What is it

The pre-ferment cryomaceration is an innovative technology, which provides that the grapes are placed in direct contact with a cryogenic medium to reach temperatures below 10 ° C, and therefore such as not to allow the development of alcoholic fermentation. Usually the must is led to 4 - 5 ° C, temperature that is maintained for a time variable between one and five days, with consequent organoleptic advantages to the finished wine described later.

The cryogenic liquids

By virtue of the characteristics that distinguish them and, in particular, of their non-toxicity, low reactivity, low cost and ease of retrieval, the carbon dioxide in liquid or solid state and the nitrogen in the liquid state are the most frequently employed in the cryogenic field food in general and in particular in the oenological.

Chemical and physical properties of the two cryogenic liquids considered

The Carbon dioxide (CO_2) can exist at ambient pressure, both in the solid state ($CO_{2,5}$ = carbon snow, dry ice), either gaseous, depending on the temperature employed (Landi, Frati 2000), justifying, at least in part, its widespread use in solid state (powder, pellets, or blocks) as a source of frigories in even small companies.

Carbon dioxide is also available on the market in the liquid state, inside containers where a more or less high pressure is maintained in function of the temperature used. In fact it is marketed in the liquid state in the cylinders to be kept at room temperature at a pressure equal to the corresponding value of its vapor pressure, or inside of cryogenic tanks, where it is stored at 20 ° C at a pressure of about 20 atmospheres.

Leaving freely expand the liquid carbon dioxide, by operating at atmospheric pressure, you are obtained CO_{2solid} (dry ice) and $CO_{2\ vapor}$, in equilibrium at a temperature of -80 ° C in varying proportions depending on the conditions (P and T) that characterized the liquid phase subjected to expansion.

The nitrogen (N_2), a diatomic gas, colorless, odorless, highly stable and not very reactive, is marketed as a cryogenic liquid form inside special tanks, where the temperature is equal to about -195 ° C.

Theoretical consumption

The chemical-physical characteristics shown in Table 1 allow to calculate the amount of cryogen to be operated in order to ensure the desired technological effect, for example the amount of cryogenic needed to cool to 1 ° C a hectolitre of must is equal to about 0.6kg if CO_2 is used in the solid state and becomes approximately double if using liquid CO_2, while it would take 1.9 kg of N_2 liquid nitrogen to achieve the same result. These values are largely indicative considering the variability induced by the usage conditions, and in particular, by the mode of delivery of the cryogen and the power exercised by the insulating material used in the construction of the container used in the cryomaceration phase.

Table 1
Main physical and chemical parameters of the two cryogenic liquids widely used in the food industry.

PARAMETER	**U.M.**	**$C0_2$**	**N_2**
Molecular weight	g mole-1	44,01	28,02
Temperature of the triple point	°C	-56,4	-209,9
Pressure at the triple point	Atm	5,1	92,9
Boiling point at P= 20 atm.	°C	-20,0	-195,8

Specific heat at P= 1 atm	°C	-78,5	--
Melting temperature at P= 20 atm.	°C	--	-210,5
Specific heat at P= 1 atm. e T= 0°C	Cal $*$ g^{-1} $*$ K^{-1}	0,17	0,25
Gas density at P=1 atm. e T =15°C	gl $*$ L^{-1}	1,87	5,22
Liquid density at P=1 atm. e T -195°C	calorie $*$ $mole^{-1}$	--	808,10
Latent heat of evaporation	calorie $*$ $mole^{-1}$	--	1335
	calorie $*$ $mole^{-1}$	--	172

