CAN USE OF ULTRASOUNDS IN RED WINEMAKING INCREASE POLYPHENOL EXTRACTION FROM GRAPE SKINS IRRESPECTIVE OF THE CULTIVAR?

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ABSTRACT

The aim of this study was to investigate whether ultrasound treatment used in winemaking on grape cultivars with different ripening times improves the extraction of phenolic compounds. The cultivars used were Primitivo (early ripening), Nero di Troia (medium-late) and Aglianico (late), all grown in southern Italy. The trial used four pilot plants consisting of 200-L submerged cap stainless steel horizontal rotary wine fermenters. De-stemmed Primitivo grapes were directly subjected to the ultrasound treatment, whereas de-stemmed Nero di Troia and Aglianico grapes were processed after dilution with previously extracted juice (1:1 w/v). Our results showed that ultrasound improved the extraction of flavonoids (+15%), total polyphenols (+10%) and proanthocyanidins (+100%) in Primitivo, had little effect on Nero di Troia, and actually increased all phenol classes for Aglianico. The main outcome of this research is that the effect of ultrasound treatment seems to be cultivar-dependent, and that ultrasound could therefore be useful in winemaking with Primitivo and Aglianico.

Industrial relevance: The use of ultrasounds in winemaking could increase the extraction of phenols from grapes, improving the sensory quality and health benefits of wine due to the higher content of nutraceuticals. The ultrasound generator and transducer can easily be included in existent traditional winemaking processes as an "add on" technology, without distorting the processing lines. This

innovation reduces the environmental impact of winemaking, involving lower energy consumption and reduced processing times, and it improves the quality of wines so that they can be more easily marketed.

Keywords:
Fermenter pilot plant
Ultrasounds
Red winemaking
Polyphenols
Anthocyanins

1. Introduction

Grapes are a source of phenolic compounds, which play an important role both in plant physiology and for human health. Phenols work in various reactions to protect cells against abiotic stresses like UV-light, or against biotic stresses such as attacks by predators and pathogens (Weisshaar & Jenkins, 1998; Winkel-Shirley, 2002). Moreover, many phenolic compounds, such as resveratrol, quercetin and rutin, have been reported as having biological activities, including cardio-protective, anti-inflammatory, anti-carcinogenic, antiviral and antibacterial properties, attributed mainly to their antioxidant and antiradical activity (Frankel, German, Kinsella, Parks, & Kanner, 1993; King, Bomser, & Min, 2006; Santos-Buelga & Scalbert, 2000; Teissedre, Frankel, Waterhouse, Peleg, & German, 1996).

In oenology, phenols are very important molecules, because they contribute to the wine's sensory properties, such as colour, flavour, astringency and bitterness. Anthocyanins and flavan-3-ols are flavonoids and are very important for the quality of red wine. Anthocyanins are responsible for colour, and flavan-3-ols, the so-called condensed tannins or proanthocyanidins, are responsible for astringency and bitterness (Gawel, 1998; Peleg, Gacon, Schlich, & Noble, 1999), and for their role

in long-term colour stability (Somers, 1971; Vivar-Quintana, Santos-Buelga, & Rivas-Gonzalo, 2002). It is well known that anthocyanins are located in grape skins, whereas flavans-3-ols are located in skins and in seeds. These compounds are extracted from grapes at the maceration/fermentation stage of winemaking. The phenolic composition of red wine is affected by different factors, such as the grapevine genome, winemaking technology and ageing conditions (Baiano, Terracone, Gambacorta, & La Notte, 2009; Gambacorta et al., 2011a; Gambuti, Rinaldi, Ugliano, & Moio, 2013; Gambuti et al., 2016; González-Neves, Gil, & Barreiro, 2008; Pérez-Lamela, García-Falcón, Simal-Gándara, & Orriols-Fernández, 2007). As far as winemaking technologies are concerned, in recent years ultrasound-assisted extraction has been tested to enhance the content and composition of the phenolic compounds in red wines.

In particular, ultrasound treatments are used to increase the extraction of polyphenols and volatiles from grape skins during maceration, and to accelerate and enhance aging. Ultrasound efficacy is linked to the formation of small bubbles that then collapse, generating kinetic energy that destroys the cell walls of vegetable tissues in aqueous systems. This process is known as cavitation, and its effects are mainly mechanical at frequencies of up to 20 kHz, and chemical at higher frequencies (Mason, Paniwnyk, & Lorimer, 1996). Several studies have recently explained the effects on colour and flavour of the use of ultrasound at different stages of the winemaking process (Bates & Patist, 2010; El Darra, Grimi, Maroun, Louka, & Vorobiev, 2013; Ferraretto, Cacciola, Ferran Batllò, & Celotti, 2013; Ferraretto & Celotti, 2016). The increase in tannins and anthocyanin concentration, as results of US application, gives wines with better aging potential (Coletta et al., 2013; García Martín & Sun, 2013). One of the most important changes during aging is a progressive increase and stabilization of the colour due to copigment anthocyanin complexes and the formation of both tannin–tannin and anthocyanin–tannin complexes (Boulton, 2001). Masuzawa, Ohdaira, and Ide (2000), found that the polymerization of polyphenolic compounds in red wine was promoted by ultrasound at low sound pressure levels.

