

## An inertizing and cooling process for grapes cryomaceration

Maria Carillo<sup>1</sup> ✉ · Andrea Formato<sup>1</sup> · Andrea Fabiani<sup>2</sup> · Giampiero Scaglione<sup>3</sup> · Giovanni Pio Pucillo<sup>1</sup>

<sup>1</sup> University of Naples "Federico II", Department of Agricultural Engineering, Portici, Naples, Italy

<sup>2</sup> Siprem International, Pesaro, Italy

<sup>3</sup> University of Naples "Federico II", Department of Arboriculture, Botany and Plant Pathology, Portici, Naples, Italy

✉ Corresponding author: mcarillo@fastwebnet.it

Received May 1, 2011 / Accepted July 26, 2011

Published online: November 15, 2011

© 2011 by Pontificia Universidad Católica de Valparaíso, Chile

### Abstract

**Background:** With this research an inertizing and cooling process for grapes cryomaceration has been set up. The process in question has been performed by an innovative plant that cooled the grapes rapidly in about 8 sec until they reached the set cryo-maceration temperature, using direct injection of liquid CO<sub>2</sub>. It works with a grape flow of approximately 2-3 tons/h, with a maximum thermal gradient of 20 K between the grape inlet and outlet temperature. For this plant a vibrating device was set up that allowed that only one grape cluster layer to be formed on the ribbon conveyor after the grapes had been put into the feedbox. A numerical model was set up for the cooling tunnel, and numerical simulations were performed to investigate the operative parameters of the machine in question. The numerical results were validated by means of experimental tests. **Results:** The wines obtained by using the considered plant (IW) were chemically analysed, and a comparison was performed with wines obtained with the same grape without the use of the plant (TW). All phenolic parameters were higher in IW wines, while other substances such as alcohol, reducing sugars, acids, and volatile acidity were less affected by the different winemaking technique. A deeper yellow colour was a direct consequence of the higher phenolic content of IW wines. Panelists preferred the IW wines, which had a richer, more delicate aroma. **Conclusions:** The study showed that careful exclusion of air combined with preventing oxidation during the cooling process, that is realized with the considered innovative cooling plant, effectively yields pleasing wines with more character.

**Keywords:** cryogenic liquid CO<sub>2</sub>, cryomaceration process, inertizing process

### INTRODUCTION

One of the most important techniques used during wine-making is cryo-maceration. It consists in submitting the mashed grapes to rapid cooling, up to 281-283 K, and maintaining this temperature for several days, in order to improve the extraction of the compounds contained in the grape skins, such as phenolic compounds and the primary aromas (Parenti et al. 2004; Salinas et al. 2005; Gómez-Míguez et al. 2007a; Gómez-Míguez et al. 2007b; Maggu et al. 2007; Kechagia et al. 2008; Baiano et al. 2009; Gordillo et al. 2010; Lukić et al. 2010; Piombino et al. 2010).

It is thus possible to obtain a limited passage of tannic polyphenols into the must and its enrichment with aromatic and volatile substances.

Low-temperature pre-fermentative maceration is frequently used in elaborating white wines to encourage contact between grape skins and juices in order to extract the greatest amount of aromas

and their precursors, both mainly located in the skin of grape berries (Peinado et al. 2004; Salinas et al. 2005).

Wine prepared with grapes by the use of cryomaceration show more aroma intensity, stability of taste properties of the wine than wines prepared traditionally by maceration without cooling. The use of cryomaceration presents white wines showing high stability to oxidation and typical intensive varietal taste and aroma.

Cryomacerating process is used by the winemaker to enhance the varietal character of white wines. This procedure provides, acceptable, well-balanced, better-rounded wines, with a stronger body in the mouth (Peinado et al. 2004).

Therefore, by means of this process, a final product can be obtained with better characteristics than the wines made with traditional processes. (Parenti et al. 2004; Peinado et al. 2004; Salinas. et al. 2005; Hernanz et al. 2007; Kechagia et al. 2008; Baiano et al. 2009; Antonelli et al. 2010; Gordillo et al. 2010).

The cryomaceration process can be performed in different ways. One of these requires the use of large-capacity refrigerator groups to achieve the rapid cooling required. These refrigerator groups involve high energy consumption and very expensive plant costs that must be amortized only during seasonal utilization. Besides, they very often damage the cooled product due to friction generated in the pipes during the passage of the mashed grapes which, in turn, produces dregs formation (Ribereau-Gayon et al. 2000; Ferreira et al. 2002).

Another cooling system that avoids such drawbacks is based on using cryogenic gas (CO<sub>2</sub> or N<sub>2</sub>). The utilization of plants for cryomaceration of grapes by means of liquid CO<sub>2</sub> represents an important technological innovation in the wine-making process. It consists in injecting liquid CO<sub>2</sub> directly into the line which, during its transition to the gaseous state, removes heat from the product to be cooled.

In the authors' opinion, this process is more advisable than that using the traditional refrigerating systems because, in the field of wine-making, it is well known that the use of liquid CO<sub>2</sub> ensures cryomaceration of the mashed grapes without requiring large refrigerating systems (and all their relative management and maintenance costs); secondly, cryomaceration by means of liquid CO<sub>2</sub> prevents mistreatment of the product due to the mechanical interaction between the mashed grapes and the heat exchanger; finally, although the operating costs related to cooling through liquid CO<sub>2</sub> can be higher than those of traditional refrigeration systems, the technique allows a great reduction in terms of fixed investment.

The process considered is inert, because the CO<sub>2</sub>, which is present in the plant, is heavier than air and protects the product to be cooled by avoiding any oxidation problems.

Considering the whole winemaking process, the most insidious step is grape crushing. During this phase, the rate of oxygen consumption can be very high. The presence of grape polyphenol oxidases makes polyphenol oxidation extremely rapid (Hansen et al. 2001; Bueno et al. 2010; Cejudo-Bastante et al. 2011). The use of an inert atmosphere is a very effective way to prevent undesired oxidation. Gibson (2004), gave a thorough description of the critical points in this technique, and all the due precautions were explained.