Induced effects on the grapes and grape must from cryomaceration

As a result of direct contact of cryogen with the epidermis of the fruit, it causes the partial freeze of the intracellular water present in intact or partially grapes become must. The resulting increase in volume, due to the passage of state of water causes the tearing of the cell membranes (cellular crash) and thus facilitates the diffusion in the liquid phase of the compounds (phenolic substances, flavorings polysaccharides) present in the still intact cells of the grape, in particular, those of the skins and seeds (Cuenat et al. 1996). Moreover, the absence of ethanol in the solvent medium limits the extraction of the more complex tannins and astringent from grape seeds, allowing to produce more balanced wines and soft to the palate. The large quantity of gas that is released as a result of the must-cryogen contact allows to limit the oxygen absorption within the liquid mass and to preserve in this way from oxidation not only the phenolic compounds in general and in particular anthocyanins, but also the aromatic components which are released, thanks to the intervention of enzymes with β -glucosidasica activity, from the glucose to which they are joined to move into the gas phase, where they can interact with our olfactory receptors. The particular situation of cellular compartmentalization that is established as a result of cell crash would cause the high activity expressed by this enzyme component typically inhibited by the imposing sugar concentration of the must (Di Stefano 1989). At this particular mechanism it would also be due the expression of those typical varietal aromas of non-aromatic grapes (Castino 1989). Furthermore, operating at these temperatures is to be more significantly slowed the kinetics connected to the oxidation of anthocyanins than it is that relative to their grape extraction from the epidermis, and then the wort and the finished wine tend to assume shades of color more lives and intense (Andrich et al. 2004).
The cryomaceration therefore represents an oenological technique able to put in evidence the chemical-organoleptic and compositional characteristics of the grapes used (Cuenat et al. 1996) although these do not seem particularly valuable to produce richer wines color and perfumes (Blouin et al. 2000, Feuillat 2000, Heatherbell et al. 1996). This capability tends to adapt to the current demands of the consumer who prefers easily identifiable wines, strongly linked to the production area, with respect to products which, although free of defects, should fail personality and originality.

Experimental activity, the purpose

The Sangiovese grapes that are characterized by a remarkable aromatic content and phenolic components but lacking in colored components (anthocyanins) are particularly suitable to enhance the winemaking potential of cold maceration, and were then vinified using this innovative technology.
the main compositional and organoleptic characteristics of the wines obtained using two different cryogenic (solid CO_2, liquid N_2) employed in most temperatures (-5, 0, + 5 ° C for CO_2, 0, + 5 ° C for N_2) and inside of tanks made of different materials (wood, steel), they were compared with those obtained for wines produced in accordance with the vinification protocol normally adopted as shown in table 2, in the two companies involved in the testing and located in the production area of Chianti classic (A) and in that the Brunello (B).

Table 2
Adopted experimental design

COMPANY	TYPE OF MACERATION	USED CRYOGEN	TEMPERATURE MACERATION	MATERIAL OF TANK
A	Traditional	/	23-26°C	Stainless steel
	Cryomaceration	CO_2	-5°C	Stainless steel
	Cryomaceration	N_2	+5°C	Stainless steel
	Cryomaceration	N_2	0	Stainless steel
B	Traditional	/	25-28°C	Wood
	Cryomaceration	CO_2	+5°C	Wood
	Cryomaceration	CO_2	+5°C	Stainless steel

Materials and methods

The experimental tests have been conducted, according to the experimental protocol shown in Table 2, in cooperation with the VinoVigna study of Empoli, in the cellars of two notes Tuscan wineries located respectively in the Chianti Classico production zone (A) and Brunello di Montalcino (B), producing wines that are placed in very different price ranges (B>A). While in the company A were employed two cryogens ($CO_{2\ solid}$ and $N_{2\ liquid}$) at three different temperatures of cryomaceration (-5, 0, + 5 ° C), at the company B were performed two tests cryomaceration using the same cryogen (CO_2) at the same temperature (5 ° C), but inside of tanks of different material (wood, steel).