78 The aim of this study was to evaluate the effectiveness of ultrasound treatment on wine grape
79 cultivars grown in southern Italy, which have different ripening times: Aglianico, Nero di Troia and
80 Primitivo.
81
82 **2. Materials and methods**
83 *2.1. Grape sampling*
84 The research was conducted in September-October 2014 on Primitivo (early ripening), Nero di
85 Troia (medium-late ripening) and Aglianico (late ripening) grape cultivars from three different
86 vineyards in southern Italy. Primitivo from the Gioia del Colle area (Puglia Region) was harvested
87 on 19th September, Nero di Troia from the Corato area (Puglia Region) was harvested on 1st October,
88 and Aglianico from the Avellino area (Campania Region) was harvested on 4th November.
89 Approximately 1,000 kg of grapes were hand-picked for each cultivar, packed in 20 kg perforated
90 plastic boxes and transferred to Agricole Pietraventosa winery at Gioia del Colle for the
91 winemaking trials.
92
93 *2.2. Winemaking*
94 The grapes were processed using four "Gioiello" pilot plants, consisting of 200-L stainless steel
95 horizontal rotary wine fermenters with a submerged cap (Industrie Fracchiolla, Adelfia, Italy). Two
96 of the fermenters, were equipped when necessary with an ultrasonic delivery system consisting of a
97 Sonic Digital LC 1500 SD 25-P ultrasonic generator (25 kHz frequency, 1500 W power output) and
98 Sonopush HD Double Twin 1500 titanium transducer (WEAL, Milan, Italy). The schematic
99 drawing of the fermenter fitted with the ultrasonic delivery system is shown in Fig. 1. The power
100 indicated on the display of the ultrasound generator was 100% when the fermenter was loaded with
101 water.
102 Primitivo was the first cultivar to be vinified, and was also used for setting up the ultrasound
103 parameters and the screening effectiveness of this technique in comparison with an alternative

technology already in use (cryomaceration). Grapes were de-stemmed and divided into four aliquots (approximately 250 kg each), then vinified using four different technologies: traditional maceration (P-C, control), cold pre-fermentative maceration (P-CM, cryomaceration), pre-fermentative ultrasound maceration (P-UBF) and post-fermentative ultrasound maceration (P-UAF), as described in Table 1. During ultrasound treatments, the display of the ultrasound generator indicated that the power of the delivery system decreased to 30%. This may be explained by the large quantity of skins which collected around the transducer, thus acting as a "screen" reducing the propagation of the ultrasound waves. Consequently, the experimental protocol was modified: in particular, ultrasounds were applied only in pre-fermentation, and the solid/liquid ratio was reduced in order to increase power. This was done by diluting de-stemmed grapes with juice of the same cultivar in the ratio of 1:1 (w:v). It was impossible to repeat winemaking with Primitivo because no grapes were available, but the modified ultrasound protocol was used with the other two cultivars: Nero di Troia and Aglianico. Specifically, approximately 85 kg of de-stemmed grapes were diluted with 85 L of juice (obtained with a manual wine-press), and vinified by traditional (NT-C, Nero di Troia control; A-C, Aglianico control) and pre-fermentation ultrasound maceration (NT-U, Nero di Troia ultrasound; A-U, Aglianico ultrasound), as described in Table 1. The trials were done in duplicate using the four pilot plants described above (2 controls + 2 ultrasounds for each cultivar). The reduction in the solid/liquid ratio limited the decrease of the power of the ultrasonic delivery system (the value indicated on the display was 60%). At the end of maceration (7 days), free-run wine was unloaded from the fermenter, and the pomace was transferred into the hand press to recover press-run wine by gentle pressing. Free-run and press-run wines were blended and transferred into 200-L stainless steel vats. One week later, the wines were transferred to other stainless steel vats in order to remove gross lees. After six months, the wines were finally bottled without any post-treatment, and then analysed.

2.3. Chemical analysis

For each cultivar, a representative 300-berry sample was picked from the top, middle and bottom of bunches taken from the perforated plastic boxes at the winery. A sub-sample of 150 berries (divided into three 50-berry replicates) was submitted to chemical analysis, while the remaining 150 berries were used to analyse phenols. The berries were pressed and the juice obtained was analysed for total soluble solids (TSS, °Brix), pH and titratable acidity (TA, g/L tartaric acid), according to EEC 2676 standard procedure (EEC, 1990).

The chemical characteristics of wines were assessed by determining ethanol (E, % v/v), pH, titratable acidity (TA, g/L), volatile acidity (VA, g/L acetic acid), malic acid (MA, g/L) and lactic acid (LA, g/L), dry reduced extract (DRE, g/L) and ashes (g/L) using an AutoAnalyzer FOSS WineScan FT 120 FT-MIR spectrometer (FOSS, Padua, Italy).

2.4. Analysis of phenolic compounds

2.4.1. Extraction from skins

From each lot of 150 berries, 90 were selected and divided into three sub-samples (30 berries per replicate), then subjected to extraction of phenols according to the method of Di Stefano and Cravero (2001) with some modifications. Briefly, skins were manually separated from the pulp, gently dried on filter paper and then macerated in 75 mL of ethanol/water/HCl solution (70/30/1 v/v) for 24 h at room temperature in the dark. Then, the extract was filtered through filter paper and immediately analysed.

2.4.2. Assessment of phenol composition

Phenol composition of skin extracts and wines was determined according to Di Stefano and Cravero (2001), whereas the colour indices (CI, colour intensity; T, tonality) were assessed according to the Glories procedure (1984), using an UV-visible spectrophotometer (Beckman Coulter DU 800, USA). Detailed procedures for the analysis of flavonoids (F), anthocyanins (A), total polyphenols

(TP), proanthocyanidins (P) and flavans reactive with vanillin (FRV) of grape skin extracts and wines have been reported in a previous work (Gambacorta et al., 2011b).