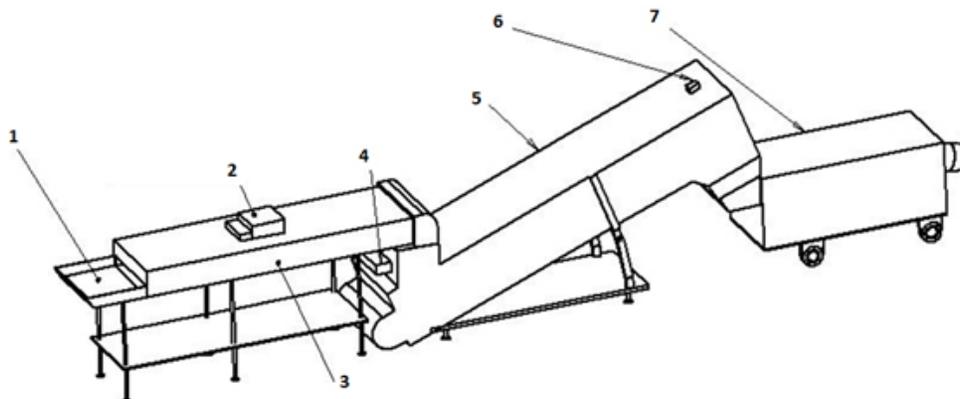
Some plants are now available on the market, in which the liquid CO<sub>2</sub> is injected after grape crusher destemmer. As the liquid CO<sub>2</sub> is injected at about 20·10<sup>5</sup> Pa, such plants present all the problems and complications of those that work with high-pressure fluids, in compliance with the regulations governing these types of plants (Formato et al. 2010).

Given such constraints, an innovative plant prototype for rapid grape cooling was designed and constructed, together with a device allowing the formation of only one layer of grape clusters to avoid thermal gradients between the different grape clusters considered.

## MATERIALS AND METHODS

The plant prototype (Figure 1) was designed and constructed as part of collaboration with Air Liquide and Siprem International. The plant is able to achieve for the treated grapes (up to 10 tons/hr) a thermal gradient,  $DT = (T_{inlet} - T_{outlet})$ , adjustable up to 20 K. The treated cooled grapes are then sent to a grape crusher destemmer machine. The main components of the plant are:

- a cooling tunnel, formed by a 4000 mm long and 1000 mm wide vibrating table, built with AISI 304 stainless steel plates 40/10 thick. The tunnel is mounted on four feet (1800 mm long) with adjustable height;
- in-line infra-red temperature transducers located on the upper part of the cooling tunnel, connected to the PLC system, that continuously detect the temperature of the grape grains on the vibrating table;
- a liquid CO<sub>2</sub> injection system with a series of nozzles, working with an on/off cycle, depending on grape temperatures, detected by temperature transducers. A control panel operates the injectors of liquid CO<sub>2</sub> so as to ensure the quantity of liquid CO<sub>2</sub> required to achieve the set thermal gradient;
- a stainless steel belt elevator 5500 mm x 1000 mm, 2650 mm in height with inclination that can be adjusted by hydraulic jack. The whole plant is completely insulated towards the outside in order to avoid any heat exchanges.



**Fig. 1 Grape rapid cooling machine:** (1) grape inlet hopper; (2) CO<sub>2</sub> injector; (3) vibrating table; (4) CO<sub>2</sub> injector; (5) belt elevator; (6) temperature drill; (7) crusher-destemmer machine.

The vibrating table is managed by means of two vibrators, each 0.35 kW. They operate using two shafts 26 mm in diameter and with eccentric rotating masses with a radius of 73 mm, able to generate a centrifugal force of 5 kN at 150 rad/s, with a vibration frequency of 50 Hz.

During the operational phase, the grapes are introduced into the vibrating table through the inlet hopper. The vibrating action of the table arranges the grape clusters next to one another so as to form a layer of only one grape cluster located on the vibrating table.

As the vibrating table has a forward speed between 0.3 and 1 m/s, the full length of the vibrating table (4 m) can be travelled by the grapes in different times, with different residence times in the cooling tunnel. The thermal exchange  $DQ$  occurs according to:

$$DQ = C \cdot m \cdot DT$$

[Equation 1]

where  $C$  is the thermal capacity;  $m$  = mass (kg) and  $DT$  is the thermal gradient (K).

The grape cooling process takes place on the vibrating table, inside the cooling tunnel, and depends both on the liquid  $CO_2$  flow injected into the plant and on the residence time of the grapes in the cooling tunnel.

In order to determine the values of these cooling process parameters, a numerical simulation was carried out and experimental tests were then performed to validate the numerical data.

### **Numerical simulation**

The main purpose of the numerical analysis was to determine the machine parameters (speed of the grapes on the vibrating table and mass flow of liquid  $CO_2$ ) in order to achieve the set temperature gradient and minimize both the process duration and  $CO_2$  consumption.

Indeed, knowing the thermodynamic parameters of the  $CO_2$  and of the grapes, having set the desired temperature reduction to be achieved, it is then possible to determine the calories to remove from the treated grapes and hence the quantity of liquid  $CO_2$  to inject into the plant.

In order to ascertain the grape cooling time and hence the forward speed of the grapes, so that they travel the four metres on the vibrating table in sufficient time to achieve complete grape cooling up to the set temperature, it was necessary to perform a numerical simulation due to the complexity of the considered phenomenon.

The main complexities are due, above all, to the non-stationary  $CO_2$  flow, due to the presence of the on/off valves, the three-dimensional aspect of the problem and the presence of localized heat transfer phenomena.

The main phenomena analyzed were the convection and conduction heat transfer mechanisms between the grapes, which at the moment of the input into the cooling tunnel are at a temperature of 298 K, and the  $CO_2$ , initially contained in a tank at a temperature of about 253 K and at a pressure of  $20 \cdot 10^5$  Pa. Calculations were performed with the ANSYS CFX 11 program code.

Because of the symmetry, only half the volume was analysed, by applying suitable boundary conditions to the *cutting surface*, namely with null thermal flux. It was thus possible to considerably reduce the computational time required.

Adiabatic boundary conditions were applied to the boundary surfaces of the model, excluding those related to the orifices of the nozzles and those related to the inlet and outlet of the grapes.

Further, to define the model mesh, a mesh sensitivity analysis was performed. Moreover, to achieve good confidence with the numerical results, a simple model and its validation with an analytical solution found in the literature were considered.

The model consists of a 10 mm sphere immersed in a gas stream. In the technical literature, the most recognized analytical solution is reported (Incropera et al. 2007). In this simplified model, all the various heat transfer phenomena that are activated in the real condition were introduced.

Temperature and pressure contours are shown in Figure 2 for various levels of mesh refinements. In Table 1 the mesh details used in the calculations of Figure 2 are summarized. The images in the figure show good convergence, in terms of pressure and temperature distribution, such that no further mesh refinements are necessary. In particular, as was expected, a mesh refinement should be used only near the grape-fluid interface. Good analytical-numerical correlation was obtained, as is shown by the curves in Figure 3.

This model, calibrated on the analytical solution, was the starting point for a more complex model consisting of several spheres, fixed in space and enclosed in an adiabatic volume, originally occupied

by the air at room temperature and then filled with CO<sub>2</sub>. In this model, the areas surrounding each grape grain are not all occupied by the gas, which is due to the presence of other grapes and the adiabatic plane on which the grapes lie.