Technique

Previously crushed and de-stemmed grapes, were added 10g / hl of potassium metabisulfite, 20g / hl of yeast extract (fermaid E Lallemand) and 20g / hl of selected yeasts (saccharomyces cerevisiae strain D254 Lallemand), freeze-dried and rehydrated prior. After 48 h of fermentation were added 20 g / hl of Sal Ammoniac (Nutrient Lallemand). In cryomacerate theses half of yeast used was added at the beginning of the maceration phase, while the remaining half (10g / hl) was added at the beginning of the alcoholic fermentation. This second aliquot was initially subjected to a pre-adaptation to the reaction medium through the implementation of a pied de cuve, obtained by adding (T = 20 ° C; t = 30 minutes) to 500 g of rehydrated yeasts in 5 liters of water (T = 35/38 ° C), 10 liters of a pressed grapes devoid of metabisulfite and 20 g / hl of sal Ammoniac. When this solution reached 28° C, fresh must was added into three aliquots spaced apart by an hour for which the temperature of the mass of the inoculum so prepared amounted to around 13 °C. The mash was then brought to the desired temperature by means of successive additions of the cryogen used.

Vinification with cold maceration differs from the traditional one in the course of the following stages:

- reduction in the average temperature of the crushed grapes until you reach the desired temperature ($T_{desired}$ / - 1 ° C) by the use of the cryogenic chosen ($CO_{2\ solid}$, $N_{2\ liquid}$);
- thermal homogenization of the mass through delestage, pumping over and punching;
- periodic monitoring of the temperature of the mass subjected to cold maceration;
- increase of the temperature of the wort to reach cryomacerated +/- 15 ° C, to allow the development of alcoholic fermentation;
- inoculation of the second aliquot of yeast previously adapted to thermal shock

The finished wines were then analyzed to determine the main chemical and physical characteristics and composition (pH, total acidity, volatile acidity, residual reducing sugars, dry extract, glycerin, APA, proteins, color intensity, color tone, total and free anthocyanins, total phenols, proanthocyanidins), using the analytical methods reported in the literature (Lots and Galoppini 19880, DiStefano and Guidoni 1989). For each analytical determination were collected three different samples. Each reported value is, therefore, the average of three replications and this has allowed us to also assess the statistical reliability and therefore to make a comparison between the data obtained by analyzing the different experimental theses produced emphasizing the existence of statistically significant differences. Furthermore in order to establish the existence of such differences in the values obtained for the same chemical composition parameter at different steeping performed, the data obtained were submitted to analysis of variance for variable size classes. While to ascertain whether the difference elapsing between two experimental thesis was or was not significant, the $Q_{0,05}$ test, which allows to calculate the minimum statistically significant difference was used (D) which must be exceeded because the analyzed values can be held with 95% probability (p = 0.05) statistically different (Snwedecor and Cochran, 1979).

Discussion

Table 3 shows the values of the parameters analyzed (pH, total acidity, volatile acidity, residual reducing sugars, dry extract, glycerin, APA, proteins, color intensity, color tone, total and free anthocyanins, total phenols, proanthocyanidins), in wines produced according to wine-making protocol normally followed in the two companies A and B involved in this experimental activity (witness wines). The data obtained by analyzing the different wines produced at the same company in accordance with the manner of soaking prescribed by experimental protocol shown in Table 2, were subjected to analysis of variance (F test) to check the possible existence of at least one difference statistically significant between the values processed. The chemical and physical parameters and compositional found positive to the F-test, have been reported in table 4, together with the relative degree of statistical confidence (95 and 99%) and the calculated value for the parameter D, which represents the minimum value of the difference that runs between the two values so that these can be considered to be statistically different with 95% probability (p = 0.05). In order to allow an easier comparison of the data relating to the parameters considered, and in particular with that obtained for the witness wine produced in the two companies (A and B), the trial sites, the analytical values were expressed as a percentage change compared to the witness of reference; Variation %= $(X_{J,A/B}\ X_{T,A/B})*100/X_{T,A/B}$, where:
$X_{J,A/B}$ = value obtained by operating in accordance with the J-th argument provided for the company A or B;
$X_{T,A/B}$ = correspondence value obtained for the wine produced on the company A or B.