2.4.3. HPLC-PAD anthocyanin analysis

Anthocyanins were analysed by HPLC using a Waters 600 E instrument (Waters, PA, USA), consisting of a quaternary pump, a photodiode array detector and an injection valve with a 20-μL loop. Separation used a NovaPack column (150 x 3.9 mm, 4 μm particle size), 10% formic acid and acetonitrile as the mobile phase. The operative conditions and tentative identification of anthocyanins are reported in a previous work (Coletta et al., 2013). Results were expressed as mg/L of malvidin-3-*O*-glucoside equivalent.

2.5. Antioxidant activity

Antioxidant activity (AA) was measured using ABTS [2,2′-azino-bis(3- ethylbenzothiazoline-6-sulfonic acid)] assay as reported by Trani, Verrastro, Punzi, Faccia and Gambacorta (2016). The results were expressed as μmol/L TEAC (Trolox equivalent antioxidant capacity).

2.6. Statistical analysis

All measurements were carried out in triplicate, and results were expressed as means ± SD (standard deviation). Statistical analysis was performed using IBM SPSS software v 19. Significant differences between technologies for each cultivar were determined using one-way ANOVA with post-hoc analysis using the HSD Tukey test.

3. Results and discussion

3.1. Qualitative characteristics of grapes

The chemical characteristics and phenolic composition of grapes are reported in Table 2. Aglianico showed the highest TSS value and the strongest acidic structure, as indicated by the lowest pH and

181 the highest TA. The strong acidic structure of Aglianico could be related to the altitude of the
182 vineyard (about 600 m a.s.l.) and to climatic conditions. As expected, Nero di Troia had the poorest
183 acidic structure, since this is a peculiar characteristic of the cultivar when it is fully ripe
184 (Gambacorta et al., 2011a; Lovino, Baiano, Pati, Faccia, & Gambacorta, 2006). Regarding the
185 phenolic composition, Primitivo had the highest F and P values, and the lowest TP and AA values;
186 Nero di Troia had the highest TP, FRV, FRV/P ratio and AA values, and the lowest A value;
187 Aglianico contained the most F, A and AA and the least FRV. Anthocyanin composition is reported
188 in Table 3. The three cultivars presented different anthocyanin contents and compositions,
189 confirming that the anthocyanin profile is typical of each cultivar, although the concentration of
190 single anthocyanins may change as a consequence of environmental and pedoclimatic conditions
191 and vineyard management (Gambacorta et al., 2011a; Gonzáles-Neves et al., 2004; Lovino, Baiano,
192 Pati, Faccia, & Gambacorta, 2006; Revilla, Garcia-beneytez, Cabello, Martin-Ortega, & Ryan,
193 2001; Tamborra & Esti, 2010). Aglianico was the richest in anthocyanins, followed by Primitivo (-
194 11%) and Nero di Troia (-24%). Regarding anthocyanin composition, Aglianico contained the
195 highest percentage of non-acylated forms (81.9%), followed by Primitivo (73.4%) and Nero di
196 Troia (49.9%). Of the non-acylated forms, malvidin-3-*O*-glucoside was the most prevalent,
197 accounting for 57.3% of the total for Aglianico, 49.4% for Primitivo and 31.6% for Nero di Troia. It
198 is noteworthy that Nero di Troia contained a relatively large amount of malvidin acylated forms
199 such as *trans*-malvidin-3-*O*-coumarylglucoside, malvidin-3-*O*-acetylglucoside and malvidin-3-*O*-
200 caffeylglucoside, which accounted for 22.9%, 12.7% and 3.1% of the total, respectively. This could
201 be explained as a genetic expression of the cultivar. Primitivo's anthocyanin profile was
202 intermediate between Aglianico and Nero di Troia, except for greater quantities of cyanidin-3-*O*-
203 glucoside, peonidin-3-*O*-glucoside and peonidin-3-*O*-coumarylglucoside. In general, these results
204 indicate that there are great differences between the phenol profiles of the three cultivars.
205
206 *3.2. Characteristics of Primitivo wines in relation to technologies*

207 The chemical characteristics, phenolic composition and colour indices of Primitivo wines are
208 reported in Table 3. Cryomaceration (P-CM) and ultrasound treatments, both in pre- (P-UBF) and
209 post-fermentation (P-UAF), slightly enhanced the ethanol content by nearly half a degree compared
210 with the control. P-CM wine had the highest TA, confirming data reported for Primitivo wines from
211 the Manduria area of Puglia (Baiano, Terracone, Gambacorta, & La Notte 2009). The technologies
212 used did not influence VA, because this depends on grape quality and correct management of the
213 winemaking process. It is noteworthy that malolactic fermentation was almost completed in wine
214 deriving from the ultrasound treatment in pre-fermentation, was in progress in P-C wine, and did
215 not start in P-UAF and P-CM wines. P-CM wine had the highest values of DRE and ashes.
216 With regard to phenolic composition, cryomaceration led to F, A, TP, FRV and P enrichment in
217 comparison with the control wine. These results agree in part with those reported by Coletta et al.,
218 (2013) for Negroamaro wines. In contrast, ultrasound treatments before and after fermentation
219 increased F (+15%), TP (+10%) and P (+100%), but had no effects on A and FRV. The greatest
220 effect of sonication was that it reduced the FRV/P ratio by 50%, due to the greater extraction of
221 proanthocyanidins. A low FRV/P ratio indicates a predisposition to chromatic and tannic
222 stabilization of wine (Suriano, Alba, Tarricone, & Di Gennaro, 2015). This result is relevant, since
223 it suggests that ultrasound could accelerate the colour stabilization of wine, thus allowing early
224 marketing of the product. Cryomaceration led to a smaller reduction in the FRV/P ratio than
225 ultrasonic treatment (-13%). As observed for TP, AA also increased with both cryomaceration and
226 ultrasound treatment. This was expected, since it is well known that phenols are strictly correlated
227 with antioxidant activity (Fernández-Pachón, Villaño, García-Parrilla, & Troncoso, 2004). With
228 regard to colour indices, the technological variables increased colour intensity, but did not have any
229 effect on tonality. Ultrasound after fermentation was more effective than traditional winemaking
230 (+21%), cryomaceration (+15%), and ultrasound before fermentation (+4%).
231 Anthocyanin composition is reported in Table 4. From a quantitative point of view, cryomaceration
232 promoted a greater extraction of anthocyanins (+22%) than for the control, in accordance with the