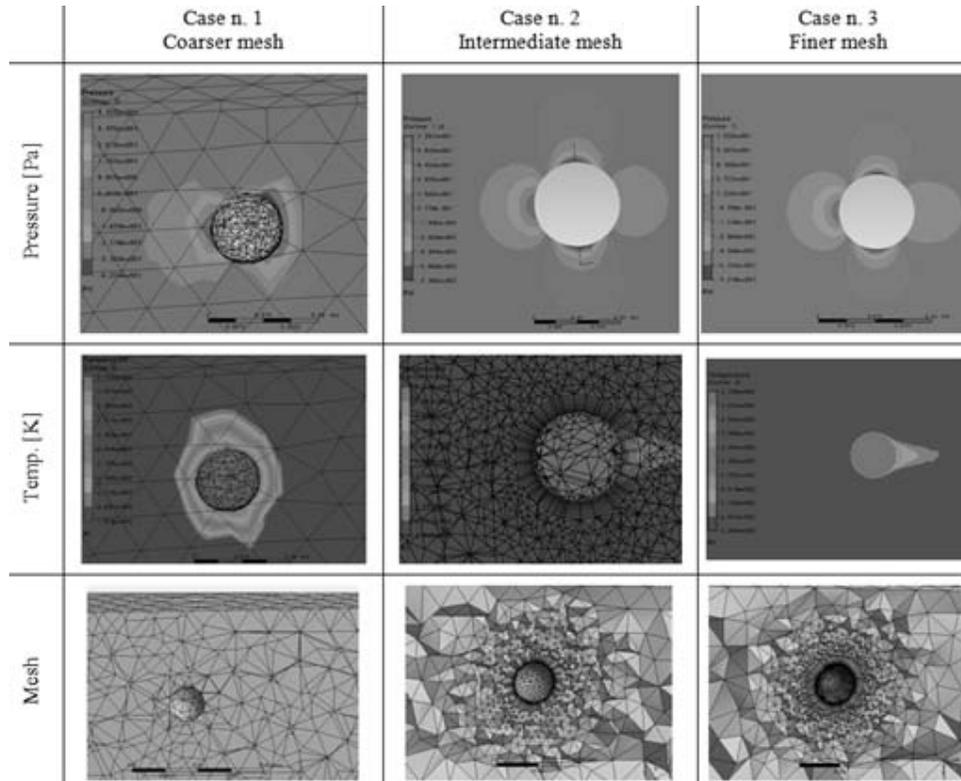


Fig. 2 CFD results for different mesh refinements.

Figure 4 shows the discretization used for the calculations, in particular the size of the elements in the area nearest to the grape grains. The characteristic diameter of the grape grains was assumed equal to 10 mm. The physical properties of the grapes considered in the calculations are reported in Table 2.

Simulation of the CO<sub>2</sub>, instead, was carried out by considering it at the state of vapour and assigning to it suitable thermodynamic characteristics in order to make it equivalent, in terms of thermal exchange, to the mixture of vapour and carbonic snow which, in reality, occurs when the nozzles open.

From some previous experimental research, it was detected that to perform, as in our case, a temperature gradient of 15 K per 100 kg of grapes, about 15 kg of CO<sub>2</sub> are necessary. The equivalent physical characteristics used for the CO<sub>2</sub> were calculated, as previously stated, in order to verify that relation.

The whole cooling system was thus modelled, taking into account the results of the first two models considered. In this model, as occurs in the real plant, the nozzles that ensure CO<sub>2</sub> injection have a relative motion with respect to the layer of grapes placed on the vibrating table. The relative motion between nozzle and grapes was simulated, in a fully equivalent way, keeping the grapes in a fixed position on the vibrating table, and translating the nozzles with an artifice.

Table 1. Mesh details.

Domain	Nodes			Elements		
	Case n. 1	Case n. 2	Case n. 3	Case n. 1	Case n. 2	Case n. 3
ambient	2405	10471	31106	10891	44991	118919
sphere	402	1020	2959	1210	3199	9695
total	2807	11491	34065	12101	48190	128614

Specifically, the model was constructed to have a series of nozzles mounted near the top face of the tunnel, with the peculiarity of not being activated simultaneously. The activation law of the nozzles was simulated, imposing that:

- carbon dioxide comes out of a pair of nozzles (at a distance equivalent to that between the nozzles of the system) with a square function of time;
- when a pair of nozzles close, the nozzles immediately preceding open, according to the direction of advancement of the vibrating table.

### Experimental tests

To validate the numerical results obtained, experiments were performed on a sample of 3 tons of Bianchetto del Metauro grapes, grown in Pesaro (Italy) and picked in the month of September 2010. The grapes had an inlet temperature of 298 K, and underwent the cryomaceration process using our rapid grape cooling plant prototype.

To verify the homogeneity of the final temperature obtained for all the grape clusters treated, 10 temperature infra-red transducers were set up and linked to a data acquisition system with a frequency sample of 1 Hz, located along the outlet cross section of the vibrating table. The distance between the temperature transducers on the same line was 100 mm, while it was 50 mm from the outer edge. It was thus possible to evaluate the temperature distribution in the zone near the outlet section, and then assess the efficiency of the machine in question. Further, a chronometer was used to evaluate the time for each grape cluster to travel along the cooling tunnel.