In the graph 2 shows the percentage values that attest as to the analytical data related to the protein content present in wines obtained following the protocols of production under the different experimental thesis analyzed and in particular the cryogen employed and the operating conditions follow the container used during the maceration of the grapes, diverges from the value provided by the corresponding witness. While in the company A, the values obtained for the cryomacerated wines while not diversifying between them in a statistically significant way, are significantly different from the witness, in the company B no difference between the data obtained exceeds the threshold (D) of indicating significance as in this case the use of maceration has not made any significant increase in protein content. This shows that the basic technology of winemaking used in the control wine production markedly influence the results of experimentation. In fact the winemaking protocol used to produce the more expensive wine witness B provides for a long period of rest on fermentation lees which induces the accumulation, inside the finished product, of proteins liberated as a result of the yeast cell lysis (Table 3). This does not happen in the witness wine A company that, not having had the contribution of products of the yeast lysis, it significantly differs in this parameter from the corresponding criomacerated products, which appear richer in protein resulting from the crash of the added yeast cells before proceeding to treatment with the cryogen. Following the cell crashes also the most valuable components of grapes are released in the liquid phase and among these are the phenolic components which, in criomacerate thesis, accumulate in solution, protected from the atmosphere inert ensured by the passage in the vapor phase of substantial volumes of cryogen employed at this stage ($CO_{2\ solid}$ e $N_{2\ liquid}$). It is thus justified the significant percentage increase shown in the graph 3, that also in this case is more evident for wine A with respect to the more expensive wine B. In fact, while in the case of A all evaluated thesis differ

significantly from its witness, one conducted at the company B, using a wooden tank, does not appear even if by little, other than the witness, probably because of the greater presence of O2 that this type of tank aimed to obtain. The different mean value reached by the temperature, the type of cryogen employed and the material used in the realization of the vat fermenter (stainless steel, wood) do not seem able to significantly affect the extraction of these components from the solid parts of the grapes, since the differences between the various tests conducted in the two companies of the trial sites never exceed the values calculated for the parameter D. Even data on concentrations of tannins to smaller molecular weight (proanthocyanidins, graphic 4) appear to be significantly different from their respective witnesses. But contrary to what observed for the content in total phenols statistically significant differences are noted to vary the cryogen employed ($N_{2\ liquid\ T=0°C}$; $CO_{2\ solid\ T=5°C}$), the adopted temperature ($N_{2\ liquid\ T=-5°C}$; $N_{2\ liquid\ T=-0°C}$) and the type of fermenter used ($CO_{2\ solid\ T=5°C\ stainless\ steel}$; $CO_{2\ solid\ T=5°C\ wood}$). While a comparison of the two cryogenic used during this experiment is complicated by concomitant variation, as well as their physical state, also on the working temperature, the use of the same cryogen ($N_{2\ liquid}$) at 5° it seems to provide better results because it happens by operating at 0° C. Probably the more reduced temperatures not only tend to slow down the extraction processes but also reduce the solubility of these compounds in the must. Confirming what was observed previously the use in other conditions being equal, a more oxidizing environment (vat wood than stainless steel) significantly reduces the concentration with which proanthocyanidins are present in solution. Similar conclusions may be repeated for total anthocyanins (graphic 5), being more sensitive to oxidation of proanthocyanidins, do not differ significantly from the control wine in company B and therefore do not show the use of tanks made of different materials a chance significant source of variability. Even in this case, the different type of product normally marketed by the two companies makes account of the differences, for which the wine of higher value (B) is to be normally produced using all the devices that preserve the color, and then located in 'use of cryomaceration a qualitative increase in percentage lower than that shown by the lower commercial value of product (A). The values found for the free anthocyanins (graphic 6) tend to corroborate these considerations, although in this case there was no significant differences using the same cryogen at different temperature values ($N_{2\ liquid\ T=-5°C}$; $N_{2\ liquid\ T=-0°C}$). The marked sensitivity to oxidation of its phenolic fraction this is well highlighted by the concurrence presence of a statistically difference between cryomacerated and witness, when operating using a vat made of stainless steel, difference that is lacking making wine in a more typical oxidizing atmosphere of a wooden tank. But the most encouraging notes for the use of this technique are from data collected for the different thesis analyzed relatively to the volatile acidity (Graphic 7), whose percentage decrease compared to the relative witnesses always appear to be significant, even if you do not notice statistically conclusive differences to vary the cryogen, the temperature and the type of fermenter used. Probably that introduction of partial yeasts performed before treatment with the cryogen allows to operate in a microbiological safety conditions such as to avoid that it achieves a substantial accumulation of acetic acid. In addition, at the tasting, the wines obtained by maceration have a content of primary aromatic components (aromas of fruity) definitely more intense of its witnesses.

