. At the end of the fermentation/maceration, the wine of each tank was drawn off and kept separately, adopting the winery procedure of stabilization and filtering. In both years, the same described procedure was carried out. No malolactic fermentation was performed on the obtained wines.

The control vinification followed the protocol of the Famiglia Cotarella cellar for red wine, involving the use of an irroration pump, daily pump-over, and "delestage". During the winemaking process in both years, between two and six pump-overs were carried out per day, lasting 12 min each, for a total pump-over time of about 1280 min. Only one "delestage" (80–90 hL), lasting 30 min, was carried out at the halfway point of alcoholic fermentation. The oxygenation (Parsec s.r.l., Sesto Fiorentino (FI), Italy) was maintained along the whole maceration time, always with the same oxygen amount (5 mg/L/day), for a total of 55 mg/L. The setting temperature was 27 °C, and the detected temperature in the three sections of the vessel is reported in Figure S1; the three sensors were controlled by SAEn5000). The temperature measurement started on the 1st day, but its stabilization in the mass occurred by the end of the second day; thus, the third day as the one with constant temperature was considered.

In the ADCF (NECTAR-ADCF™, Parsec s.r.l., Sesto Fiorentino, Italy) technique, the tank pressure was kept at 100 mbar; 2–8 pump-overs each day were carried out, lasting 10 min, for a total time of pump-over of about 1220 min in both years. The oxygenation procedure provided in total 18 mg/L of oxygen, while the setting temperature was increased progressively starting from 27 ± 1 °C (Figure S2).

The AIR MIX (AIRMIXING M.I.™, Parsec s.r.l., Sesto Fiorentino, Italy) technique consisted of injection of air jets from three nozzles connected to each other through a pipe and laterally placed inside the container in the lower part. These nozzles were timed to inject the air jets sequentially. The programmed injection sequence generated a liquid movement that created, in turn, a disrupting wave responsible for hindering the cap formation and favoring the uniform heat distribution into the tank. Neither pump-over nor delestage were carried out. The air injection from the three nozzles lasted from 20 up to 90 s, every 3–6 h, with a total from 2 to 12 min per day. Oxygenation was 18 mg/L total, and the setting temperature was the same as in the other vessels (Figure S3).

For each wort stirring operation (control and ADCF), the time (minutes) was considered because the energy power of the equipment was the same: the pump consumption was 1 kW/h, irroration pump for delestage 0.17 kW/h, racking pump 2.0 kW/h, and cooling equipment compressor 20 kW/h.

2.2. Chemical Analysis

The sampled berries at harvest, coming from three set of bunches representative of the whole vineyard, were hand squeezed, and the must was obtained by means of a juice extractor (JU3701 Frutelia Centrifuge Moulinex, ÉcullyFrance). The extracted must was centrifuged (6869 g for 5 min at 22 °C) and filtered (paper filter 0.82 µm). These last steps, centrifugation and filtering were performed also for the samples coming from fermenting musts. Juices, musts, and final wines were analyzed by a calibrated Fourier transform infrared WineScan™ FT 120 (Foss Analytics, Hillerod, Denmark) to determine the following oenological parameters: sugars (g/L hexoses), pH, titratable acidity (tartaric acid g/L), volatile acidity (g/L acetic acid), malic acid (g/L), tartaric acid (g/L), citric acid (g/L), total extract (g/L), ashes (g/L), YAN (g/L), total anthocyanins (mg/L malvidin), and total polyphenols (mg/L gallic acid). Each sample was analyzed in triplicate; thus, three flasks (three set of berries or three bottles of must or three bottles of wine) were prepared, and three WineScan™ analyses were performed. The accuracy of the WineScan™ analyses was

confirmed by destructive analyses performed by OIV methods as previously reported [27]. Sampling of the fermenting musts was done every day at the same time; three 500 mL bottles of liquid must and each sampling time from each tank were sampled after mixing the fermentation mass with a pump-over or with air injection in the case of AIR MIX technique.

