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#### **GOfermentor trials in Piedmont**

2016 harvest

**FINAL REPORT** 



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#### Introduction

The GOfermentor technology was developed by Dr. Vijay Singh in USA in order to achieve a better vinification process:

- automation of cap management and easily control of maceration-fermentation parameters;
- 2. control of air exposure effects with reduction of air damage on wine quality;
- 3. reduction of wash water use (very important in warm areas).

## Purpose

The goal of trials was the comparison between the GOfermentor technology and the traditional winemaking of autochthonous red grapes grown in South Piedmont (Italy):

- 1. Barbera
- 2. Nebbiolo

Nebbiolo grapes are characterized by a relatively low anthocyanin content and a high tannin content. Instead, Barbera grapes are rich in anthocyanins with an average content of tannins. In both cases the maceration must be managed very carefully. These grapes cultivars are generally harvested at different times: Barbera before and then Nebbiolo.

## Experimental Design

The trials were conducted in the "Azienda Agricola Castello di Neive" wine cellar in Neive village (CN, Italy). The vinifications were made during 2016 harvest period (September-October).

For each variety (Barbera or Nebbiolo) we worked separately using two GOfermentor units, using grapes harvested in different vineyards (Table 1). Traditional vinification into stainless steel fermentors (c.a. 5'000 kg) were done for each vineyard sample, while for GOfermentor vinifications it was used about 850 kg of grapes of same lot.

Table 1 – Trials samples and sample codes

Grape cultivar	ultivar Vineyard		Control (traditional vinification)
Barbera	B1 -Santo Stefano SC	B1F	B1C
barbera	B2 – Santo Stefano AP	B2F	B2C
Nebbiolo	N1 – Santo Stefano VV	N1F	N1C
Neppioio	N2 – Val Torta N	N2F	N2C

In all trials, were carried out the same additives for the control and GOfermentor trial (Table 2). The cap management (punch down/pump over) on traditional vinifications were those scheduled by the winery and traditional for the variety: Table 3 summarize the cap management cycle used. The GOfermentor equipment was set up with a punch schedule of twelve punches per day (one punch every 2 hours), a punch duration of 300 seconds with 10-seconds off cycles. These parameters were evaluated and tuned after preliminary tests in order to enhance the extraction for the subsequent Barbera and Nebbiolo vinifications, and may differ depending on the vintage and on the grape variety. The maximum temperature was set at 28 °C for all trials. The racking was performed when the control density was less than 1 Brix, and the run-off wine obtained.

The monitoring of trials was carried out by analytical tests summarized in Table 4. Moreover, wines were evaluated by a sensory panel to assess the organoleptic characteristics (visual, aroma and taste).

Table 2 Additives used during fermentation.

Trial	Туре	Description
B1C, B1F	SO <sub>2</sub>	NO
	LSA yeast	150 g/t (Uvaferm 43)
	Nutrient product	150 g/t (Fermoplus)
	Tannins	30 g/t (Grapetan)
B2C, B2F	SO <sub>2</sub>	50 mg/kg
	LSA yeast	150 g/t (Uvaferm 43)
	Nutrient product	150 g/t (Enovit)
	Tannins	50 g/t (Grapetan)
N1C, N1F	SO <sub>2</sub>	50 mg/kg
	LSA yeast	150 g/t (Uvaferm 43)
	Nutrient product	150 g/t (Enovit)
	Tannins	30 g/t (Grapetan)
N2C, N2F	SO <sub>2</sub>	50 mg/kg
	LSA yeast	150 g/t (D254)
	Nutrient product	150 g/t (Fermoplus)
	Tannins	30 g/t (Grapetan)

Table 3 Cap management schema (punch down: PD; pump over: PO).

Trial	Type	Cycle schema
All GOfermentor (F)	PD	5 minutes every 2 hours
B1C	РО	5 minutes every 5 hours per 3 days, after 12 minutes every 5 hours per 7 day
B2C	РО	5 minutes every 5 hours per 3 days, after 15 minutes every 5 hours per 8 days
N1C	PO	5 minutes every 5 hours per 7 days, after 15 minutes every 5 hours per 10 days
N2C	РО	5 minutes every 8 hours per 5 days, after 10 minutes every 8 hours per 7 days

Table 4 - Analytical Tests performed.

Parameters	Product*	Symbol	Unit	References
Grape indices				
Cell maturity index	g	Ea	1	Glories and Augustin, 1993; Cagnasso et al., 2008
Seed maturity index	g	Мр	1	Glories and Augustin, 1993; Cagnasso et al., 2008
Anthocyanin potential	g	A1	mg/L	Glories and Augustin, 1993; Cagnasso et al., 2008
Easy extractable anthocyanin	g	A3.2	mg/L	Glories and Augustin, 1993; Cagnasso et al., 2008
Technological indices				
Density Index (Brix scale)	f	DI	°Bx	
Alcoholic strength % vol.	w	TAV	1	OIV, 2016
Glucose and fructose	w		g/L	OIV, 2016
Dry matter g/L	w	EST	g/L	OIV, 2016
Total acidity (as tartaric acid)	g, f, w	TAc	g/L	OIV, 2016
Volatile acidity (as acetic acid)	w	VA	g/L	OIV, 2016
Tartaric acid	w	Ta	g/L	Schneider et al., 1987
Malic acid	w	Ma	g/L	Schneider et al., 1987
Lactic acid		La	g/L	Schneider et al., 1987
Total Sulfur dioxide	w	TSO	mg/L	OIV, 2016
Polyphenol indices	*			
Total anthocyanins index	f, w	TA	mg/L	Di Stefano et al., 1989, 1991
Total polyphenols (Folin Ciocalteu) as (+) catechin	w	FC	mg/L	al., 1989, 1991
Total flavonoids index	f, w	TF	mg/L	Di Stefano et al., 1989
Absorbance to 280 nm	f, w	A280	1	Ribereau-Gayon, 1970
Flavanol Vanillin Assay index (as (+) catechin)	w	VAN	mg/L	Di Stefano et al., 1989
Proanthocyanidines (as cyanidin chloride)	w	PRO	mg/L	Di Stefano et al., 1989
Anthocyanin profile by HPLC	w		1	OIV, 2016
Color indices				
Color density (OP=10 mm)	f, w	CD	1	OIV, 2016; Glories, 1984
Color tone	f, w	СТ	1	OIV, 2016; Glories, 1984
CIELAB parameters	w			OIV, 2016
<ul> <li>red-green</li> </ul>		a*		
<ul> <li>yellow-blue</li> </ul>		b*		
• Croma		C*		
• Hue		H*	rad	
<ul> <li>Clarity</li> </ul>		L*		
Cofactors	w	A365	1	Boulton, 1996
Copigmentation color fraction	w	CC	1	Boulton, 1996
Anthocyanin color fraction	w	ACF	1	Boulton, 1996
Polimeric pigment color fraction	w	PPC	1	Boulton, 1996