Results

This innovative technology of vinification, allowing to preserve both the coloring components and the aromatic ones of the grapes used, appears to be able to ensure a high level of quality to the finished wine, which thus becomes softer, more round and balanced, as well as richer than perfumes varietal. The production of wines strongly linked to the grape variety used and the territory, which differ from the standard products available on the market so as to be easily identifiable by the consumer, can find in this new technology a valuable tool to highlight the special features. Also it is the lower price range products to benefit most from this increase in quality, to produce a wine that is placed at higher levels than normally placed on the market, thus justifying the financial and operational burden that this oenological practice involves.

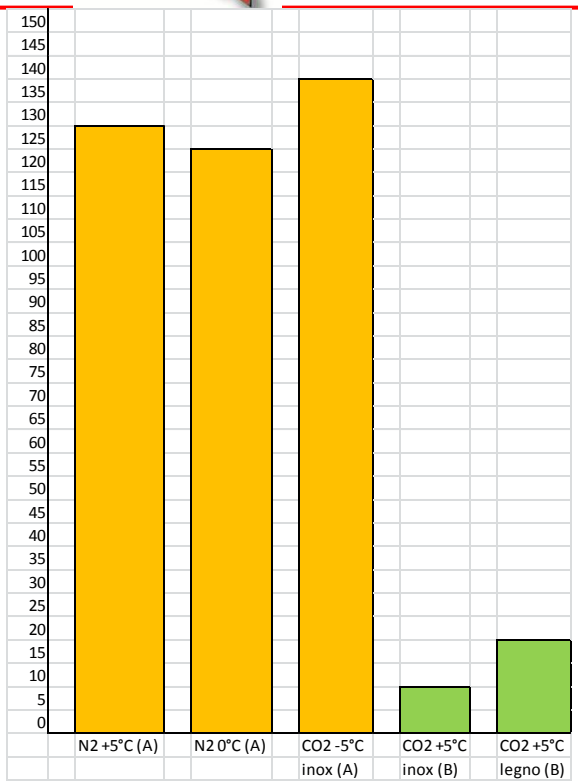
Table 3
Values of the parameters analyzed

ANALYTICAL PARAMETER	WITNESS WINE A	WITNESS WINE B
pH	3.53 +/-0.05	3.27 +/- 0.02
Total acidity (g/L ac. Tartarico)	6.62 +/- 0.20	8.05 +/- 0.20
Volatile acidity (g/L ac. Acetico)	0.37 +/- 0.03	0.45 +/-0.02
Residual sugar (g/L)	Traces	Traces
Dry extract (g/L)	19.88 +/- 0.50	25.54 +/- 0.50
Ashes(g/L)	2.83 +/- 0.07	2.19 +/- 0.50
Glycerine (g/L)	8.34 +/- 0.21	7.81 +/- 0.19
A.P.A. (mg/L)	78.96 +/- 2.22	100.52 +/- 2.51
Protein (mg/L)	130.41 +/- 27.32	342.43 +/- 8.64
Color intensity	15.87 +/- 0.20	24.92 +/- 0.62
Color tone	0.97 +/- 0.03	0.75 +/- 0.02
Total anthocyanins (g/L malvidin)	0.19 +/- 0.04	0.62 +/- 0.05
Free anthocyanins (g/L malvidin)	0.17 +/- 0.02	0.48 +/- 0.04
Total phenols (g/L catechins)	1.90 +/- 0.16	3.33 +/- 0.23
Total proanthocyanidins (g/L catechins)	1.05 +/- 0.02	2.10 +/- 0.13