As previously reported [28], for the color determination, a spectrophotometer (Cary 4000 UV–Vis, Agilent Technologies, Santa Clara, CA, USA) working in the range 300–700 nm was used. Using detected absorbance at 420, 520, and 620 nm, tonality (according to the formula Abs 420 nm/Abs 520) and color intensity (according to the formula Abs 420 nm + Abs 520 nm + Abs 620 nm) were calculated.

Main wine anthocyanins were characterized by HPLC consisting of a PU-2089 Plus quaternary pump (Jasco International Co., Ltd., Tokyo, Japan) equipped with a degasser, an AS-2057 Plus autosampler (Jasco International Co., Ltd., Tokyo, Japan), and a CO-2060 Plus column oven (Jasco International Co., Ltd., Tokyo, Japan). Detection was carried out with an UV-2070 Plus visible detector (Jasco International Co., Ltd., Tokyo, Japan). The data were processed with ChromNAV (software version 2.3).

As previously reported [29], for analytical determination of anthocyanins, a quantity of 1 mL of wine 1:1 diluted with phase A (see follow) was taken from the samples. The sample thus obtained was filtered through a 0.45 µm diameter PVDF (polyvinylidene fluoride) filter before being injected into the HPLC. The separation was carried out through a DionexAcclaim® 120 C18 column, 5 µm, 4.6 × 250 mm, and thermostated at 30 °C.

The mobile phase consisted of a ternary gradient: solvent A = 50 mM ammonium dihydrogen phosphate adjusted to pH 2.6 with acid phosphoric; solvent B = 20% solvent A and 80% acetonitrile; solvent C = 0.2 M orthophosphoric acid adjusted to pH 1.5 with NaOH. The phenolic compounds were identified based on their elution order, the retention times of pure compounds, and the characteristics of their UV–Vis spectra at the wavelength of 520 nm for anthocyanins.

2.3. Statistical Analysis

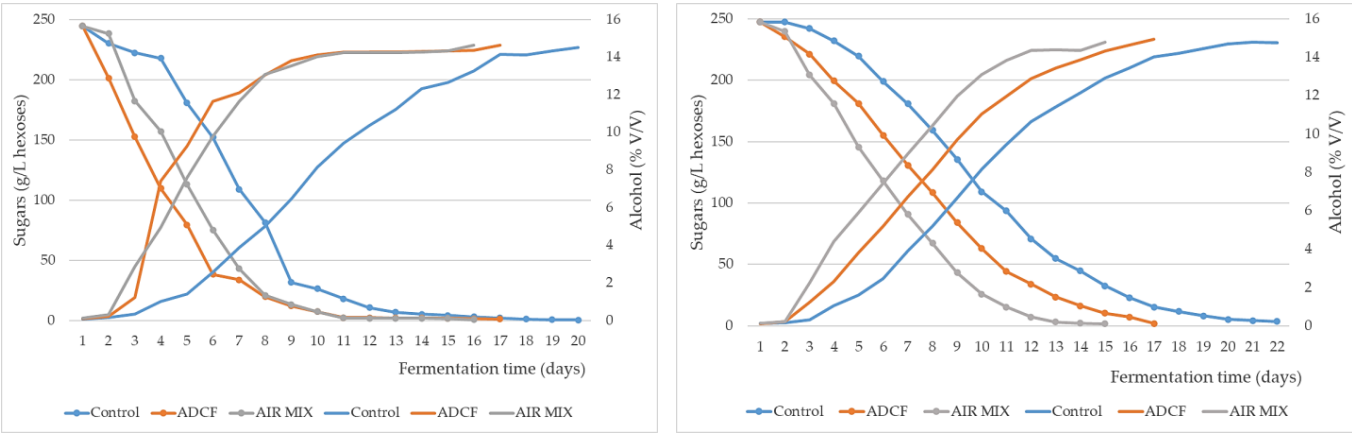
One-way ANOVA was run (CoStat, Version 6.451, CoHort Software, Pacific Grove, CA, USA), and Tukey's honestly significant difference (HSD) test with $p \leq 0.05$ for multiple comparison was used.

Principal component analysis (PCA) was carried out on the chemical dataset of wine. This analysis was performed on correlation matrix using the software Xlstat2022 (Addinsoft, New York, NY, USA), and the averaged acquisitions of the two years were considered.