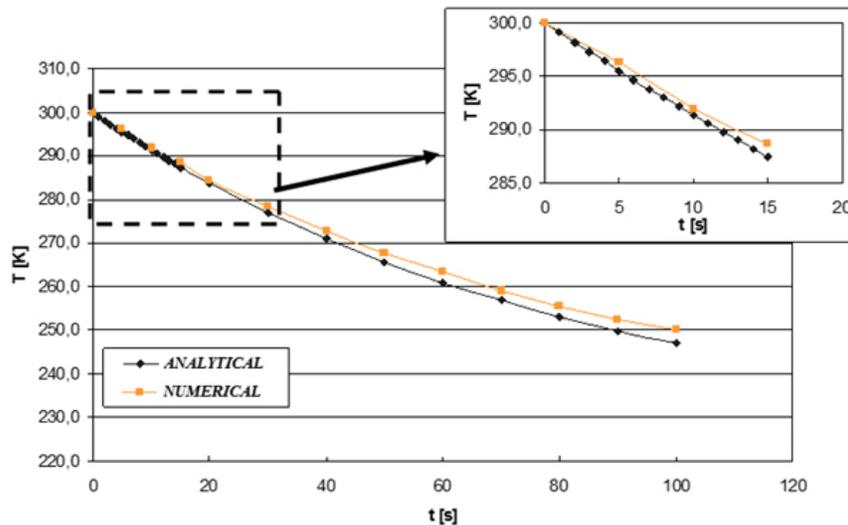
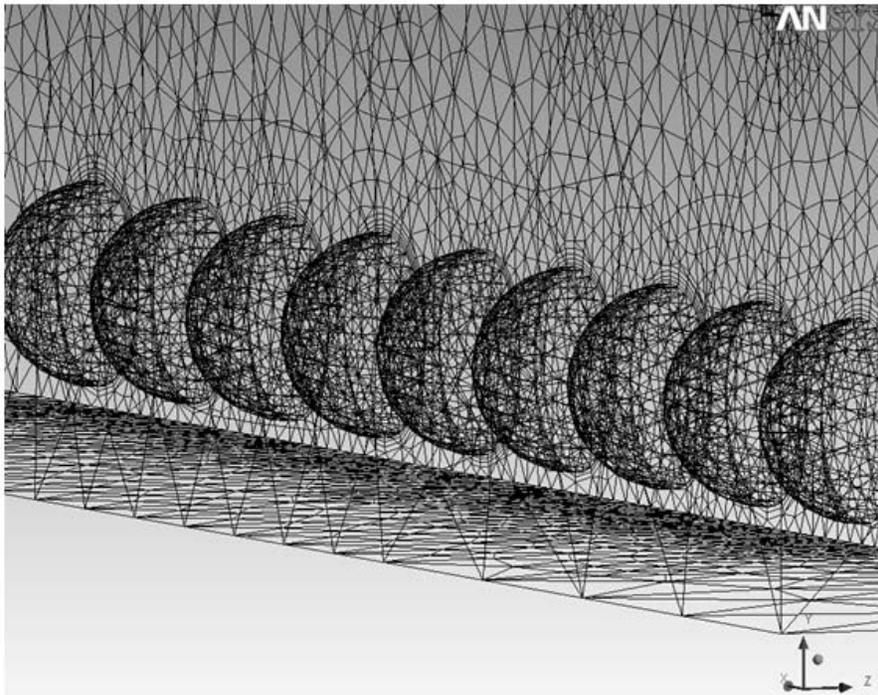


Fig. 3 Comparison of analytical-numerical CFD results.

The grapes in question were in optimal health, they had an ageing level of 24-26°Brix and a total acidity of 6-7 g/l. A temperature gradient of 15 K was chosen for the considered machine. Thus the grapes in question have to be submitted to a temperature decrease from 298 to 283 K by means of the grape cooler.

The cooled grapes were then sent to the crusher-destemmer machine, which operated in an inert environment and at the cryo-macerating temperature. After destemming and crushing, the mash was transferred to closed 100 l stainless steel tanks with an inert and CO<sub>2</sub>-controlled environment.

Three tanks were used for the mash obtained with the considered plant, and three tanks were used for the mash obtained without it. The grapes were processed separately to obtain two batches of must, one (IW), by using the plant and the other without it (TW). The temperature was maintained constant and checked at 283 K.



**Fig. 4 Mesh detail near the grape-CO<sub>2</sub> interface.**

Hence the grapes were subjected to maceration immediately after crushing, at 283 K for 24 hrs. Potassium metabisulphite (120 mg/kg grape) was added to the grapes prior to pressing. All the musts obtained after pressing were inoculated with 30 g/hl commercial yeast (*Saccharomyces cerevisiae*) (Bonilla et al. 2001; Razmkhab et al. 2002; Fornairon-Bonnefond and Salmon, 2003; Mazauric and Salmon, 2005; Hierro et al. 2006). Three samples for the two batches considered (TW and IW) were thus obtained.

Fermentation was performed at the temperature of 295 K. Manual punching down was carried out twice a day by "pigeage". Then the wines were strained off and the mash was pressed.

Malolactic fermentation was then induced by inoculation with *Oenococcus oenilactic* acid bacteria. When the second fermentation was finished, the wines were supplied with 20 mg/l sulphur dioxide, and analyzed after 4 weeks (Gómez-Míguez et al. 2007c).

**Table 2. Thermodynamic properties of the grape grains.**

symbol	description	value	U.M.
$r$	density	1300	kg/m <sup>3</sup>
$c_p$	specific thermal capacity	3600	J/kg/K
$k$	thermal conductivity	0.61	W/m/K

The sample from bottling was obtained by using an isobaric level filler through counter pressure equipped with an air pre-evacuation system. In our conditions, the dissolved oxygen rose to 0.6 mg/l after bottling (Girard et al. 2001; Lopes et al. 2005; Brotto et al. 2010). The bottles were closed using crown caps. Musts and wines were analyzed following the methods listed in Table 3.

Musts were analyzed after clarification treatments, while wines were analyzed 30 days after bottling. Determination of iron and copper was carried out with an atomic absorption spectrophotometer equipped with a model AA240FS Varian air-acetylene burner (Palo Alto, CA, USA) (Flamini, 2003).

### Statistical analysis

The data for each variable were analysed with a multifactor analysis of variance (ANOVA). Statistical significance of each factor under consideration was calculated using the LSD test. The data were statistically analysed using Statgraphic Plus 5.1 software.

### Sensory analysis

A triangle test on each considered wine (TW vs. IW) was carried out separately with a panel of 16 trained judges. The preference test was carried out as indicated by Bueno et al. (2010) using 26 non-trained judges asked for the global preference (Ferreira et al. 2003).

**Table 3. Methods used in the study.**

determination	literature	musts	wines
Reducing sugars	European Community Official Journal, 1990	*	*
pH	European Community Official Journal, 1990	*	*
Titrateable acidity	European Community Official Journal, 1990	*	*
Volatile acidity	European Community Official Journal, 1990		*
Malic acid	European Community Official Journal, 1990		*
Total and reduced extract	European Community Official Journal, 1990		*
Alcoholic strength	European Community Official Journal, 1990		*
SO <sub>2</sub>	European Community Official Journal, 1990	*	
Iron	European Community Official Journal, 1990	*	
Copper	European Community Official Journal, 1990	*	
Free amino nitrogen (FAN)	Shively and Henick-Kling, 2001	*	
Tartaric acid	Vidal and Blouin, 1978		*
Ascorbic acid	AOAC, 1990	*	*
Total phenols	Singleton and Rossi, 1965		*
Optical density (420 nm)	Somers and Ziemelis, 1985		*
Optical density (320 nm)	Somers and Ziemelis, 1985		*
Optical density (280 nm)	Ribéreau-Gayon et al. 2000		*
Catechins	Fuleki and da Silva, 2003		*
Phenolic compounds	Castellari et al. 2002		*

\*Indicates in which samples the determination was carried out.