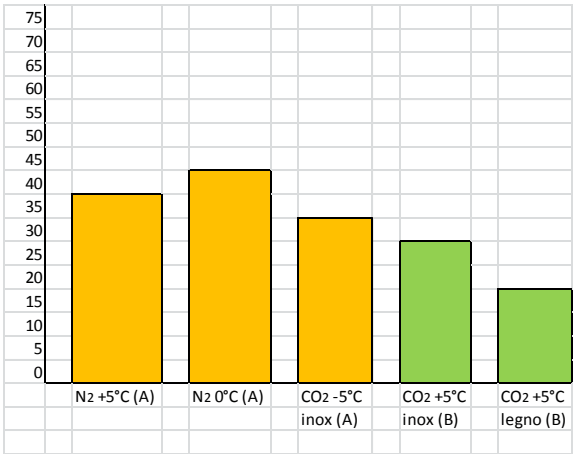
Table 4
Value taken by F of Fischer, its reliability and minimum value of the difference between the analyzed data so that they can be assume as statistically different with 95% probability (D test)

	A		B	
ANALYTICAL PARAMETER	**VALUE TAKEN BY F AND ITS STATISTIC RELIABILITY**	**D**	**VALUE TAKEN BY F AND ITS STATISTIC RELIABILITY**	**D**
Volatile acidity	F=14.00 (99)	0.07	F=22.75 (99)	0.05
Total phenols	F=22.20 (99)	0.31	F=14.63 (99)	0.47
Total anthocyanins	F=31.40 (99)	0.08	F=4.82 (n.s.)	0.10
Free anthocyanins	F=31.24 (99)	0.04	F=5.50 (95)	0.08
Proanthocyanidins	F=58.45 (99)	0.04	F=22.58 (99)	0.27
Protein	F=45.10 (99)	5.46	F=0.27 (n.s.)	7.00

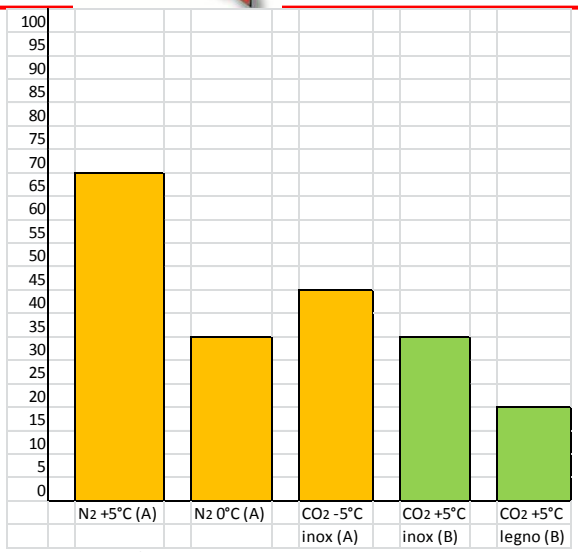
n-s. = not significant existence of at least a statistically significant difference with 95% (p = 0.05) or 99% (p = 0.01) probability