3. Results and Discussion

In the 2020 vintage (Figure 1a), the control sample ended alcoholic fermentation (sugars below 2 g/L and no CO_2 release) in 20 days, while ADCF and AIR MIX samples in 17 and 16 days, respectively, showed very similar patterns. In 2021 (Figure 1b), the fermentation kinetics were similar to 2020 ones: the control lasted 22 days, while ADCF and AIR MIX took approximately the same: 17 and 15 days, respectively, through the previous year. Differently, ADCF and AIR MIX kinetic patterns were not close to that observed in 2020. AIR MIX fermentation rate was the fastest one. In the first year, all the wines were dry (below 2 g/L sugars), while in the second year, the control had more than 3 g/L of residual sugar (Tables 1 and 2). The observed more rapid fermentation in ADCF and AIR MIX is not due to oxygen addition because, as reported in Materials and Methods, the oxygen addition was much lower. The fastest rate is due to the continuous movement of the mass (AIR MIX) or to the cap immersion and rapid mass movement when the over-pressure valve was opened (ADCF). This allows to obtain a more uniform condition in the tank volume, which means a uniform temperature (Supplement Materials). As a consequence, we also assume an uniform oxygen concentration and uniform CO_2 distribution is useful for berry enzymes (in the first day of fermentation/maceration) and yeasts. Recently, we have seen the importance of having a uniform internal temperature in the tank to have

greater polyphenol extraction and smooth fermentation [30]. In the control, the pump-over does not guarantee a uniform condition in the tank volume because the mass mixing occurs only when pump-over takes place. Pump-over volume and frequency did not have a significant effect on phenolic extraction [31]. The final alcohol concentration was, in the three samples, around 14.5° (V/V).



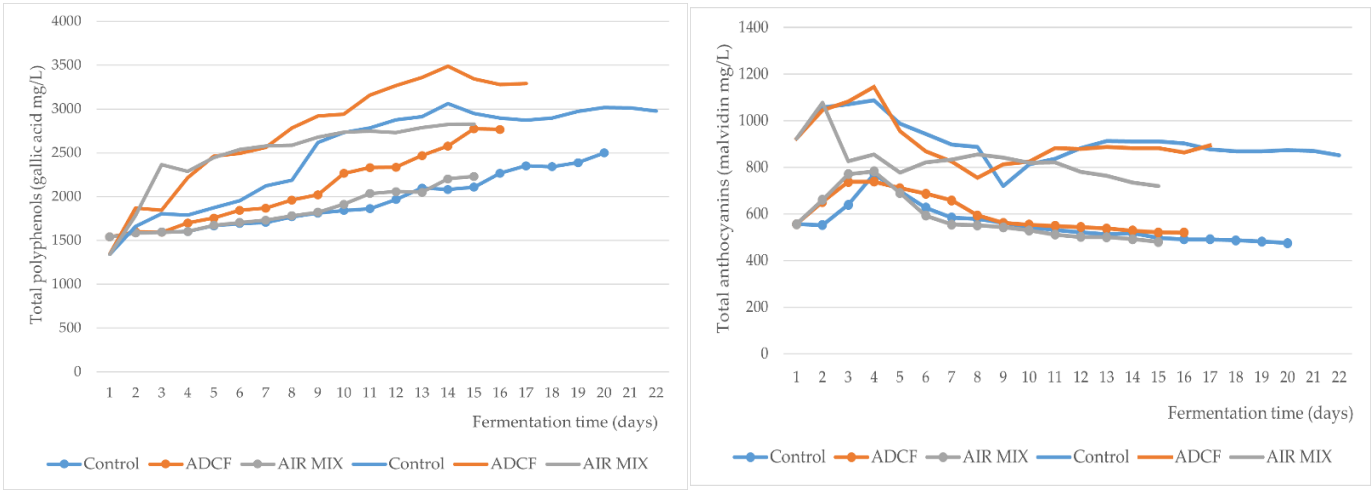
(**a**) (**b**)

Figure 1. Sugars (lines with solid dots) and alcohol content are in the Y-axes and fermentation time in the X-axis. (**a**) Fermentation kinetics 2020; (**b**) fermentation kinetics 2021.