<sup>\*</sup> grapes, f: maceration-fermentation, w: wine

# Wine-making

The grapes were picked at ripeness, and a small sample was randomly taken to assess the principal grape characteristics at harvest, which are shown in Table 5. The grapes were crushed and destemmed, and the fermentors were filled.

The winemaking operations in GOfermentor trials are shown in Figures 1.1, 1.2 and 1.3

Table 5 – Grape ripeness indices of Barbera and Nebbiolo samples at harvest

Grape sample:			B1	B2	N1	N2
Parameters		u.m.				
Harvest date			Sept. 19 <sup>th</sup>	Sept. 21 <sup>th</sup>	Oct. 3 <sup>rd</sup>	Oct. 7 <sup>th</sup>
Cell maturity index	Ea	1	43.2 %	45.5 %	25.5 %	34.5 %
Seed maturity index	Мр	1	62.9 %	67.3 %	67.0 %	63.7 %
Anthocyanin potential	A1	mg/L	1279	1214	493	548
Easy extractable anthocyanin	A3.2	mg/L	726	661	367	359
Density index (sugar content)	DI	°Bx	25.9	24.2	24.9	24.2
Total acidity (as tartaric acid)	TAc	g/L	9.2	10.3	6.0	6.3
рН			3.01	3.06	3.22	3.12





Figure 1.1 Filling operation of GOfermentor trials



Figure 1.2 GOfermentor during maceration and fermentation

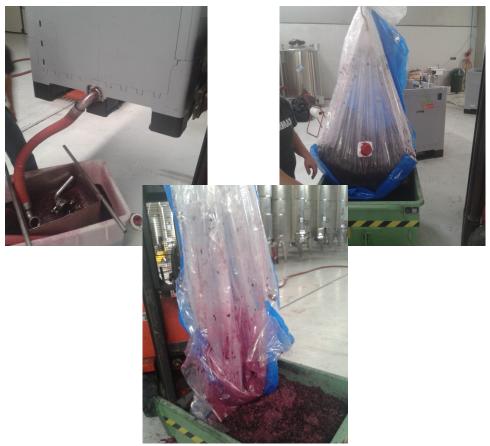


Figure 1.3 Wine running-off and racking operations of GOfermentor trials

#### Barbera Maceration

The high sugar content achieved by Barbera grapes did not cause any problems during the fermentation of B1 trial (Figure 2.1). In fact, there are no significant differences between control and GOfermentor. In B2 trial (Figure 2.2), a delay of approximately 2 days of the fermentative process in GOfermentor appears likely due to a state of stress of yeasts caused by insufficient ventilation in the second part of the process.

The extraction of the total flavonoids in trial B1 (Figure 3.1) has taken place almost in the first 6 days. Afterwards the control trial of appeared more efficient. Instead, in the B2 trial, the control extracted an higher flavonoid content after the second day of maceration (Figure 3.2).

The extraction of anthocyanins using GOfermentor (B1, Figure 4.1) is superior to the control in the first 5 days, then the control continues the extraction while the GOfermentor trend remains almost stable. The control reached the maximum total anthocyanins value between the sixth and seventh maceration day, while the GOfermentor trial reached the maximum with one day of delay. Figure 4.2 shows the anthocyanin extraction of B2: after the first 24 hours the control tends to extract more, and the control curves and the GOfermentor show a near parallel pattern. The maximum value of total anthocyanins was reached by both at the end of the maceration. The color intensity of the B1 trials (Figure 5.1) shown a similar pattern to that

of anthocyanin extraction with prevalence in control trial after the first 5 days. Similar behavior with increased differences was found in B2 experiment (Figure 5.2).

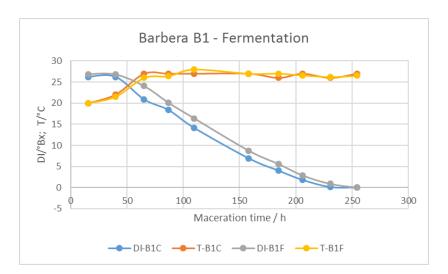


Figure 2.1. Evolution of density (DI) and temperature (T) during skin contact in control (C) and GOfermentor (F) trials.

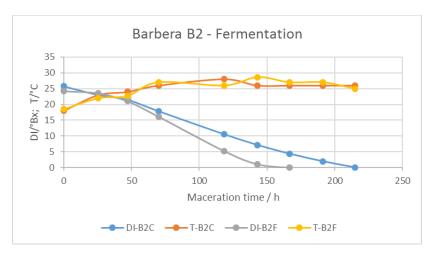


Figure 2.2. Evolution of density (DI) and temperature (T) during skin contact in control (C) and GOfermentor (F) trials.

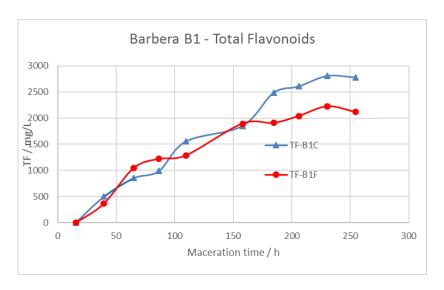


Figure 3.1. Evolution of total flavonoids (TF) during skin contact in control (C) and GOfermentor (F) trials.

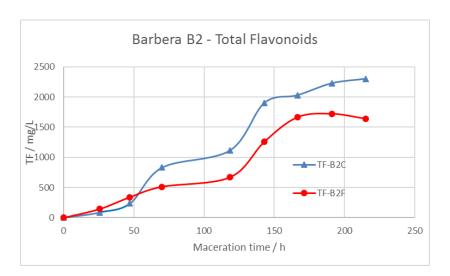


Figure 3.2. Evolution of total flavonoids (TF) during skin contact in control (C) and GOfermentor (F) trials.