## RESULTS AND DISCUSSION

### Numerical results

By means of numerical simulations, it was possible to obtain temperature distributions in the cooling tunnels at the beginning, during and at the end of the process.

The carbonic anhydride, injected through the nozzles, fills all the volume of the tunnel, also filling to the top the spaces between the grape grains. Injection of CO<sub>2</sub>, at a temperature of 253 K, inside the tunnel, in which the initial temperature is 298 K, thus causes a gradual temperature reduction in the entire environment, until a practically uniform thermal rate is reached inside the tunnel.

Moreover, numerical analysis showed that 8 sec is required for complete grape cooling (also considering the inner part of the grape grain). Therefore, at a speed of 0.5 m/s, the treated grapes left the vibrating table after 8 sec, and were completely cooled to the desired temperature.

### Experimental results

Experimental tests were performed to validate the numerical data obtained. Therefore, tests were carried out by setting the speed of the vibrating table at 0.5 m/s. We were able to verify, by means of the temperature distribution detected by the temperature transducers in the outlet cross-section of the vibrating table, that the final outlet temperature for the grapes was uniform and equal to 283 K. Further, the time range required for a grape cluster to cross the whole vibrating table estimated to be 8 s. For all the grapes treated by our rapid grape cooling plant, at an inlet temperature of 298 K, with a machine set point of 15 K for the temperature gradient to perform, the final temperature measured was 283 K, with a complete absence of thermal gradients among the considered grape grains. During the experimental tests, we observed that, in order to obtain a temperature gradient of 15 K in 100 kg of grapes, about 15 kg of CO<sub>2</sub> are required. This agrees with other results obtained from some previous experimental tests (Formato et al. 2010).

### Analytical results

The most important analytical characteristics of musts are reported in Table 4. The level of sugars (26.10°Brix and 257.6 g/l total sugars), of pH (3.27), of acidity (7.20), and of the parameters Fe, Cu and Fan respectively 8.51; 1.18 and 136, are satisfactory in connection with the wine and grape type considered. The composition of the must stresses the perfect maturation of the grape at the time when it is delivered in the wine cellar (Table 4), data result also confirmed by the “potential alcoholic strength” of 15.20.

**Table 4. Musts composition (data average over 2010 of three samples considered).**

Parameters	Mean ± dxm
°Brix	26.10 ± 0.42
Total sugars (g/L)	257.6 ± 0.35
Potential alcoholic strength (%) (v/v)	15.20 ± 0.43
Titrateable acidity <sup>a</sup> (g/L)	7.20 ± 0.39
pH	3.27 ± 0.08
Fe (mg/L)	8.51 ± 0.07
Cu (mg/L)	1.18 ± 0.04
FAN (Free amino nitrogen) (mg/L)	136 ± 0.3

<sup>a</sup>As tartaric acid.

The analytical characteristics of the wines (Table 5) attest that in both cases of traditional and criomaceration process, the evolution of musts into wines has been carried out regularly. In this paper, similarly to what was found by Piombino et al. (2010) on the Lipari Malvasia grapes, and by Antonelli et al. (2010), on “Sauvignon Blanc” and “Trebiano Romagnolo” wines, the crio-maceration process, compared with the traditional method, has not meaningfully conditioned the alcoholic degree of the wine, that resulted to be almost similar in the two processes considered (12.90 for TW, 12.85 for IW), also the potential alcoholic degree has not greatly influenced, it was not statistically distinguishable between the two methods (13.36 vs 13.40) and the pH of the wines obtained with the two processes considered (3.07 vs 3.09). No statistically appreciable influence moreover has been found either for titratable acidity (6.9 vs 7.5) or for volatile acidity (0.35 for both methods) in agreement with what was obtained by other authors (Antonelli et al. 2010).

**Table 5. Comparison between the different winemaking techniques.**

Treatment	TW	IW
	Mean $\pm$ dxm	Mean $\pm$ dxm
Alcohol (%) (v/v)	12.90 $\pm$ 0.2a	12.85 $\pm$ 0.17a
Potential alcohol (%) (v/v)	13.40 $\pm$ 0.68a	13.36 $\pm$ 0.58a
Reducing sugars (g/L)	8.63 $\pm$ 3.6a	11.11 $\pm$ 4.3a
Reduced extract (g/L)	18.73 $\pm$ 0.57a	19.02 $\pm$ 0.34a
Titratable acidity (g/L)	7.5 $\pm$ 0.08a	6.9 $\pm$ 0.31a
Volatile acidity (g/L)	0.35 $\pm$ 0.01a	0.35 $\pm$ 0.01a
Tartaric acid (g/L)	1.92 $\pm$ 0.04a	1.81 $\pm$ 0.07a
Malic acid (g/L)	1.08 $\pm$ 0.07a	1.70 $\pm$ 0.40b
pH	3.07 $\pm$ 0.01a	3.09 $\pm$ 0.08a
Fe (mg/L)	0.640 $\pm$ 0.06a	0.632 $\pm$ 0.07a
Cu (mg/L)	0.061 $\pm$ 0.012a	0.051 $\pm$ 0.009a
O.D. 420 nm	0.093 $\pm$ 0.020a	0.122 $\pm$ 0.060b
O.D. 320 nmb	4.598 $\pm$ 0.650a	8.317 $\pm$ 0.490b
O.D. 280 nmc	6.790 $\pm$ 0.710a	10.61 $\pm$ 0.630b
Total phenols (mg/L)	184 $\pm$ 16.43a	258 $\pm$ 10.66b
Catechins (mg/L)	7.06 $\pm$ 0.19a	15.42 $\pm$ 1.09b
(+)-Catechin (mg/L)	3.18 $\pm$ 0.04a	3.22 $\pm$ 0.02b
(-)-Epicatechin (mg/L)	17.16 $\pm$ 3.40a	21.05 $\pm$ 3.58b
Gallic acid (mg/L)	12.51 $\pm$ 4.07a	22.36 $\pm$ 5.05b
Caftaric acid (mg/L)	28.32 $\pm$ 6.81a	58.67 $\pm$ 6.38b
Coutaric acid (mg/L)	5.60 $\pm$ 1.02a	10.90 $\pm$ 2.04b
Caffeic acid (mg/L)	2.23 $\pm$ 0.81a	3.38 $\pm$ 1.35b
Syringic acid (mg/L)	2.51 $\pm$ 0.41a	3.37 $\pm$ 0.63b
Proanthocyanidins (mg/L)	23 $\pm$ 3a	44 $\pm$ 4b

Instead statistically meaningful differences between the two methods have been found for numerous other parameters. In particular, the criomacerating process has meaningfully increased the level of malic acid (1.70 vs 1.08), and of acids: gallic (22.36 vs 12.51), caftaric (58.67 vs 28.32), coutaric (10.90 vs 5.60), caffeic (3.38 vs 2.23), syringic (3.37 vs 2.51), increasing also the level of proanthocyanidins (44 vs 23) (Table 5).