As regards the wines, in 2020 and 2021, titratable acidity was higher in the control, while pH was not significantly different among the samples in 2020 and lower in the control in 2021. The higher acidity of the control was mainly due to the higher level of tartaric acid in both years, while malic acid was significantly slighter than in ADCF (2020, 2021) and AIR MIX (2021). Volatile acidity was significantly higher in both years in the control wines. This higher content of volatile acidity was due to the cap presence on the surface of the must/wine in fermentation, which stands between one pump-over and the other; this event could have favored the activity of lactic bacteria or acetic bacteria present in the fermenting mass and equipment [32–34].

An important difference among the three systems was related to the phenolic fraction, mainly in the kinetics of extraction. In 2021, the initial content of grape polyphenols was significantly higher than the year before not only due to a more advanced ripening but also to a drought stress (Table 1). In both years, a very deep anthocyanin extraction in AIR MIX and ADCF yielded an increase rate of 3- and 6-fold than the control in the first 2–4 days (Figure 2a,b). Polyphenol extraction was stronger in 2021, probably due to riper berries, and, also in this case, AIR MIX and ADCF showed a higher rate of extraction (Figure 2a). The observed greater extraction is due to the used techniques but also to the contribute of a more rapid ethanol formation, which facilitated the extraction. By the end, total polyphenol content was significantly higher in ADCF in both years, while AIR MIX values were similar to the control. Total anthocyanins were similar in concentration among ADCF, control, and AIR MIX. In 2021, the AIR MIX wine showed slightly lower values.

The ADCF system was very efficient in the extraction because the cap was submerged by means of overpressure, and the sudden reduction of the pressure created an intense cap movement with a significant leaching effect. In addition, the maintenance of a slightly higher CO_2 concentration favored a stronger extraction, and this confirms what was observed by Ichikawa et al., 2012 [35] and Bosso et al., 2011 [36] regarding tannins.



(**a**) (**b**)

Figure 2. (**a**) Behaviors of total polyphenols extraction in 208 hL tank, 2020 and 2021 years; (**b**) behaviors of total anthocyanins extraction in 208 hL tank, 2020 and 2021 years; 2020 (line with solid dots).

The slight reduction in polyphenols and anthocyanins in AIR MIX wine by the end, notwithstanding a greater initial extraction, especially in 2021, was due to two main factors: a mismanagement of maceration temperature and an unwanted prolonged maceration phase beyond the end of fermentation (3–4 additional days). Indeed, temperature was the same (27 °C) in AIR MIX, in ADCF (27 °C), and in control, but the AIR MIX technique temperature was real and uniform in all the volumes (no hot cap was formed), while in the other two techniques, the temperature of 27 °C was approximately maintained in the lower section of the vessel, whereas in the other parts (middle and upper), the temperature rose significantly above 30 °C (Supplemental Materials). This temperature difference in the upper section of the vessel provoked a greater extraction during fermentation progress. The second factor is related to the non-immediate racking as soon as the fermentation ended. As the cap was not formed, the high presence of lees needed to be immediately removed because otherwise, they could adsorb anthocyanins and other phenols, also reducing the color intensity. The described effect is very well-known [37], and it is affected by alcohol content and temperature [38].

To confirm what is reported above, ADCF wine had a higher color intensity and hue than the two other wines in both years (Tables 1 and 2), suggesting that the higher the anthocyanin and tannin extracts during fermentation, the higher the wine color density [39].

As regards specific anthocyanins, only data of malvidin and its derivatives, considering it is the most significant anthocyanin in Cabernet Sauvignon (in our case, about 60% out of the total), are reported (Table 3). Confirming what was measured in total anthocyanins, ADCF wines had the highest content in anthocyanins, both single and bound, and AIR MIX the lowest overall in 2021 due to temperature and the long-lasting effect on the lees before racking, as aforementioned. The other anthocyanins (not reported) behaved as malvidin.