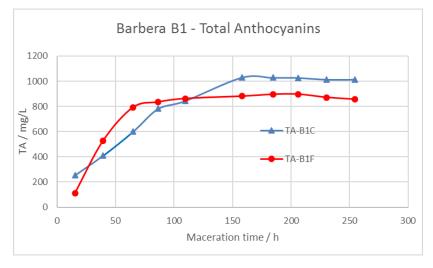


Figure 4.1. Evolution of total anthocyanins (TA) during skin contact in control (C) and GOfermentor (F) trials

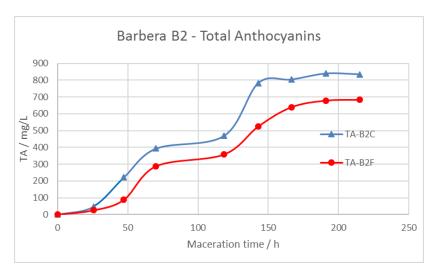


Figure 4.2. Evolution of total anthocyanins (TA) during skin contact in control (C) and GOfermentor (F) trials

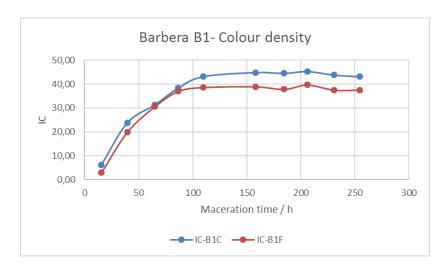


Figure 5.1. Evolution of colour density (IC) during skin contact in control (C) and GOfermentor (F) trials.

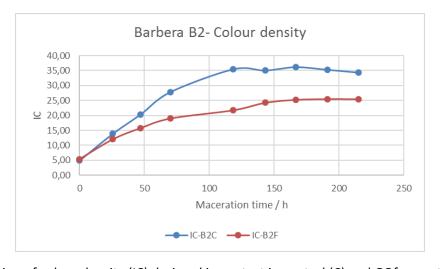


Figure 5.2. Evolution of colour density (IC) during skin contact in control (C) and GOfermentor (F) trials.

#### **Nebbiolo Maceration**

The fermentation process (Figures 6.1 and 6.2) shown for both Nebbiolo trials a delay for the GOfermentor in the consumption of sugars, probably due to lower ambient temperature, especially in the N2 test.

Extraction of the total flavonoids of the N1 test (Figure 7.1) shown a prevalence in control trial over the GOfermentor after the first 4 days. The differences persist also by prolonging maceration of the GOfermentor. In the N2 trial (figure 7.2) there is an extraction similar to the N1 test but with the continuation of the maceration the differences with the control almost disappear.

Anthocyanin extraction (Figure 8.1) gave better results for the GOfermentor in N1 trial until the fourth day. After that point, the control continued the slow extraction of anthocyanins for two days, while the GOfermentor trial was stable with very little increases. The control and the GOfermentor have both reached the maximum on the sixth day. In the N2 test, the GOfermentor was better in the first 4 days, then the extraction curves overlapped and the GOfermentor reached the maximum in the eighth day extraction. The control achieved maximum extraction after about 10 days with values similar to the GOfermentor (Figure 8.2).

The color density in the N1 test shown a similar pattern as the anthocyanin extraction. GOfermentor showed higher values over the first 4 days. Afterwards, the color density values were almost comparable between control and GOfermentor after 12 days of maceration (Figure 9.1). The GOfermentor in the N2 test (Figure 9.2) showed higher or equal color density values throughout maceration. The maximum color density in the GOfermentor trial was found on the eleventh day. The color density of the control at the 13<sup>th</sup> day was slightly less than that of the GOfermentor.

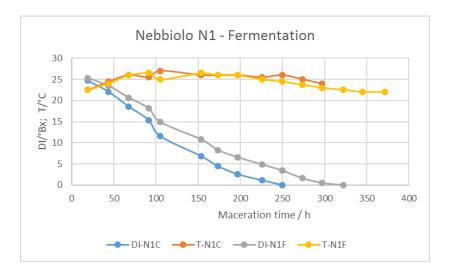


Figure 6.1. Evolution of density (DI) and temperature (T) during skin contact in control (C) and GOfermentor (F) trials.

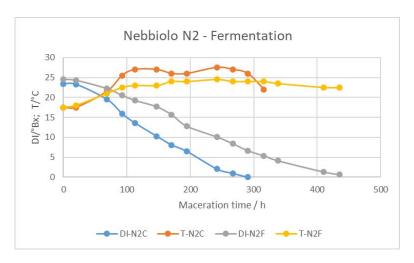


Figure 6.2. Evolution of density (DI) and temperature (T) during skin contact in control (C) and GOfermentor (F) trials.

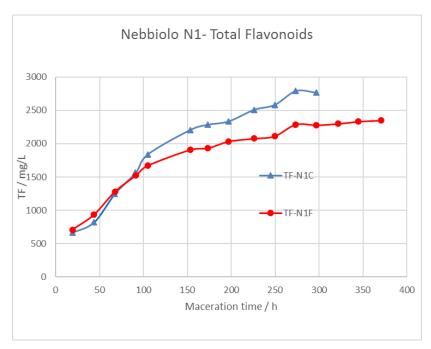


Figure 7.1. Evolution of total flavonoids (TF) during skin contact in control (C) and GOfermentor (F) trials.

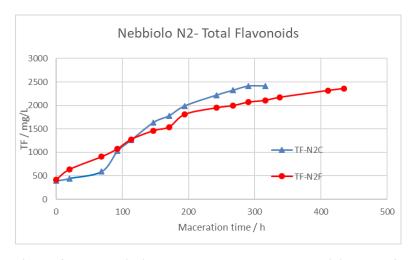


Figure 7.2. Evolution of total flavonoids (TF) during skin contact in control (C) and GOfermentor (F) trials.