233 results of spectrophotometric analysis (Table 3). The anthocyanin increase in cryomacerated wine is
234 a controversial topic in the literature (Álvarez, Aleixandre, García, & Lizama, 2006; Gambacorta et
235 al., 2011a; Gil-Munõz et al., 2009; Gómez-Míguez, González-Miret, & Heredia, 2007; González-
236 Neves, Gil, Favre, & Ferrer, 2012; Reynolds, Cliff, Girard, & Kopp, 2001; Soto Vásquez, Río
237 Segade, & Orriols Fernández, 2010), and our results suggest that the effectiveness of
238 cryomaceration in anthocyanin extraction is cultivar-dependent. Ultrasound used in pre-
239 fermentation had no effect, but caused a reduction in post-fermentation (-37%). This disagrees with
240 results previously reported for Negramaro wine (Coletta et al., 2013), according to which
241 ultrasound increased total anthocyanins, and this may suggest that anthocyanin extraction is also
242 cultivar-dependent for ultrasound treatment. With regard to anthocyanin composition, the profile
243 observed was typical of Primitivo cultivar (Baiano, Terracone, Gambacorta, & La Notte 2009;
244 Suriano, Alba, Tarricone, & Di Gennaro, 2015; Trani, Verrastro, Punzi, Faccia, & Gambacorta,
245 2016). The differences detected between grapes and wines are related both to the different
246 molecular structure of each anthocyanin and to the degradation reactions that occur during
247 winemaking (González-Neves, Gil, & Barreiro, 2008; Gambacorta et al., 2011a). In particular,
248 wines contained a lower proportion of acylated forms than grapes (13.3-17.1% *vs*. 26.6%), and the
249 greatest reduction was observed for coumarate forms (7.6-8.6% *vs*. 21%). Regarding the effect of
250 technology, cryomaceration determined a lower extraction of non-acylated forms than the control,
251 while the effect of ultrasound treatment was negligible.
252
253 *3.3. Characteristics of Nero di Troia and Aglianico wines in relation to ultrasound*
254 The chemical characteristics, phenolic composition and colour indices of Nero di Troia and
255 Aglianico wines produced using the modified ultrasound protocol are reported in Table 5. The
256 treatment favoured an E increase for both cultivars, and an increase in DRE and ashes for Aglianico.
257 As expected, the addition of juice to de-stemmed grapes reduced the concentration of phenols in
258 both experimental and control wines. The amounts detected in our samples were much lower than

259 those reported in a previous work on traditionally-made wines using the same cultivars
260 (Gambacorta et al., 2011b). Ultrasound had poor effects on phenol extraction for Nero di Troia, as
261 indicated by the slight increase in TP (+5%) and slight decrease in P (-6%), and by the absence of
262 changes in other compounds. This may be due to this cultivar's tough skin, which could have
263 reduced the release of polyphenols. In contrast, when ultrasound was applied to Aglianico, it
264 promoted high levels of phenol extraction, except for FRV. The lower level of FRV extraction in
265 connection with the higher level of P extraction led to a significant decrease in the FRV/P ratio (of
266 approximately 12%). This must be considered a positive result, because a low FRV/P ratio favours
267 the colour stabilization of wine (Suriano, Alba, Tarricone, & Di Gennaro, 2015). Ultrasound also
268 led to increased antioxidant activity in both wines, although it was statistically significant only in
269 Aglianico (+12%). Regarding the colour indices, the treatment increased CI only for Aglianico
270 (+20%), whereas no effect was observed on T for both cultivars.
271 Anthocyanin composition is reported in Table 7. As expected, and in accordance with
272 spectrophotometric analysis, ultrasound increased anthocyanin extraction for both cultivars,
273 although to different extents (+4% for Nero di Troia and +20% for Aglianico). As previously
274 observed for Primitivo, the anthocyanin composition of wine was also different from the
275 corresponding grapes. For Nero di Troia, the non-acylated and acetate forms increased (64% *vs.*
276 49.9% and 24.2% *vs.* 18.1%, respectively) and coumarate forms decreased (10.2% *vs.* 26.9%),
277 while for Aglianico non-acylated forms did not significantly change, acetate forms increased (about
278 10% *vs.* 3.3%) and coumarate forms decreased (5.5-6.4% *vs.* 13%). Finally, the anthocyanin profile
279 remained unchanged in both cultivars, suggesting that sonication had only a quantitative effect.
280 Overall, the results showed that the effect of ultrasound treatment on chemical-physical and phenol
281 parameters is cultivar-dependent, and that Aglianico was the most sensitive cultivar to this
282 treatment.
283
284