PCA was used as an unsupervised pattern recognition technique to compare the tested maceration techniques to highlight differences and similarities among the fermentation days and correlations among variables. The resulting score plot for the first two principal components is shown in Figure 3. The score plot, accounting for 90.41% of the total variance, highlights that the first principal component discriminates between the first week (PC1 positive values) and the next two weeks of fermentation (PC1 negative values), while the second principal component discriminates between AIR MIX (PC2 negative values) and ADCF and control (PC2 positive values). PC1 is positively correlated to sugar content and titratable acidity (TA) and negatively correlated to alcohol and volatile acidity (VA).

Table 3. HPLC determination of malvidin and derivatives (mg/L) at the end of alcoholic fermentation in the 2020 and 2021 years. Data are expressed as mean (±SD) of three 500 mL bottles, each one taken immediately after wine mixing in the tank. Different letters (each year) in a row indicate statistically different data (Tukey, $p \leq 0.05$).

	2020			2021		
Compound	**Control**	**ADCF**	**AIR MIX**	**Control**	**ADCF**	**AIR MIX**
Malvidin	200.98 ± 7.28 ab	220.33 ± 8.23 a	189.77 ± 10.28 b	463.43 ± 23.13 a	481.56 ± 11.19 a	421.84 ± 16.19 b
Malvidin acetate	132.25 ± 6.15 b	154.61 ± 8.12 a	141.26 ± 8.12 b	202.47 ± 10.17 a	222.48 ± 13.18 a	171.32 ± 9.12 b
Malvidin coumarate	58.68 ± 6.18 ab	62.24 ± 4.19 a	57.98 ± 1.14 b	62.45 ± 1.05 b	68.78 ± 2.08 a	56.64 ± 2.06 c