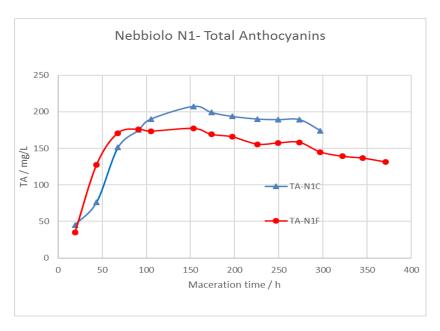
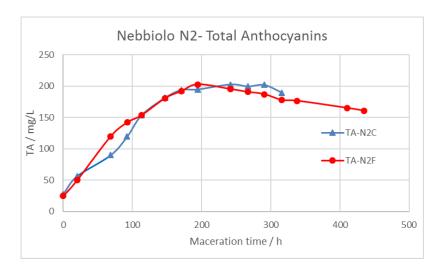


Figure 8.1. Evolution of total anthocyanins (TA) during skin contact in control (C) and GOfermentor (F) trials



Figue 8.2. Evolution of total anthocyanins (TA) during skin contact in control (C) and GOfermentor (F) trials.

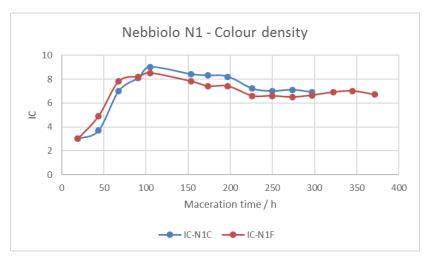


Figure 9.1. Evolution of colour density (IC) during skin contact in control (C) and GOfermentor (F) trials.

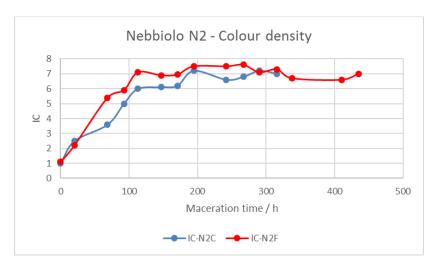


Figure 9.2. Evolution of colour density (IC) during skin contact in control (C) and GOfermentor (F) trials.

#### Wine characteristics

After the first racking the wines were placed in 1-hL stainless steel vats. The analytical characteristics of Barbera (B1 and B2) test wines in two points, one week after racking (V1) and before bottling (V2), are summarized in Table 6.1. The analogue Nebbiolo test parameters (N1 and N2) are given in Table 6.2.

Barbera wines are characterized by a high alcohol content and a high total acidity, in line with the characteristics of the cultivar. The high degree of ripeness of grapes has also been demonstrated by low concentrations of malic acid (<1.2 g/L). The volatile acidity content after racking did not exceed 0.48 g/L and did not show substantial differences between the control and the GOfermentor produced wines.

In Nebbiolo wines (N1 and N2) we noticed the typical alcohol values of the variety, and in the N2 test there is still a high sugar residue, also present in the N1FV1 sample. The presence of a high sugar residue has been the consequence of the slowing down of the fermentation process, probably caused by the difficulty of controlling the temperature or by a vinification in a atmosphere with low oxygen content. The volatile acidity of Nebbiolo wines is slightly higher than that of the Barbera wines and seemed to be a consequence of the slowest fermentation at the end of the process.

After the racking, a weekly control of the MLF was carried out using HPLC analysis. The MLF process took place spontaneously and was completed on December 19<sup>th</sup> in the following wines: B1C, B2C, B2F, N1C and N2F. At the same date the MLF was not completed in B1F, N1F and N2F wines. The process in the subsequent controls did not complete and the wine storage at 18-20 °C was interrupted to avoid spoilages. With increasing spring temperatures, the MLF did not start. Therefore, as the residual malic acid content was not high and to avoid possible microbiological spoilage, we have added sulfur dioxide in all wines and we proceeded with the stabilization and bottling. Tartaric stabilization was carried out by cold storage at a temperature of 2°C for 4 weeks before bottling.

Table 6.1 Chemical parameters of of Barbera wines: control (C) and GOfermentor (F), after running-off (V1) and before bottling (V2).

	Wine Samples							
Parameters	B1CV1	B1FV1	B2CV1	B2FV1	B1CV2	B1FV2	B2CV2	B2FV2
Alcohol by volume (%)	15.53	14.99	15.22	14.83	15.73	15.55	15.29	14.95
Dry matter g/L	36.5	35.9	35.9	35.3	32.9	32.5	30.1	29.4
Sum of glucose and fructose g/L	2.7	2.9	2.6	1.8	1.4	1.2	1.2	0.6
Titratable acidity (as tartaric acid)								
g/L	9.23	9.19	9.26	9.11	8.03	7.80	7.50	8.63
Volatile acidity (as acetic acid) g/L	0.48	0.45	0.41	0.30	0.49	0.53	0.63	0.85
Total SO <sub>2</sub> mg/L	50	67	43	45	43	34	31	41
рН	3.23	3.25	3.32	3.28	3.26	3.22	3.33	3.30
Tartaric acid g/L	4.3	4.4	4.3	4.8	3.7	3.7	3.1	3.9
Malic acid g/L	1.1	1	1.2	nd	nd	0.9	nd	nd
Lactic acid g/L	< 0.15	< 0.15	< 0.15	1.16	0.83	0.18	1.10	1.18

nd: not detectable

Table 6.2 Chemical parameters of Nebbiolo wines: control (C) and GOfermentor (F), after running-off (V1) and before bottling (V2).

	Wine Samples							
Parameters	N1CV1	N1FV1	N2CV1	N2FV1	N1CV2	N1FV2	N2CV2	N2FV2
Alcohol by volume (%)	14.38	14.65	13.71	13.79	14.46	14.80	14.20	14.57
Dry matter g/L	28.1	30.7	31.8	32.6	26.5	26.80	24.6	26.6
Sum of glucose and fructose g/L	2.3	6.0	6.9	13.1	0.6	0.7	0.7	1.0
Titratable acidity (as tartaric acid) g/L	6.04	6.30	5.89	6.68	6.75	7.95	8.10	7.65
Volatile acidity (as acetic acid) g/L	0.59	0.67	0.52	0.59	0.60	0.73	0.51	0.80
Total SO <sub>2</sub> mg/L	43	40	38	46	33	47	43	40
рН	3.50	3.53	3.48	3.38	3.57	3.48	3.57	3.44
Tartaric acid g/L	1.7	1.7	2.0	2.1	2.6	2.4	2.5	2.3
Malic acid g/L	0.7	0.7	1.0	1.0	nd	0.6	nd	0.7
Lactic acid g/L	< 0.15	< 0.15	< 0.15	< 0.15	0.83	0.37	0.91	0.46

nd: not detectable

#### Wine phenolic profile

Tables 7.1 (Barbera) and 7.2 (Nebbiolo) shown the relative analyzed parameters of the phenolic substances.