As it is known, the malic acid quantity is very important in white wines since it generally is considered a favourable element to perceive a feeling of freshness, contributing meaningfully to the drinkability of the wine. As for as the content of polyphenols is concerned, many authors have studied the effect of the cryomacerating process on their content in the wine. (Vasquez et al. 2010).

Gómez Míguez et al. (2007a) compared the cold-maceration with the traditional winemaking on Shiraz wine concluding that the two wines are quite different, because the cold maceration period prior to the beginning of the alcoholic fermentation significantly affected the colour and phenolic composition of the wines. Further, the cryo-macerating process can influence the polyphenols content of the wine in connection, above all, with the heating of musts after the application of this technique. Probably as a consequence of phenol protection and the oxidation prevention of the reducing environment used in this paper, the IW process increased the phenol content of the wines (258 vs 184).

This result confirms what was obtained by numerous other authors. Among these Antonelli et al. (2010), on cultivars Sauvignon Blanc and "Trebiano Romagnolo" found an increase of the phenolic parameters comparing the cryo-macerating and traditional processes. Analogous results Alvarez et al. (2006), reached first with Monastrell and after with Tempranillo wines (Alvarez et al. 2009).

In this paper, we have not been found statistically appreciable differences, in reducing sugar (11.11 vs 8.63), reduced extract (19.02 vs 18.73), in the content in tartaric acid (1.81 vs 1.92), in monomer of catechine (+) - catechin (3.18 vs 3.22).

While for the (-)-epicatechin (17.16 vs 21.056), gallic acid (12.51 vs 22.36) and syringic acid (2.51 vs 3.37) were found behaviours in agreement at what was found by Vazquez et al. (2010), on the Mencia cultivar, for which a global increment of the colour was detected by using the IW process compared with the TW process.

In our paper the total increase of the colour seems to be well correlated to the increase of the Gallic and Syringic acids and of the (-) epicatechin.

Like pointed out previously, in this paper we have denoted the greater colouring intensity of the wine induced by the IW process.

The global colour increase that occurred was probably, also a direct consequence of a higher phenolic and anthocianin content: for OD 420 (yellow) 320 and 280 values increased respectively of 31%, 81% and 56% versus TW values. Our results seem to confirm what was found by numerous authors about the colour increase caused by the cryo-macerating process (Gómez Míguez et al. 2007a).

TW traditional winemaking, IW Inert winemaking, OD optical density, dxm = standard deviation of the mean. Analysis of variance (ANOVA): mean values within different columns designated by different letters are significantly different by the Fisher's tests at  $p \leq 0.005$ ; mean values within different columns designated by the same letter are not significantly different by the Fisher's tests at  $p \leq 0.005$

### **Sensory analysis**

The wines obtained were submitted to a sensory evaluation by two panel of tasters from the Agricultural Faculty of the University of Naples. The results of the panelists were submitted to ANOVA analysis and the averages compared by Fisher Test at  $p \leq 0.05$ . A sensory analysis of the wines was conducted approximately 3 months after finishing alcoholic and malolactic fermentation. The expert tasting panel in this study was composed of 16 members (Holt et al. 2008; Parpinello et al. 2009); all of them part of the tasting wine group with a long experience in identifying differences in wines which are produced in experimental research performed at the University. A second group of 26 non expert tasters was utilized only for a preference test between wines. Our expert panelist was able to distinguish between TW and IW ( $p \leq 0.05$ ) for IW and TW when the triangle test was used. However for 11 expert tastersthere were appreciable colour differences between the considered processes, and TW wine had less colour than the IW, which was confirmed by analyzing the total phenol concentration and the lowest value of gallic acid. Moreover, non-trained judges (preference test) preferred IW wines.

### **CONCLUDING REMARKS**

The results obtained with the grape cooler were very satisfactory because it succeeds in bringing about uniform rapid cooling of all the grapes considered. This does not occur when cooling was effected by

other types of equipment, such as fermenters, which had been previously used by the farm in question. Moreover, since the entire process was performed in an inert environment, better high quality wine could be obtained. This is a major result for producers. For the above reasons, sensory evaluation, as well as chemical analyses, confirm that IW wine is a real opportunity to diversify and enhance the number of products obtainable from a single cultivar. IW wines were found to achieve a good quality standard, most probably able to satisfy consumer preferences. In conclusion, these results are highly encouraging for further studies and applications of this technique.

## ACKNOWLEDGMENTS

The authors would like to thank Dr. Mark Walters for his painstaking editing of the original English version.