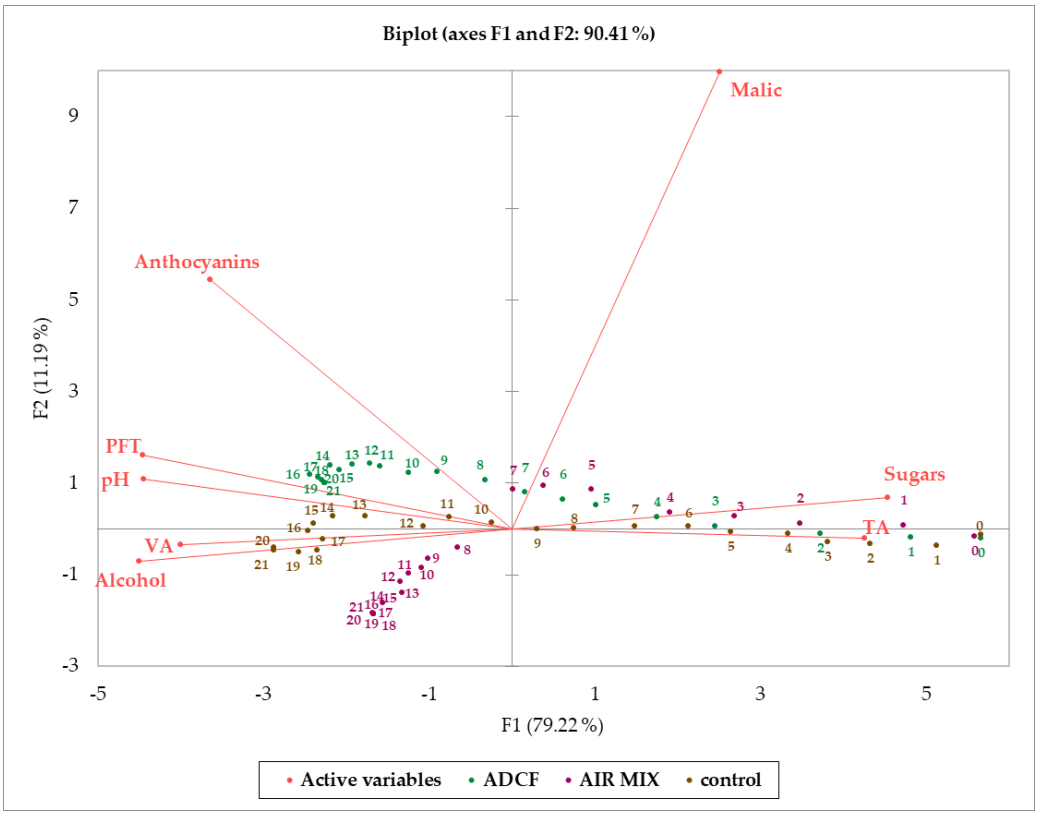


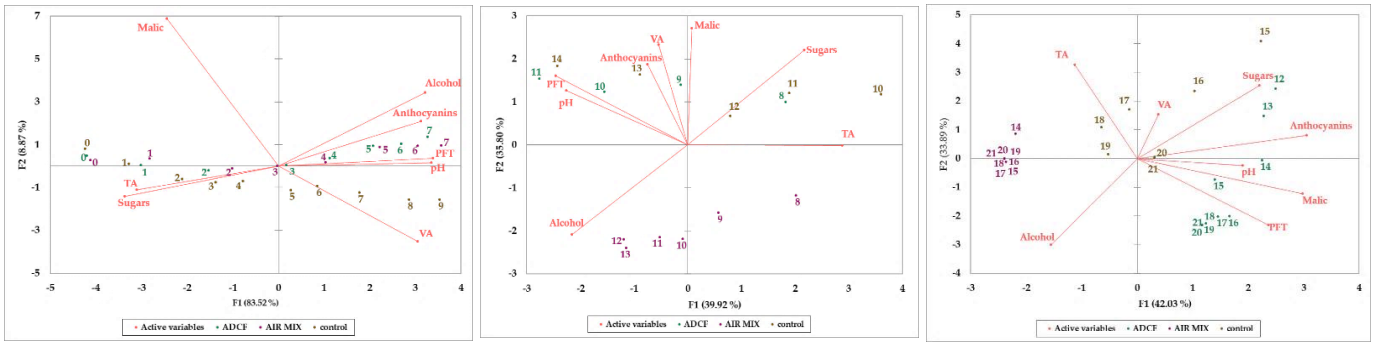
Figure 3. Principal component analysis (PCA) biplot of the fermentation process using the three different maceration techniques (average of the data of 2020 and 2021 year). TA, titratable acidity; VA, volatile acidity; PFT, total polyphenols. The numbers indicate the days of fermentation/maceration.

Thus, PC1 gives a quick view on the consume of sugar and alcohol formation and is therefore an indication of the fermentation stage. It is worth noting that this first step of fermentation is prolonged for a further two days in the traditional maceration technique.

The results reported in Figure 3 led to observation of a data set separation in three stages of fermentation: stage I, the beginning; stage II, the middle; and stage III, the ending.

A more detailed clustering of the scores (days) can be observed in the response of single PCA computation at each fermentation stage (Figure 4a–c), also including loadings (eno-chemical variables) influencing those segregations. The results of PCA referred to stage I of fermentation (Figure 4a) and clearly show how a high sugar content, which on average exceeded 200 g/mL and, together with TA, significantly affected the first three days of fermentation/maceration of all the three vinification techniques tested. The alcohol production started from the third day in AIR MIX and ADCF techniques and from the fifth in the control. It is interesting to note that the PC2 distinguishes the control from the other techniques by the volatile acidity (VA) effect, confirming what was observed above by discussing the chemical results. On the other hand, AIR MIX and ADCF are closer

in the same quadrant and significantly affected by alcohol, pH, total polyphenols, and anthocyanins.



(**a**) (**b**) (**c**)

Figure 4. Principal compounds analysis biplot of the three-fermentation process phases using three different maceration techniques (average of the data of 2020 and 2021 year). TA, titratable acidity; VA, volatile acidity; PFT, total polyphenols. The numbers indicate the days of fermentation/maceration. (**a**) Stage I; (**b**) stage II; and (**c**) stage III.