In particular, as expected a significant decrease in total anthocyanins (TA) was observed between V1 and V2 points in all Barbera and Nebbiolo wines. The anthocyanin profile of the wines, represented in Tables 8.1 (Barbera) and 8.2 (Nebbiolo), is typical of varieties tested: high content of peonidin-3-G and low content of acylated anthocyanins in Nebbiolo wines, high content of malvidin-3-G and acylated anthocyanins in Barbera wines. There were not significant differences between the control and the GOfermentor trials.

In the case of Barbera, the levels of total phenols (FC) and proanthocyanidins (PRO) reached high values that allowed a possible long wine aging process. FC and PRO values were lower in GOfermentor tests. This difference is justified by the type of grape which is poor in tannins in the skins. In the vinification of Barbera grapes, tannins must be extracted from the grapes to obtain structured wines. Barbera's GOfermentor tests showed a lower concentration of low molecular weight flavanols (VANs) which are present in particular in the seeds. This aspect is positive, because it favors a higher degree of polimerizarion of higher tannins as they show the higher values of the PRO/VAN ratio. Comparison between sampling points (V2 vs V1) showed a slight increase in PRO/VAN ratio values, indicating an evolution of the tannins structure during the first period of aging.

All Nebbiolo produced wines have reached very important levels of total phenols (FC) and proanthocyanidin (PRO), which is typical of the variety. For Nebbiolo, a grape variety used for long-aging Barbaresco and Barolo wines, is essential to extract these phenolic substances from the grape skin during maceration. Consequently, the differences between the control and GOfermentor tests are smaller compared to what was observed in the Barbera trials.

The PRO/VAN ratio, a parameter correlated with the average degree of polymerization of tannins, is low for Nebbiolo (always less than 2), but higher in GOfermentor N2 trials with respect to control. This ratio has grown slightly over time (V2 vs V1). The evolution of tannins is indispensable for these wines and it will require aging in oak casks or other means to perform. In fact, permeation of micro-doses of oxygen is crucial for the evolution of wine sensory properties.

Table 7.1 Phenolic parameters of Barbera wines: control (C) and GOfermentor (F), after running-off (V1) and before bottling (V2).

	Wine Samples								
Parameters	B1CV1	B1CV1 B1FV1 B2CV1 B2FV1 B1CV2 B1FV2 B2CV2 B2FV2							
Total anthocyanins (TA) mg/L	913	872	779	612	507	449	513	303	
Total polyphenols (FC) mg/L	3267	3033	2859	2205	2803	2500	2634	1984	
Total flavonoids (TF) mg/L	2772	2249	2326	1638	1967	1451	1849	1111	
Absorbance to 280 nm	82.25	72.15	74.6	57.4	74.25	66.75	68.15	52.5	
Proanthocyanidines (PRO) mg/L	3168	2631	2781	1835	2638	2478	2620	1670	
Flavanol Vanillin Assay (VAN) mg/L	1434	865	1067	573	999	711	1051	518	
PRO/VAN ratio	2.21	3.04	2.61	3.20	2.64	3.48	2.49	3.22	

Table 8.1 Anthocyanin profile (%) of Barbera wines: control (C) and GOfermentor (F), after running-off (V1) and before bottling (V2).

	Wine Samples								
Parameters	B1CV1	B1FV1	B2CV1	B2FV1	B1CV2	B1FV2	B2CV2	B2FV2	
Delphinidin 3-O-glucoside	10.6	11.0	8.2	10.6	11.1	12.9	10.8	9.6	
Cyanidin 3-O-glucoside	3.5	4.2	3.2	4.6	4.7	5.6	5.2	3.9	
Petunidin 3-O-glucoside	13.1	13.1	11.0	12.9	13.4	14.0	13.3	11.8	
Peonidin 3-O-glucoside	5.7	6.9	6.4	7.7	6.8	8.5	7.9	6.3	
Malvidin 3-O-glucoside	43.7	42.2	47.2	43.2	43.5	41.4	43.2	47.7	
Delphinidin 3-O-glucoside acetate	2.1	2.2	1.6	1.8	2.0	2.4	1.8	1.4	
Cyanidin 3-O-glucoside acetate	1.2	1.4	1.2	1.4	2.4	1.6	1.4	1.3	
Petunidin 3-O-glucoside acetate	2.2	2.1	1.9	2.0	1.9	2.1	2.2	1.9	
Peonidin 3-O-glucoside acetate	1.0	1.2	1.3	1.3	0.9	1.1	1.2	1.3	
Malvidin 3-O-glucoside acetate	8.4	7.7	8.6	7.1	6.1	4.9	6.5	7.6	
Delphinidin 3-O-glucoside p-coumarate	0.6	0.6	1.1	1.0	1.1	0.9	0.8	0.8	
Peonidin 3-O-glucoside caffeate	nd	nd	nd	nd	nd	nd	nd	nd	
Malvidin 3-O-glucoside caffeate	0.1	0.1	0.1	0.1	0.3	0.1	0.1	0.2	
Cyanidin 3-O-glucoside p-coumarate	0.8	0.9	0.9	0.8	0.9	0.7	0.8	0.6	
Petunidin 3- <i>O</i> -glucoside <i>p</i> -coumarate	1.4	1.1	1.1	1.0	1.1	1.0	0.9	1.1	
Peonidin 3- <i>O</i> -glucoside <i>p</i> -coumarate	0.7	0.8	0.9	0.7	0.6	0.4	0.6	1.0	
Malvidin 3-O-glucoside p-coumarate	4.9	4.3	5.3	3.7	3.3	2.2	3.3	3.6	
$\boldsymbol{\Sigma}$ monomer anthocyanins	76.5	77.4	76.0	79.1	79.5	82.4	80.5	79.3	
$\boldsymbol{\Sigma}$ acetylglucoside anthocyanidin	14.9	14.7	14.6	13.6	13.3	12.1	13.1	13.5	

nd: not detectable

Table 7.2 Phenolic parameters of Nebbiolo wines: control (C) and GOfermentor (F), after running-off (V1) and before bottling (V2).