4. Conclusion

The results of this study demonstrate that both cryomaceration and ultrasound treatment applied to de-stemmed Primitivo grapes improve the extraction of some phenolic compounds, decreasing the FRV/P ratio. As a consequence, both technologies can be recommended for this cultivar, in order to favour wine colour stabilization. The results for Nero di Troia and Aglianico suggest that the effect of ultrasound is cultivar-dependent under our experimental conditions. In conclusion, the main outcome of this work is that the usefulness of ultrasound in red winemaking should be thoroughly tested on single cultivars. Our study shows that good results can be obtained when ultrasound is applied to Primitivo and Aglianico, which appeared more sensitive to cavitation.

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418 Legend to figure

419 **Fig. 1** - Schematic drawing of the fermenter equipped with the ultrasonic delivery system.

420

Table headings

Table 1

Winemaking technologies tested using the four pilot plants.

Table 2

Chemical characteristics and phenolic composition of grapes (mean values ± SD).

Table 3

Anthocyanin composition of grapes (mg/kg of berries, mean values ± SD).

Table 4

Chemical characteristics, phenolic composition and colour indices of Primitivo wines (mean values ± SD).

Table 5

Anthocyanin composition of Primitivo wines (mg/L, mean values ± SD).

Table 6

Chemical characteristics, phenolic composition and colour indices of Aglianico and Nero di Troia wines (mean values ± SD).

Table 7

Anthocyanin composition of Aglianico and Nero di Troia wines (mg/L, mean values ± SD).

446 **Table 1**

Winemaking technology	Action
P-C	Seven days 'maceration at 25°C. Addition of potassium metabisulphite (20 g/100 kg); yeast (*Saccharomyces cerevisiae* var. *Bayanus*, Mycoferm CRU 05, 20 g/100 kg, Everintec, Pramaggiore, Italy); yeast activator (preparation based on ammonium sulphate, diammonium phosphate, chemically inert filter and as dispersing agent, Vitamin B1, Enovit, 20 g/100 kg, AEB); O_2, 10 mg/L/day after 2 days from the beginning of fermentation; yeast activator, 20 g/100 kg after 3 days from the beginning of fermentation; without any further oenological treatment.
P-CM	As P-C, but with the cooling of de-stemmed grapes until 5°C using cooling jacket, and maintenance of the sample at 5°C for 48 h.
P-UBF	As P-C, but with 2 h of ultrasound treatment before the start of fermentation as the drum rotated.
P-UAF	As P-C, but with 2 h of ultrasound treatment at the end of fermentation as the drum rotated.
NT-DC*	As P-C, but using about 85 kg of de-stemmed grapes + 85 L of juice.
NT-DU*	As NT-DC, but with 2 h of ultrasound treatment before the start of fermentation as the drum rotated.
A-DC*	As NT-DC.
A-DU*	As A-DC, but with 2 h of ultrasound treatment before the start of fermentation as the drum rotated.

447 P-C, Primitivo control; P-CM, Primitivo cryomaceration; P-UBF, Primitivo ultrasound before
448 fermentation; P-UAF, Primitivo ultrasound after fermentation; NT-DC, Nero di Troia diluted
449 control; NT-DU, Nero di Troia diluted ultrasound; A-DC, Aglianico diluted control; A-DU,
450 Aglianico diluted ultrasound. *Two winemaking replicates.
451

Table 2

Parameters	Primitivo	Nero di Troia	Aglianico
TSS (°Brix)	†19.4±0.01^{c}	20.2±0.01^{b}	21.9±0.02^{a}
pH	3.35±0.01^{b}	3.45±0.01^{a}	2.92±0.02^{c}
TA (g/L of juice)	7.08±0.04^{b}	5.22±0.04^{c}	11.71±0.08^{a}
F (mg/kg)	2988±136^{a}	2725±115^{b}	2986±110^{a}
A (mg/kg)	1670±54^{b}	1519±72^{c}	1920±45^{a}
TP (mg/kg)	1662±57^{c}	2019±29^{a}	1809±78^{b}
FRV (mg/kg)	755±22^{b}	1046±66^{a}	510±31^{c}
P (mg/kg)	1578±102^{a}	1230±86^{b}	1118±114^{b}
FRV/P	0.48	0.85	0.46
AA (μmol/kg)	3579±297^{b}	5777±519^{a}	5772±355^{a}

TSS, total soluble solids; TA, titratable acidity; F, flavonoids: as (+)-catechin; A, anthocyanins: as malvidin-3-*O*-glucoside; TP, total polyphenols: as gallic acid; FRV, flavans reactive with vanillin: as (+)-catechin; P, proanthocyanidins: as cyanidin chloride; AA, antioxidant activity. †In rows, data followed by different letters indicate statistically significant differences at $P < 0.05$.