**Financial support:** Siprem International and the Office of the Italian Scientific Research- PRIN 2007-2010.

## REFERENCES

- ÁLVAREZ, I.; ALEIXANDRE, J.L.; GARCÍA, M.J. and LIZAMA, V. (2006). Impact of prefermentative maceration on the phenolic and volatile compounds in Monastrell red wines. *Analytica Chimica Acta*, vol. 563, no. 1-2, p. 109-115. [\[CrossRef\]](#)
- ÁLVAREZ, I.; ALEIXANDRE, J.L.; GARCÍA, M.J.; LIZAMA, V. and ALEIXANDRE-TUDÓ, J.L. (2009). Effect of the prefermentative addition of copigments on the polyphenolic composition of Tempranillo wines after malolactic fermentation. *European Food Research and Technology*, vol. 228, no. 4, p. 501-510. [\[CrossRef\]](#)
- ANTONELLI, A.; ARFELLI, G.; MASINO, F. and SARTINI, E. (2010). Comparison of traditional and reductive winemaking: Influence on some fixed components and sensorial characteristics. *European Food Research and Technology*, vol. 231, no. 1, p. 85-91. [\[CrossRef\]](#)
- AOAC (1990). *Official Methods of Analysis* (15<sup>th</sup> edition), Association of Official Analytical Chemists, Washington, DC, USA. p. 1049-1060.
- BAIANO, A.; TERRACONE, C.; GAMBACORTA, G. and LA NOTTE, E. (2009). Phenolic content and antioxidant activity of *Primitivo* wine: Comparison among winemaking technologies. *Journal of Food Science*, vol. 74, no. 3, p. C258-C267. [\[CrossRef\]](#)
- BONILLA, F.; MAYEN, M.; MERIDA, J. and MEDINA, M. (2001). Yeasts used as fining treatment to correct browning in white wines. *Journal of Agricultural and Food Chemistry*, vol. 49, no. 4, p. 1928-1933. [\[CrossRef\]](#)
- BROTTO, L.; BATTISTUTTA, F.; TAT, L.; COMUZZO, P. and ZIRONI, R. (2010). Modified nondestructive colorimetric method to evaluate the variability of oxygen diffusion rate through wine bottle closures. *Journal of Agricultural and Food Chemistry*, vol. 58, no. 6, p. 3567-3572. [\[CrossRef\]](#)
- BUENO, M.; CULLERÉ, L.; CACHO, J. and FERREIRA, V. (2010). Chemical and sensory characterization of oxidative behavior in different wines. *Food Research International*, vol. 43, no. 5, p. 1423-1428. [\[CrossRef\]](#)
- CASTELLARI, M.; SARTINI, E.; FABIANI, A.; ARFELLI, G. and AMATI, A. (2002). Analysis of wine phenolics by high-performance liquid chromatography using a monolithic type column. *Journal of Chromatography A*, vol. 973, no. 1-2, p. 221-227. [\[CrossRef\]](#)
- CEJUDO-BASTANTE, M.J.; PÉREZ-COELLO, M.S. and HERMOSÍN-GUTIÉRREZ, I. (2011). Effect of wine micro-oxygenation treatment and storage period on colour-related phenolics, volatile composition and sensory characteristics. *LWT Food Science and Technology*, vol. 44, no. 4, p. 866-874. [\[CrossRef\]](#)
- EUROPEAN COMMUNITY OFFICIAL JOURNAL. (1990). Commission Regulation (EEC) No 2676/90 determining Community methods for the analysis of wines. *Official Journal*, No L 272, p. 1-192.
- FERREIRA, A.C.S.; GUEDES DE PINHO, P.; RODRIGUES, P. and HOGG, T. (2002). Kinetics of oxidative degradation of white wines and how they are affected by selected technological parameters. *Journal of Agricultural and Food Chemistry*, vol. 50, no. 21, p. 5919-5924. [\[CrossRef\]](#)
- FERREIRA, A.C.S.; OLIVEIRA, C.; HOGG, T. and GUEDES DE PINHO, P. (2003). Relationship between potentiometric measurements, sensorial analysis, and some substances responsible for aroma degradation of white wines. *Journal of Agricultural and Food Chemistry*, vol. 51, no. 16, p. 4668-4672. [\[CrossRef\]](#)
- FLAMINI, R. (2003). Mass spectrometry in grape and wine chemistry. Part I: Polyphenols. *Mass Spectrometry Reviews*, vol. 22, no. 4, p. 218-250. [\[CrossRef\]](#)
- FORMATO, A.; FABIANI, A.; PUCILLO, G.P. and SCAGLIONE, G. (2010). An optimized machine for fast grape cooling by cryogenic fluid. In: *Proceedings of International Conference of Agricultural Engineering AgEng 2010*. Clermont Ferrand, France, (September 6<sup>th</sup>-8<sup>th</sup>, 2010).
- FORNAIRON-BONNEFOND, C. and SALMON, J.M. (2003). Impact of oxygen consumption by yeast lees on the autolysis phenomenon during simulation of wine aging on lees. *Journal of Agricultural and Food Chemistry*, vol. 51, p. 2584-2590. [\[CrossRef\]](#)
- FULEKI, T. and DA SILVA, R.J.M. (2003). Effects of cultivar and processing method on the contents of catechins and procyanidins in grape juice. *Journal of Agricultural and Food Chemistry*, vol. 51, no. 3, p. 640-646. [\[CrossRef\]](#)