	Wine Samples							
Parameters	N1CV1 N1FV1 N2CV1 N2FV1 N1CV2 N1FV2 N2CV2 N2FV2							N2FV2
Total anthocyanins (TA) mg/L	163	123	176	154	121	82	121	100
Total polyphenols (FC) mg/L	3661	3193	3306	3098	3583	2981	3189	2955
Total flavonoids (TF) mg/L	2879	2476	2569	2469	2956	2477	2606	2429
Absorbance to 280 nm	70.5	61.4	62.9	61.25	67.6	57.55	59.7	56.05
Proanthocyanidines (PRO) mg/L	4882	3975	3995	4012	4858	3858	4288	4371
Flavanol Vanillin Assay (VAN) mg/L	3125	2567	2800	2608	2873	2393	2690	2532
PRO/VAN ratio	1.56	1.55	1.43	1.54	1.69	1.61	1.59	1.73

Table 8.2 Anthocyanin profile (%) of Nebbiolo wines: control (C) and GOfermentor (F), after running-off (V1) and before bottling (V2).

	Wine Samples								
Parameters	N1CV1	N1FV1	N2CV1	N2FV1	N1CV2	N1FV2	N2CV2	N2FV2	
Delphinidin 3-O-glucoside	5.1	5.0	5.1	2.7	5.6	5.0	4.1	2.2	
Cyanidin 3-O-glucoside	2.7	2.3	2.4	0.9	3.4	2.9	1.9	1.5	
Petunidin 3-O-glucoside	6.8	6.3	6.3	5.9	7.0	7.2	6.6	6.5	
Peonidin 3-O-glucoside	22.3	23.5	20.2	16.6	23.1	22.4	18.5	13.7	
Malvidin 3-O-glucoside	46.5	47.9	51.6	56.3	48.4	48.1	57.6	59.8	
Delphinidin 3-O-glucoside acetate	1.7	0.8	1.0	1.3	0.9	0.8	0.6	1.2	
Cyanidin 3-O-glucoside acetate	1.4	1.2	1.0	1.3	0.6	2.3	0.6	1.2	
Petunidin 3-O-glucoside acetate	0.8	0.8	0.8	0.7	0.3	0.5	0.3	1.4	
Peonidin 3-O-glucoside acetate	2.4	2.6	1.9	2.6	2.5	3.8	1.9	3.4	
Malvidin 3-O-glucoside acetate	3.7	3.2	3.1	3.7	3.1	2.8	2.6	3.5	
Delphinidin 3-O-glucoside p-coumarate	0.5	0.3	0.4	0.6	0.5	1.0	0.3	0.9	
Peonidin 3-O-glucoside caffeate	nd	nd	nd	nd	nd	nd	nd	nd	
Malvidin 3-O-glucoside caffeate	0.4	0.5	0.3	0.4	nd	nd	nd	nd	
Cyanidin 3- <i>O</i> -glucoside <i>p</i> -coumarate	0.7	0.7	0.6	0.9	0.6	0.5	0.6	0.8	
Petunidin 3- <i>O</i> -glucoside <i>p</i> -coumarate	0.5	0.5	0.5	0.6	nd	nd	nd	nd	
Peonidin 3- <i>O</i> -glucoside <i>p</i> -coumarate	2.4	2.4	2.5	3.2	2.0	1.6	2.1	2.0	
Malvidin 3-O-glucoside p-coumarate	2.2	1.9	2.2	2.5	2.0	1.2	2.4	1.8	
$\Sigma$ monomer anthocyanins	83.3	85.1	85.5	82.3	87.5	85.5	88.7	83.7	
$\boldsymbol{\Sigma}$ acetylglucoside anthocyanidin	10.0	8.6	7.8	9.5	7.5	10.1	6.0	10.8	

nd: not detectable

#### Wine color parameters

The color parameters are shown in Tables 9.1 (Barbera) and 9.2 (Nebbiolo).

Barbera wines shown a color density greater than 25 absorbance units at first check after racking (V1). The higher values are those detected for the control trials, with biggest differences in B2 wine. These differences shrunk after the first phase of evolution (V2 vs V1) for trial B2, while for B1 the color density was found higher for the GOfermentor wine. Instead, color tone did not show differences between control and GOfermentor after racking (V1), as the values shown a clear predominance of red component (a\*) compared to the yellow component (b\*). Over time the color tone grows more for B1 control than GOfermentor, and vice versa for the B2 test.

The copigmentation indices shown interesting trends. The copigmentation color fraction (CC) is slightly higher for GOfermentor trials (time V1). During the first phase of evolution, CC decreases more than control but, at the same time, increases significantly the polymeric pigment color fraction (PPC): about 28% for B1FV2 and about 54% for B2FV2 with respect to control. In fact, the value of PPC expresses the fraction of more stable pigments.

Table 9.1 Color parameters of Barbera wines: control (C) and GOfermentor (F), after running-off (V1) and before bottling (V2).

	Wine Samples										
Parameters	B1CV1	B1FV1	B2CV1	B2FV1	B1CV2	B1FV2	B2CV2	B2FV2			
Copigmentation indices											
Cofactors (A <sub>365</sub> )	14.0	12.9	13.3	9.9	13.2	12.3	11.2	8.9			
Total phenols (A <sub>280</sub> )	82.3	72.2	74.6	57.4	74.3	66.8	68.2	52.5			
Copigmentation color fraction	0.53	0.54	0.49	0.52	0.27	0.19	0.33	0.14			
Anthocyanin color fraction	0.36	0.36	0.38	0.37	0.41	0.41	0.41	0.45			
Polymeric pigment color fraction	0.11	0.10	0.13	0.11	0.32	0.41	0.26	0.40			
<u>Color indices</u>											
Color tone	0.39	0.39	0.42	0.41	0.56	0.54	0.52	0.59			
Color density (OP=10 mm)	39.3	36.1	33.9	25.9	27.0	30.0	23.7	19.8			
L*	2.17	2.97	3.24	4.19	2.06	1.63	4.19	2.23			
a*	15.5	41.9	43.7	48.4	14.6	11.8	48.9	15.7			
b*	3.7	5.1	5.6	7.2	3.5	2.8	7.2	3.8			
C*	15.9	42.2	44.1	48.9	15.0	12.1	49.4	16.2			
H*	0.24	0.12	0.13	0.15	0.24	0.23	0.15	0.24			

The color parameters of Nebbiolo wines (Table 9.2) show much lower color densities (CD) than the corresponding Barbera wines. These differences derive from the lower content of anthocyanins of Nebbiolo grapes. CD values are higher in the control wines at the first evaluation (V1). At the second evaluation (V2),

the CD values are similar for all the trials because the lost of anthocyanin content are balanced by the formation of pigment less sensitive to bleaching by pH and sulfur dioxide actions (see PPC parameter in table 9.2).