Table 3

Compounds	Primitivo	Nero di Troia	Aglianico
Dp	†33.8±7.9[b]	35.2±0.1[b]	69.8±11.3[a]
Cy	12.8±1.6[a]	8.7±1.8[b]	5.8±1.2[c]
Pt	45.0±10.4[b]	31.3±1.4[c]	68.5±10.5[a]
Pn	62.3±8.7[a]	24.4±0.5[c]	32.6±5.4[b]
Mv	316.2±37.1[b]	171.4±14.7[c]	411.0±49.7[a]
Dp-Ac	1.9±0.3[b]	8.8±0.4[a]	2.0±0.1[b]
Pt-Ac	5.7±0.7[c]	13.6±0.5[a]	7.8±0.8[b]
Pn-Ac	2.2±0.3[b]	6.9±1.2[a]	0.9±0.1[c]
Mv-Ac	12.7±1.0[b]	68.8±4.6[a]	13.2±0.5[b]
cis-Mv-Cm	3.3±0.1[a]	3.7±0.4[a]	0.7±0.1[b]
Mv-Cf	9.4±0.6[b]	16.8±1.4[a]	8.1±0.7[b]
Pt-Cm	5.1±0.2[b]	6.5±0.5[a]	3.4±0.1[c]
Pn-Cm	21.2±0.3[a]	11.7±1.3[b]	7.1±0.8[c]
trans-Mv-Cm	105.2±4.4[b]	124.3±9.8[a]	82.3±3.5[c]
Total anthocyanins	639.7±44.4[b]	542.6±33.9[c]	717.4±75.6[a]

Dp, delphinidin-3-*O*-glucoside; Cy, cyanidin-3-*O*-glucoside; Pt, petunidin-3-*O*-glucoside; Pn, peonidin-3-*O*-glucoside; Mv, malvidin-3-*O*-glucoside; Dp-Ac, delphinidin-3-*O*-acetylglucoside; Pt-Ac, petunidin-3-*O*-acetylglucoside; Mv-Ac, malvidin-3-*O*-acetylglucoside; Dp-Cm, delphinidin-3-*O*-coumarylglucoside; *cis*-Mv-Cm, *cis*-malvidin-3-*O*-coumarylglucoside; Mv-Cf, malvidin-3-*O*-caffeylglucoside; Pt-Cm, petunidin-3-*O*-coumarylglucoside; Pn-Cm, peonidin-3-*O*-coumarylglucoside; *trans*-Mv-Cm, *trans*-malvidin-3-*O*-coumarylglucoside. †In rows, data followed by different letters indicate statistically significant differences at $P < 0.05$.

Table 4

Parameters	P-C	P-CM	P-UBF	P-UAF
E (% v/v)	†12.54±0.01^{d}	13.27±0.01^{a}	13.00±0.01^{c}	13.15±0.01^{b}
pH	3.50±0.01^{a}	3.47±0.02^{a}	3.48±0.01^{a}	3.41±0.01^{b}
TA (g/L)	5.81±0.01^{c}	6.60±0.03^{a}	5.65±0.03^{d}	6.50±0.02^{b}
VA (g/L)	0.35±0.01^{b}	0.37±0.01^{b}	0.43±0.01^{a}	0.35±0.01^{b}
MA (g/L)	1.33±0.01^{c}	3.23±0.04^{a}	0.23±0.01^{d}	2.61±0.03^{b}
LA (g/L)	0.70±0.02^{b}	ND	1.16±0.02^{a}	0.02±0.01^{c}
DRE (g/L)	28.0±0.1^{b}	29.2±0.2^{a}	27.5±0.1^{c}	29.2±0.1^{a}
Ashes (g/L)	2.92±0.01^{b}	3.03±0.04^{a}	2.78±0.01^{c}	2.75±0.03^{c}
F (mg/L)	1316±11^{c}	1616±94^{a}	1472±121^{b}	1534±66^{b}
A (mg/L)	387±12^{b}	452±24^{a}	392±20^{b}	385±21^{b}
TP (mg/L)	1512±73^{d}	1815±53^{a}	1606±30^{c}	1677±34^{b}
FRV (mg/L)	429±25^{bc}	636±11^{a}	458±15^{b}	415±25^{c}
P (mg/L)	725±44^{d}	1221±24^{c}	1508±20^{a}	1457±15^{b}
FRV/P	0.59	0.52	0.30	0.28
AA (μmol/L)	8822±260^{c}	10330±389^{a}	9465±349^{b}	10230±332^{a}
CI (pathlength 1 mm)	1.01±0.01^{d}	1.15±0.02^{b}	1.05±0.01^{c}	1.22±0.03^{a}
T (pathlength 1 mm)	0.56±0.01^{a}	0.55±0.01^{a}	0.54±0.01^{a}	0.55±0.01^{a}

P-C, Primitivo control; P-CM, Primitivo cryomacerated; P-UBF, Primitivo ultrasound before fermentation; P-UAF, Primitivo ultrasound after fermentation. E, ethanol; TA, titratable acidity: as tartaric acid; VA, volatile acidity: as acetic acid; MA, malic acid; LA, lactic acid; DRE, dry reduced extract; F, flavonoids: as (+)-catechin; A, anthocyanins: as malvidin-3-*O*-glucoside; TP, total polyphenols: as gallic acid; FRV, flavans reactive with vanillin: as (+)-catechin; P, proanthocyanidins: as cyanidin chloride; CI, colour intensity; T, tonality. ND, not detected. †In rows, data followed by different letters indicate statistically significant differences at $P < 0.05$.