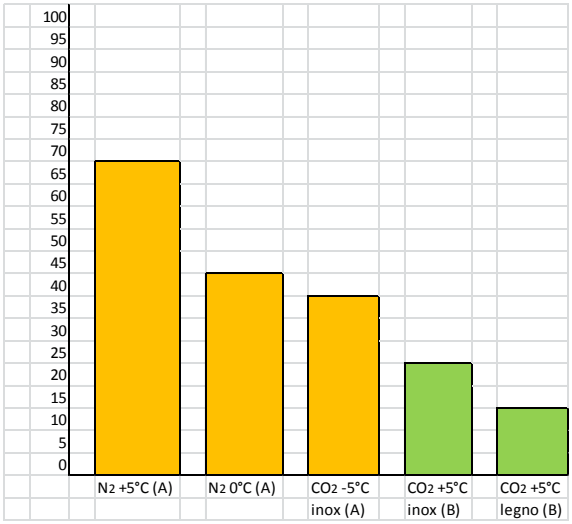
Graphic 2 percentage change of the proteins evaluated in all experimental thesis than that found for its witness and confidence intervals related to these.



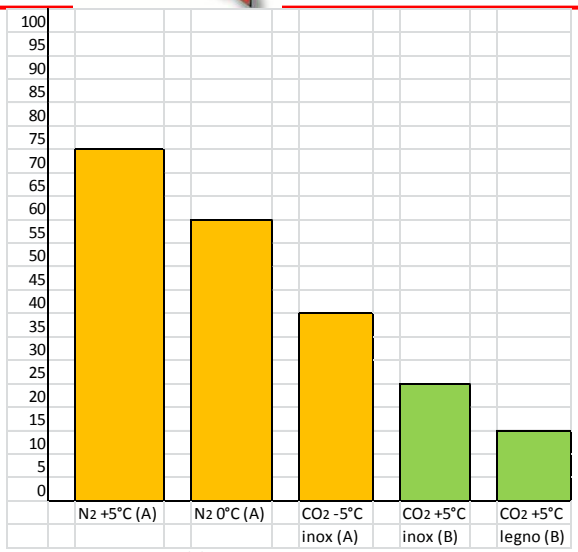
Graphic 3 percentage change in the concentration of total phenols evaluated in all experimental thesis than those found for its witness and confidence intervals related.



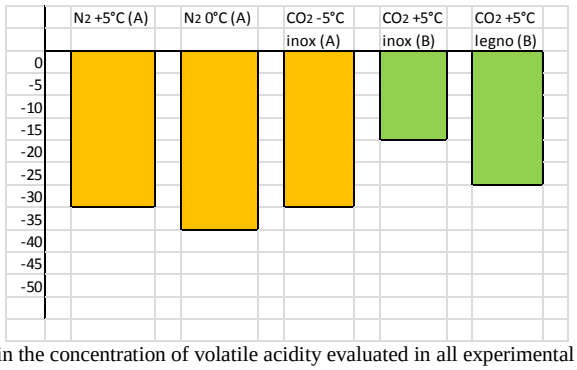
Graphic 4 percentage change in the concentration of proanthocyanidins evaluated in all experimental thesis than that found for its witness and confidence intervals in these related



Graphic 5 percentage change in the concentration of total anthocyanins evaluated in all experimental thesis than that found for its witness and confidence intervals in these related



Graphic 6 percentage change in the concentration of free anthocyanins evaluated in all experimental thesis than that found for its witness and confidence intervals in these related



Graphic 7 percentage change in the concentration of volatile acidity evaluated in all experimental thesis than that found for its witness and confidence intervals in these related

References: extracted from article published on VQ No. 2 in 2006, edited by Angela Zinnai, Francesca Venturi, Yasmine Calamita, Giampaolo Andrich (Dipartimento di chimica e biotecnologie agrarie, Università degli studi di Pisa).

Currently MIROS has produced equipment for maceration and removal quick temperature in cooperation with the manufacturers of cryogenic gases, these working systems called Polar and Krios, are able to make you get the results shown in this article, also working continuously up to 18000/20000 Kg / h production. Our machines are able to generate up to 400.000 refrigeration snapshots.

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