Color tone (CT) showed higher values than those of the respective Barbera wines. These CT values are typically of Nebbiolo wines. The difference between CT values of N1 trials at V1 vanished at V2 evaluation.

The copigmentation indices showed a low effect of copigmentation on the color of Nebbiolo young wines due to the low content of copigmentation factors and anthocyanins. In all cases, copigmentation color fraction (CC) values are higher for control wines, while polymeric pigment color fraction (PPC) values were always higher for GOfermentor trials. This last observation highlights a process of stabilization of the more intense colored pigments for the GOfermentor assays, without any influence on the color tone.

Table 9.2 Color parameters of Nebbiolo wines: control (C) and GOfermentor (F), after running-off (V1) and before bottling (V2).

	Wine Samples										
Parameters	N1CV1	N1FV1	N2CV1	N2FV1	N1CV2	N1FV2	N2CV2	N2FV2			
Copigmentation indices											
Cofactors (A <sub>365</sub> )	7.3	6.4	5.8	6.2	7.1	6.0	5.7	5.9			
Total phenols (A <sub>280</sub> )	70.5	61.4	62.9	61.3	67.6	57.6	59.7	56.1			
Copigmentation color fraction	0.11	0.06	0.14	0.11	0.16	0.09	0.20	0.09			
Anthocyanin color fraction	0.60	0.58	0.62	0.64	0.44	0.42	0.46	0.49			
Polymeric pigment color fraction	0.29	0.36	0.24	0.25	0.40	0.49	0.34	0.41			
<u>Color indices</u>											
Color tone	0.69	0.73	0.61	0.61	0.88	0.87	0.80	0.79			
Color density (OP=10 mm)	7.7	6.0	7.2	6.5	6.6	6.3	5.6	6.5			
L*	22.03	27.02	23.45	26.33	22.45	21.45	27.47	21.23			
a*	54.8	57.9	56.3	58.6	51.4	49.7	55.8	51.4			
b*	44.4	47.9	45.3	47.6	43.9	41.3	48.2	41.1			
C*	70.5	75.2	72.3	75.5	67.6	64.6	73.7	65.8			
H*	0.68	0.69	0.68	0.68	0.71	0.69	0.71	0.67			

## Sensory evaluation

The wines were preliminarly tasted by a panel of 6 assessors after racking, while a panel of 15 assessors carried out the sensory evaluation at the end of the experiment. Each parameter was evaluated on a scale from 0 to 5 (with increasing intensity of the descriptor).

For the first tasting, only the most representative descriptors of the winemaking process were selected. For the second tasting the descriptors were more complete, allowing a more detailed description. The results of the statistical elaborations are shown in the figure captions.

It is worth noting that all the tested wines have a composition and attitude for long-aging wines and, consequently, sensory assessments express only partial judgment at the time when tasting took place.

#### Barbera sensory profile

The results of the sensory analysis of Barbera wines are shown in Figures 10.1 and 10.2 (B1 trials), 11.1 and 11.2 (B2 trial). On Barbera wines, the use of GOfermentor has allowed to obtain wines with a color judged similar to control in B1 vinification, whereas color appeared less intense in the B2 trial. Wines obtained with the GOfermentor system appeared less astringent and with more intense aromatic notes.

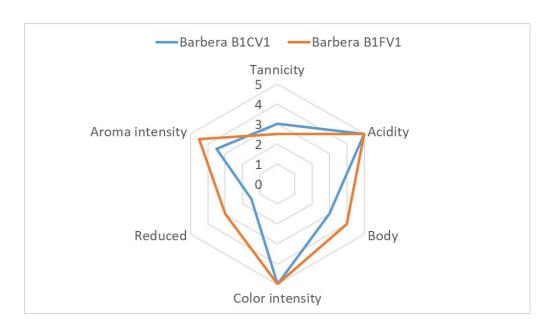


Figure 10.1 Sensory profile of Barbera wines (B1) after racking (V1)

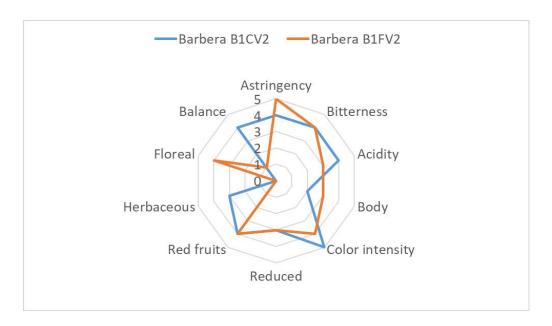


Figure 10.2 Sensory profile of Barbera wines (B1) before bottling (V2). Significance level: \* = 0.05; \*\* = 0.01.

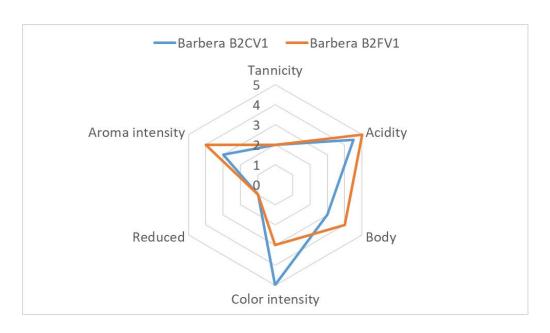


Figure 11.1 Sensory profile of Barbera wines (B2) after racking (V1)

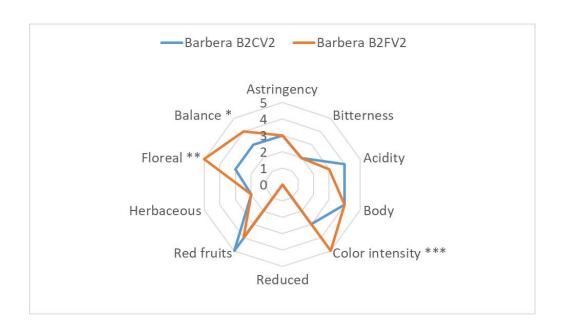


Figure 11.2 Sensory profile of Barbera wines (B2) before bottling (V2).