Table 5

Compounds	P-C	P-CM	P-UBF	P-UAF
Dp	†3.8±0.2^{b}	4.7±0.2^{a}	3.9±0.2^{b}	2.6±0.1^{c}
Cy	1.1±0.1^{a}	1.1±0.1^{a}	1.0±0.1^{ab}	0.8±0.1^{b}
Pt	6.4±0.5^{b}	8.0±0.1^{a}	6.4±0.1^{b}	4.6±0.1^{c}
Pn	6.6±0.4^{b}	8.8±0.6^{a}	7.0±0.3^{b}	4.4±0.2^{c}
Mv	57.4±4.1^{b}	65.7±1.6^{a}	56.3±0.8^{b}	40.6±0.2^{c}
Dp-Ac	1.1±0.1^{b}	2.0±0.1^{a}	1.7±0.3^{a}	2.0±0.1^{a}
Pt-Ac	0.8±0.1^{ab}	0.9±0.1^{a}	0.6±0.1^{b}	0.5±0.1^{b}
Pn-Ac	ND	0.4±0.1^{a}	0.3±0.1^{a}	ND
Mv-Ac	2.2±0.1^{b}	2.8±0.1^{a}	2.1±0.1^{b}	1.6±0.1^{c}
cis-Mv-Cm	0.6±0.1^{a}	0.5±0.1^{a}	0.6±0.1^{a}	0.6±0.1^{a}
Mv-Cf	1.2±0.1^{a}	0.7±0.2^{b}	0.6±0.1^{b}	1.2±0.2^{a}
Pt-Cm	1.1±0.1^{a}	0.9±0.2^{a}	0.5±0.1^{b}	ND
Pn-Cm	ND	1.5±0.1^{a}	1.3±0.1^{a}	0.8±0.1^{b}
trans-Mv-Cm	4.6±0.1^{c}	6.2±0.2^{a}	4.9±0.1^{b}	3.4±0.1^{d}
Total anthocyanins	86.9±6.1^{b}	106.5±2.6^{a}	88.3±2.2^{b}	63.2±0.1^{c}

P-C, Primitivo control; P-CM, Primitivo cryomacerated; P-UBF, Primitivo ultrasound before fermentation; P-UAF, Primitivo ultrasound after fermentation. Dp, delphinidin-3-*O*-glucoside; Cy, cyanidin-3-*O*-glucoside; Pt, petunidin-3-*O*-glucoside; Pn, peonidin-3-*O*-glucoside; Mv, malvidin-3-*O*-glucoside; Dp-Ac, delphinidin-3-*O*-acetylglucoside; Pt-Ac, petunidin-3-*O*-acetylglucoside; Mv-Ac, malvidin-3-*O*-acetylglucoside; Dp-Cm, delphinidin-3-*O*-coumarylglucoside; *cis*-Mv-Cm, *cis*-malvidin-3-*O*-coumarylglucoside; Mv-Cf, malvidin-3-*O*-caffeylglucoside; Pt-Cm, petunidin-3-*O*-coumarylglucoside; Pn-Cm, peonidin-3-*O*-coumarylglucoside; *trans*-Mv-Cm, *trans*-malvidin-3-*O*-coumarylglucoside. ND, not detected. †In rows, data followed by different letters indicate statistically significant differences at $P < 0.05$.

Table 6

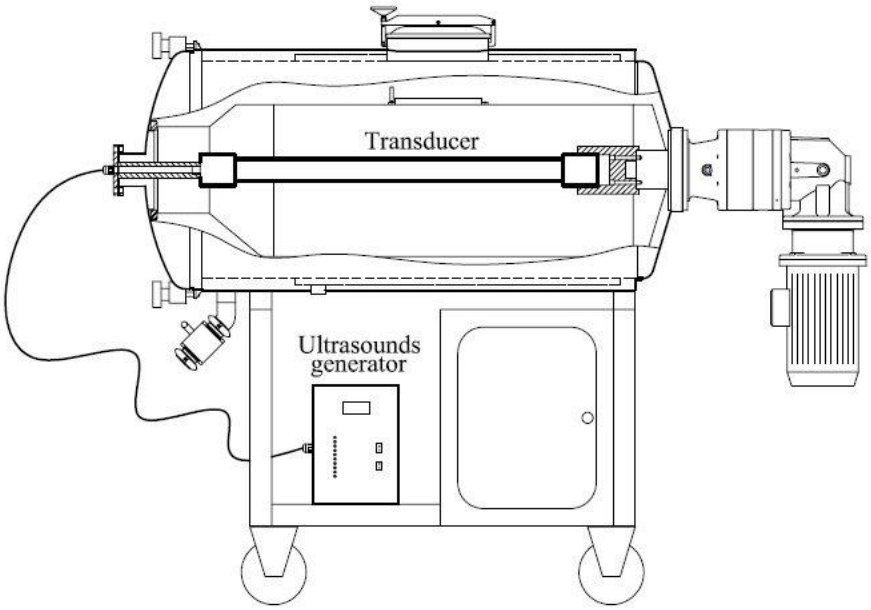
Parameters	NT-DC	NT-DU	A-DC	A-DU
E (% v/v)	†11.82±0.05^{b}	12.39±0.07^{a}	12.73±0.05^{y}	13.07±0.08^{x}
pH	3.51±0.03^{a}	3.52±0.03^{a}	3.07±0.03^{x}	3.07±0.02^{x}
TA (g/L)	5.73±0.07^{a}	5.51±0.06^{b}	8.98±0.06^{x}	9.07±0.07^{x}
VA (g/L)	0.23±0.01^{a}	0.24±0.03^{a}	0.24±0.02^{x}	0.26±0.03^{x}
MA (g/L)	2.79±0.05^{a}	2.59±0.06^{b}	4.56±0.08^{x}	4.62±0.07^{x}
LA (g/L)	ND	ND	ND	ND
DRE (g/L)	25.7±0.3^{a}	25.8±0.3^{a}	27.7±0.3^{y}	28.8±0.5^{x}
Ashes (g/L)	2.91±0.04^{a}	2.95±0.03^{a}	2.33±0.04^{y}	2.45±0.05^{x}
F (mg/L)	1553±23^{a}	1508±26^{a}	1248±22^{y}	1437±32^{x}
A (mg/L)	375±18^{a}	388±20^{a}	338±15^{y}	398±25^{x}
TP (mg/L)	1873±35^{b}	1975±47^{a}	1182±50^{y}	1343±46^{x}
FRV (mg/L)	875±35^{a}	835±37^{a}	923±33^{x}	861±27^{y}
P (mg/L)	2208±41^{a}	2072±36^{b}	1487±20^{y}	1574±26^{x}
FRV/P	0.40	0.40	0.62	0.55
AA (μmol/L)	10500±283^{a}	10819±605^{a}	8415±435^{y}	9433±260^{x}
CI (pathlength 1 mm)	0.66±0.03^{a}	0.67±0.02^{a}	1.25±0.03^{y}	1.50±0.05^{x}
T (pathlength 1 mm)	0.56±0.02^{a}	0.56±0.03^{a}	0.47±0.03^{x}	0.45±0.04^{x}