A similar segregation along PC2 occurs among AIR MIX sample and the others during the middle fermentation stage (Figure 4b). This result was affected by a high alcohol content (an average of 14% vs. 11% and 12% in control and ADCF, respectively). The last stage of fermentation confirmed what was reported above; it is the PC1 that segregates AIR MIX (with negative values along this axis) from the control and ADCF systems (Figure 4c). It is interesting to observe the clustering effect on the days, which means that fermentation was over, but the wine was kept on the lees, as aforementioned, provoking the adsorption of the pigments by the lees. Additionally, it highlights the presence in the same quadrant of day 17, 18, and 19, referring to the control wine. The localization of these scores is attributable to the influence of the loading titratable acidity (TA). In fact, TA in the control wine was comparable to what was measured in AIR MIX. At this stage, ADCF wine confirmed its richness in total anthocyanins, malic acid, and total polyphenols (PFT).

Finally, the labor-time estimation of each single method is reported in Table 4. The total lowest time was detected for AIR MIX, i.e., about 80% less, mainly because no pump-over or delestage were performed. Furthermore, the mass cooling was much lower, considering that the resonance waves, hindering the cap formation, favored an uniform internal temperature of the tank, with no differences between cap and liquid fractions.

Table 4. Labor-time (min) of each vinification method compared to each vinification techniques. Data refer to the mean of the two-year trials.

Equipment	Control	ADCF	AIR MIX
Pump-over pump (min)	1280	1220	0
Irroration pump (min)	27	0	0
Racking pump (min)	27	17	10
Cooling equipment (min)	560	460	118
Gas compressor (min)	0	0	175
Total	1894	1697	303

4. Conclusions

The ADCF, a soft technique of extraction, provided wines with the highest content in total polyphenols and total anthocyanins in both years of testing. AIR MIX, another very innovative soft extraction technique based on resonance waves, behaved as ADCF for a very rapid rate of extraction, also ending similarly to first the fermentation process. AIR MIX requires an accurate management of (i) jet air injection to create resonance waves; (ii) extraction temperature because all the mass has the same temperature, and no difference exits between cap and liquid; and (iii) rapid racking when the fermentation is over and no keeping of lees. Finally, it is possible to emphasize that AIR MIX allowed for a reduction of about 80% of energy (expressed as minutes of equipment working) compared to the control system, and no personnel is needed during fermentation-maceration but only for racking.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/beverages8040062/s1, Figure S1: Temperature kinetics of the three sensors of the control vessel during fermentation, with sensors located in the lower, middle, and upper part of the vessel; Figure S2: Temperature kinetics of three sensors of the ADCF vessel during fermentation, with sensors located in the lower, middle, and upper part of the vessel; Figure S3: Temperature kinetics of the three sensors of the AIR MIX vessel during fermentation, with sensors located in the lower, middle, and upper part of the vessel.

Author Contributions: Conceptualization, F.M. and A.B. (Andrea Bellincontro); methodology, A.B. (Alessandro Bianchi), S.P. and F.M.; validation, A.B. (Alessandro Bianchi), F.M. and A.B. (Andrea Bellincontro); formal analysis, A.B. (Alessandro Bianchi), S.P., L.P., G.D.A. and B.N.; investigation, A.B. (Alessandro Bianchi) and S.P.; resources, F.M. and G.F.; data curation, A.B. (Alessandro Bianchi), S.P., R.C. and B.N.; writing—original draft preparation, S.P., A.B. (Alessandro Bianchi), A.B. (Andrea Bellincontro) and F.M.; writing—review and editing, S.P., A.B. (Alessandro Bianchi) and F.M.; visualization, A.B. (Alessandro Bianchi), S.P. and F.M.; supervision, F.M. and A.B. (Andrea Bellincontro); funding acquisition, F.M. and G.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by TUSCANY REGION with the Project WINESENS, POR FESR Tuscany 2014–2020, Azione 1.1.5 Sub. A1. Call for tender N. 2-MSMEs Research and Development Projects, Cup cipe: D84E20003790009 and by MUR with the Project VIOLoC, FISR 2019, Grant number FISR2019-03020.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article.

Acknowledgments: We thank The Cotarella Family Winery for the availability to run the experiments.

Conflicts of Interest: The authors declare no conflict of interest.

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