Significance level: \*= 0.05; \*\* = 0.01; \*\*\* = 0.001.

#### Nebbiolo sensory profile

The results of the sensory analysis of Nebbiolo wines are shown in Figures 12.1 and 12.2 (N1 trials) and 13.1 and 13.2 (N2 trial). The extraction of the tannic component in Nebbiolo was limited with the GOfermentor vinification system, and this has led to more balanced, less astringent and tannic wines. The color, not particularly intense in all Nebbiolo wines, was judged slightly lower in both GOfermetor trials compared to the control.

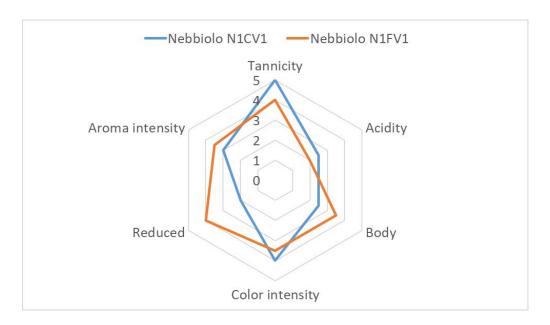


Figure 12.1 Sensory profile of Nebbiolo wines (N1) after running-off (V1)

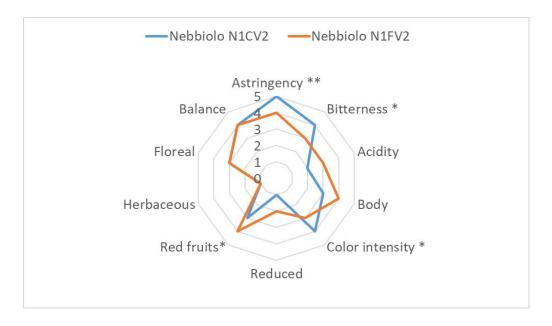


Figure 12.2 Sensory profile of Nebbiolo wines (N1) before bottling (V2). Significance level: \* = 0.05; \*\* = 0.01.

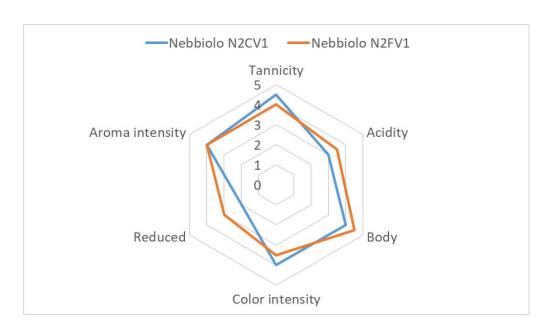


Figure 13.1 Sensory profile of Nebbiolo wines (N2) after running-off (V1)

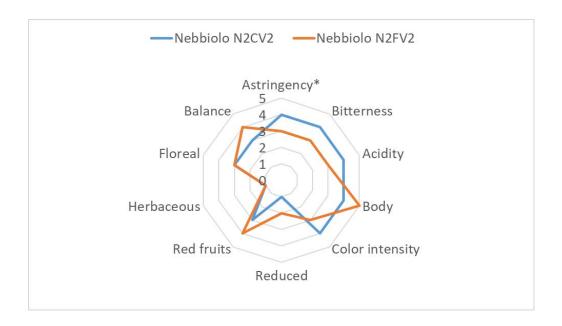


Figure 13.2 Sensory profile of Nebbiolo wines (N2) before bottling (V2).

Significance level: \* = 0.05.

### Conclusions

The winemaking experiences with the GOfermentor system, conducted in the 2016's harvest, allows us to highlight some factors related to possible uses of this system.

GOfermentor has proven to be an innovative system that allows the winemaking of little quantity of grapes, with an automatic control of maceration management operations. The system is easy to handle and to setup. It has been difficult to control the fermentation temperature by using only cold water circulation/recirculation in the GoCooler device. In unsatisfactory thermal conditions it is convenient a vinification in controlled temperature environments.

The pomace cap management was soft, a condition that favoring the extraction of tannins with a positive impact in terms of mouthfeel. In our experiences, during the punching period it was been difficulty stir about 1/3 of pomace cap. This aspect, about "difficult to handle" (necessity of an intense extraction process) grapes such as Nebbiolo and Barbera, it may limit the extraction of favorable phenolic compounds.

All wines produced in the experiments using GOfermentor, after about 6 months of aging, evidenced improved values of red color stability (correct polymerization between tannins and anthocyanins). This trait is very interesting for Nebbiolo and Barbera wines in the event of a long aging time wine production.

The system allowed to limit the negative effects of oxygen in the first step of maceration. During the next fermentation phases, limited oxygen supply can be problematic in grape musts with high sugar content. The system could be improved to automatically add little amounts of oxygen during alcoholic fermentation, to increase yeast vitality and limit some aspects of reduction notes in wines. However, this aspect must be better investigated because of the adverse effects of oxygen during the vinification process. The control of the effects of oxygen appears to be particularly useful in the production of white and *rosé* wines.

About aroma, GOfermentor has proven to be particularly effective in favoring the production of fruity aroma wines with respect to control wines.

Run-off operations were easy to operate, and removing the macerated pomace is simple by forklift and common cellar equipments.

In conclusion, the GOfermentor system can express the best potential in the production of young red wines, or aged wines from grapes which not require an intense (hard) extraction process. The system is useful also when conducting vinifications using moderate amounts of grapes (less than 900 kg). In addition, the GOfermentor system can be a valuable tool for experimental vinification thanks to the high automation that makes the technological effects reproducible, and to the single-use bag that allows a clean production environment in each vinification.