NT-DC, Nero di Troia diluted control; NT-DU, Nero di Troia diluted ultrasound; A-DC, Aglianico diluted control; A-DU, Aglianico diluted ultrasound. E, ethanol; TA, titratable acidity: as tartaric acid; VA, volatile acidity: as acetic acid; MA, malic acid; LA, lactic acid; DRE, dry reduced extract; F, flavonoids: as (+)-catechin; A, anthocyanins: as malvidin-3-*O*-glucoside; TP, total polyphenols: as gallic acid; FRV, flavans reactive with vanillin: as (+)-catechin; P, proanthocyanidins: as cyanidin chloride; CI, colour intensity; T, tonality. ND, not detected. †In rows, data followed by different letters (^{a,b}Nero di Troia, ^{x,y}Aglianico) indicate statistically significant differences at $P < 0.05$.

Table 7

Compounds	NT-DC	NT-DU	A-DC	A-DU
Dp	5.2±0.3^{a}	5.6±0.2^{a}	†5.7±0.3^{y}	6.9±0.5^{x}
Cy	0.4±0.1^{a}	0.5±0.2^{a}	0.4±0.1^{x}	0.5±0.1^{x}
Pt	6.9±0.6^{a}	7.2±0.4^{a}	6.8±0.5^{y}	8.7±0.4^{x}
Pn	2.3±0.3^{a}	2.5±0.2^{a}	2.9±0.4^{y}	3.8±0.5^{x}
Mv	48.2±0.6^{b}	49.9±0.4^{a}	50.6±0.7^{y}	59.0±0.8^{x}
Dp-Ac	1.5±0.2^{a}	1.5±0.3^{a}	5.9±0.4^{y}	6.9±0.4^{x}
Pt-Ac	2.0±0.2^{a}	2.0±0.3^{a}	0.3±0.1^{y}	0.8±0.2^{x}
Pn-Ac	1.4±0.3^{a}	1.5±0.3^{a}	0.1±0.1^{x}	0.2±0.1^{x}
Mv-Ac	19.3±0.6^{a}	19.8±0.3^{a}	1.6±0.2^{x}	2.0±0.3^{x}
cis-Mv-Cm	0.3±0.1^{a}	0.3±0.1^{a}	0.9±0.2^{x}	1.1±0.2^{x}
Mv-Cf	1.4±0.3^{a}	1.5±0.3^{a}	1.3±0.3^{y}	2.3±0.4^{x}
Pt-Cm	1.1±0.1^{a}	1.2±0.3^{a}	0.4±0.1^{x}	0.3±0.1^{x}
Pn-Cm	0.9±0.2^{a}	1.1±0.1^{a}	0.1±0.1^{x}	0.3±0.1^{x}
trans-Mv-Cm	7.7±0.8^{a}	7.9±0.6^{a}	3.0±0.5^{y}	4.5±0.6^{x}
Total anthocyanins	98.4±1.1^{b}	102.4±2.0^{a}	80.2±1.7^{y}	96.9±2.3^{x}

NT-DC, Nero di Troia diluted control; NT-DU, Nero di Troia diluted ultrasound; A-DC, Aglianico diluted control; A-DU, Aglianico diluted ultrasound. Dp, delphinidin-3-*O*-glucoside; Cy, cyanidin-3-*O*-glucoside; Pt, petunidin-3-*O*-glucoside; Pn, peonidin-3-*O*-glucoside; Mv, malvidin-3-*O*-glucoside; Dp-Ac, delphinidin-3-*O*-acetylglucoside; Pt-Ac, petunidin-3-*O*-acetylglucoside; Mv-Ac, malvidin-3-*O*-acetylglucoside; Dp-Cm, delphinidin-3-*O*-coumarylglucoside; *cis*-Mv-Cm, *cis*-malvidin-3-*O*-coumarylglucoside; Mv-Cf, malvidin-3-*O*-caffeylglucoside; Pt-Cm, petunidin-3-*O*-coumarylglucoside; Pn-Cm, peonidin-3-*O*-coumarylglucoside; *trans*-Mv-Cm, *trans*-malvidin-3-*O*-coumarylglucoside. †In rows, data followed by different letters (^{a,b}Nero di Troia, ^{x,y}Aglianico) indicate statistically significant differences at $P < 0.05$.



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