- GIBSON, R. (2004). Reductive winemaking for white wines. *Internet Journal of Enology and Viticulture* [online] [cited march 2010]. Available from Internet: <http://www.infowine.com/default.asp?scheda=1240&provenienza=16>.
- GIRARD, B.; YUKSEL, D.; CLIFF, M.; DELAQUIS, P. and REYNOLDS, A. (2001). Vinification effects on the sensory, colour and GC profiles of Pinot noir wines from British Columbia. *Food Research International*, vol. 34, no. 6, p. 483-499. [\[CrossRef\]](#)
- GÓMEZ-MÍGUEZ, M.J.; GONZÁLEZ-MIRET, M.L.; HERNANZ, D.; FERNÁNDEZ, M.Á.; VICARIO, I.M. and HEREDIA, F.J. (2007a). Effects of prefermentative skin contact conditions on colour and phenolic content of white wines. *Journal of Food Engineering*, vol. 78, no. 1, p. 238-245. [\[CrossRef\]](#)
- GÓMEZ-MÍGUEZ, M.J.; CACHO, J.F.; FERREIRA, V.; VICARIO, I.M. and HEREDIA, F.J. (2007b). Volatile components of Zalema white wines. *Food Chemistry*, vol. 100, no. 4, p. 1464-1473. [\[CrossRef\]](#)
- GÓMEZ-MÍGUEZ, M.J.; GÓMEZ-MÍGUEZ, M.; VICARIO, I.M. and HEREDIA, F.J. (2007c). Assessment of colour and aroma in white wines vinifications: Effects of grape maturity and soil type. *Journal of Food Engineering*, vol. 79, no. 3, p. 758-764. [\[CrossRef\]](#)
- GORDILLO, B.; LOPEZ-INFANTE, M.I.; RAMIREZ-PEREZ, P.; GONZALEZ-MIRET, M.L. and HEREDIA, F.J. (2010). Influence of prefermentative cold maceration on the color and anthocyanic copigmentation of organic tempranillo wines elaborated in a warm climate. *Journal of Agricultural and Food Chemistry*, vol. 58, no. 11, p. 6797-6803. [\[CrossRef\]](#)
- HANSEN, H.J.; NISSEN, P.; SOMMER, P.; NIELSEN, J.C. and ARNEBORG, N. (2001). The effect of oxygen on the survival of non-*Saccharomyces* yeasts during mixed culture fermentations of grape juice with *Saccharomyces cerevisiae*. *Journal of Applied Microbiology*, vol. 91, no. 3, p. 541-547. [\[CrossRef\]](#)
- HERNANZ, D.; RECAMALES, Á.F.; GONZÁLEZ-MIRET, M.L.; GÓMEZ-MÍGUEZ, M.J.; VICARIO, I.M. and HEREDIA, F.J. (2007). Phenolic composition of white wines with a prefermentative maceration at experimental and industrial scale. *Journal of Food Engineering*, vol. 80, no. 1, p. 327-335. [\[CrossRef\]](#)
- HIERRO, N.; GONZÁLEZ, Á.; MAS, A. and GUILLAMÓN, J.M. (2006). Diversity and evolution of non-*Saccharomyces* yeast populations during wine fermentation: Effect of grape ripeness and cold maceration. *FEMS Yeast Research*, vol. 6, no. 1, p. 102-111. [\[CrossRef\]](#)
- HOLT, H.E.; FRANCIS, I.L.; FIELD, J.; HERDERICH, M.J. and ILAND, P.G. (2008). Relationships between berry size, berry phenolic composition and wine quality scores for Cabernet Sauvignon (*Vitis vinifera* L.) from different pruning treatments and different vintages. *Australian Journal of Grape and Wine Research*, vol. 14, no. 3, p. 191-202. [\[CrossRef\]](#)
- INCROPERA, F.P.; DE WITT, D.P.; BERGMAN, T.L. and LAVINE, A.S. (2007). *Fundamentals of heat and mass transfer*, 6<sup>th</sup> edition. John Wiley and Sons, 997 p. ISBN 978-0470055540.
- KECHAGIA, D.; PARASKEVOPOULOS, Y.; SYMEOU, E.; GALIOTOU-PANAYOTOU, M. and KOTSERIDIS, Y. (2008). Influence of prefermentative treatments to the major volatile compounds of Assyrtiko wines. *Journal of Agricultural and Food Chemistry*, vol. 56, no. 12, p. 4555-4563. [\[CrossRef\]](#)
- LOPES, P.; SAUCIER, C. and GLORIES, Y. (2005). Nondestructive colorimetric method to determine the oxygen diffusion rate through closures used in winemaking. *Journal of Agricultural and Food Chemistry*, vol. 53, no. 18, p. 6967-6973. [\[CrossRef\]](#)
- LUKIĆ, I.; MILIČEVIĆ, B.; BANOVIĆ, M.; TOMAS, S.; RADEKA, S. and PERŠURIĆ, Đ. (2010). Characterization and differentiation of monovarietal grape marc distillates on the basis of varietal aroma compound composition. *Journal of Agricultural and Food Chemistry*, vol. 58, no. 12, p. 7351-7360. [\[CrossRef\]](#)
- MAGGU, M.; WINZ, R.; KILMARTIN, P.A.; TROUGHT, M.C.T. and NICOLAU, L. (2007). Effect of skin contact and pressure on the composition of Sauvignon Blanc must. *Journal of Agricultural and Food Chemistry*, vol. 55, no. 25, p. 10281-10288. [\[CrossRef\]](#)
- MAZURIC, J.P. and SALMON, J.M. (2005). Interactions between yeast lees and wine polyphenols during simulation of wine aging: I. Analysis of remnant polyphenolic compounds in the resulting wines. *Journal of Agricultural and Food Chemistry*, vol. 53, no. 14, p. 5647-5653. [\[CrossRef\]](#)
- PARENTI, A.; SPUGNOLI, P.; CALAMAI, L.; FERRARI, S. and GORI, C. (2004). Effects of cold maceration on red wine quality from Tuscan Sangiovese grape. *European Food Research and Technology*, vol. 218, no. 4, p. 360-366. [\[CrossRef\]](#)
- PARPINELLO, G.P.; VERSARI, A.; CHINNICI, F. and GALASSI, S. (2009). Relationship among sensory descriptors, consumer preference and colour parameters of Italian Novello red wines. *Food Research International*, vol. 42, no. 10, p. 1389-1395. [\[CrossRef\]](#)
- PEINADO, R.A.; MORENO, J.; BUENO, J.E.; MORENO, J.A. and MAURICIO, J.C. (2004). Comparative study of aromatic compounds in two young white wines subjected to pre-fermentative cryomaceration. *Food Chemistry*, vol. 84, no. 4, p. 585-590. [\[CrossRef\]](#)
- PIOMBINO, P.; GENOVESE, A.; GAMBUTI, A.; LAMORTE, S.A.; LISANTI, M.T. and MOIO, L. (2010). Effects of off-vine bunches shading and cryomaceration on free and glycosylated flavours of Malvasia delle Lipari wine. *International Journal of Food Science and Technology*, vol. 45, no. 2, p. 234-244. [\[CrossRef\]](#)
- RAZMKHAB, S.; LOPEZ-TOLEDANO, A.; ORTEGA, J.M.; MAYEN, M.; MERIDA, J. and MEDINA, M. (2002). Adsorption of phenolic compounds and browning products in white wines by yeasts and their cell walls. *Journal of Agricultural and Food Chemistry*, vol. 50, no. 25, p. 7432-7437. [\[CrossRef\]](#)
- RIBÈREAU-GAYON, P.; GLORIES Y.; MAUJEAN, A. and DUBOURDIEU, D. (2000). *Trattato di enologia* vol. I-II. Microbiologia del vino. Vinificazioni. Edagricole, Bologna, 592 p. ISBN 9788850651955.
- SALINAS, M.R.; GARIJO, J.; PARDO, F.; ZALACAIN, A. and ALONSO, G.L. (2005). Influence of prefermentative maceration temperature on the colour and the phenolic and volatile composition of rosé wines. *Journal of the Science of Food and Agriculture*, vol. 85, no. 9, p. 1527-1536. [\[CrossRef\]](#)
- SHIVELY, C.E. and HENICK-KLING, T. (2001). Comparison of two procedures for assay of free amino nitrogen. *American Journal of Enology and Viticulture*, vol. 52, no. 4, p. 400-401.

- SINGLETON, V.L. and ROSSI, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, vol. 16, no. 3, p. 144-158.
- SOMERS, T.C. and ZIEMELIS, G. (1985). Spectral evaluation of total phenolic components in *Vitis vinifera*: Grapes and wines. *Journal of the Science Food and Agriculture*, vol. 36, no. 12, p. 1275-1284. [\[CrossRef\]](#)
- VAZQUEZ, E.S.; SEGADE, S.R. and FERNANDEZ, I.O. (2010). Effect of the winemaking technique on phenolic composition and chromatic characteristics in young red wines. *European Food Research and Technology*, vol. 231, no. 5, p. 789-802. [\[CrossRef\]](#)
- VIDAL, M. and BLOUIN, J. (1978). Dosage colorimetrique rapide de l'acide tartrique dans les mout et les vins. *Revue Francais Oenologie*, vol. 70, p. 39-46.

**How to reference this article:**

CARILLO, M.; FORMATO, A.; FABIANI, A.; SCAGLIONE, G. and PUCILLO, G.P. (2011). An inertizing and cooling process for grapes cryomaceration. *Electronic Journal of Biotechnology*, vol. 14, no. 6. <http://dx.doi.org/10.2225/vol14-issue6-fulltext-10>