CHARACTERIZATION OF "TERRITOIRES" THROUGHOUT THE PRODUCTION OF WINES OBTAINED WITH WITHERED GRAPES: THE CASES OF "TERRA DELLA VALPOLICELLA" (VERONA) AND "TERRA DELLA VALLE DEL PIAVE" (TREVISO) IN NORTHERN ITALY

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Key words: over-ripening, dehydration techniques, post-harvest, organoleptic quality, sensory evaluation.

Abstract

In the definition and description of a "territoire" ("terra", in Italian), together with environmental and genetic factors, an important role is also played by agronomic, technical, and cultural aspects that contribute to characterize the produce of the specific area. The production of wines obtained following partial dehydration of harvested grapes may be considered as an interesting example of "territoire" characterization. Valpolicella, a hilly area North-West of Verona (Italy), is famous not only for its homonymous wine but also for the Recioto and Amarone that are obtained following dehydration of harvested grapes. The withering process is accomplished with traditional methods, or, in recent years, with new drying systems differently affecting the loss of water process (rate, intensity) with consequences on berry physiology and wine quality traits. Slow and rapid dehydration rates have been compared and some biochemical and molecular parameters linked to quality aspects (anthocyanins, resveratrol) have been monitored in the cv Corvina. A second example is represented by "Terra della Valle del Piave" and its Raboso wine, characterized by a strong and sometimes unpleasant taste, due to unbalanced polyphenol content. The application of the DMR technique (cluster bearing canes detached and berries allowed to over-ripen in the field) solves this problem: results concerning organoleptic evaluations of grapes and wines obtained using this technique are reported.

Resumè

Dans la définition et la description d'un "territoire" ("terra" en italien), avec les facteurs du milieu et génétiques, un rôle important est joué par ceux agronomiques, techniques et de culture qui contribuent à caractériser le produit d'une zone spécifique. La production des vins obtenus à la suite d'une déshydratation partielle des raisins peut être considérée un intéressant exemple de caractérisation d'un «territoire». La Valpolicella, une région collinaire au nord-ouest de Vérone (Italie) est célèbre non seulement pour le vin qui porte le même nom, mais aussi pour le Recioto et l'Amarone qui sont obtenus à la suite d'une déshydratation des raisins en post-récolte. Le procédé de la déshydratation est obtenu avec des méthodes traditionnelles ou, plus récemment, avec de nouveaux systèmes de perte d'eau (intensité et vitesse) avec des conséquences sur la physiologie de la baie et les aspects qualitatifs du vin. Une comparaison entre une déshydratation rapide et une lente a été effectuée sur la variété Corvina et on reporte des données biochimiques et moléculaires liées à des paramètres qualitatifs (anthocyanine, resvératrol). Un deuxième exemple est représenté par la «Terra della Valle del Piave» et son vin Raboso Piave, souvent caractérisé par un goût assez désagréable dû aux polyphénols qui ne sont pas équilibrés et mûrs. L'application de la technique DMR (Doppia Maturazione Ragionata -

Double Maturation Raisonnée) permet de résoudre ce problème: on reporte les données concernant l'effet de l'application de cette technique sur les propriétés organoleptiques du vin.

Introduction

Besides the interaction between environmental and genetic factors (that undoubtedly play a paramount role in determining the success of grape cultivation and represent the basis for the viticultural terroir zoning), viticulture is also related to social, cultural, and traditional aspects that, together, define the wider concept of "territoires" ("terra", in Italian) (Cargnello 2003a, 2003b).
In this context, agronomic practices and handling techniques of grapes before and after harvest markedly contribute to characterize wines, that, for their unique organoleptic traits, are representative of specific "territories". The production of wines obtained following over-ripening or partial dehydration of grapes (on- or off-plant) may be considered as an interesting example of "territoire" characterization.
It is well known that qualitative parameters and organoleptic traits of wines strictly depend upon physico-chemical properties of berries and that these properties are continuously evolving during the ripening stage. The most important changes that characterize this developmental process are sugar accumulation, decrease of organic acid, metabolism of polyphenols, synthesis of aromatic compounds, firmness decrease. The fruit ripening syndrome is strictly regulated at genetic level (Giovannoni, 2004) and a number of genes, potentially involved in the previously mentioned changes, have been isolated in grape and their expression studied throughout ripening (Davies and Robinson, 2000; Nunan et al., 2001; Burger and Botha, 2004). Besides the developmental regulation, gene expression and the related biochemical pathways are influenced by environmental factors (temperature, R.H., etc.) and several kind of stresses, including dehydration that occurs during the over-ripening (e.g., late harvest) and the post-harvest phase. The amount of water lost and the rate of dehydration markedly affect several fruit physiological processes (Kays, 1991) with consequences on the quality of the wines.
Grape dehydration techniques applied in several viticultural zones vary in relation to local traditions, environmental aspects, and the wine type to be produced. For example, in Valpolicella, a hilly area north-west of Verona (Italy), typical Recioto and Amarone wines are obtained following dehydration (up to 35-40% of water loss) of harvested grapes (mainly cvs Corvina, Corvinone, Rondinella and Molinara) placed in drawers which are stored in specialized areas (called "fruttai") where withering take place due to favourable meteorological conditions. For these specific wines, aim of grape withering is to obtain musts with more concentrated components, in particular for sugar and extracts, and enhanced aromatic traits. In recent years the withering techniques of grapes are evolving with the use of technology aimed to control the process (through modulation of environmental parameters as temperature, R.H. and ventilation) thus affecting the dehydration rate.
An other technique has been introduced in "Terra della Valle del Piave" (Treviso Province, North-East Italy) for the production of Raboso Piave wine. This wine is in general characterized by strong and unpleasant polyphenols that negatively affects the consumer approach. Cargnello (1992) proposed the application of the DMR ("Doppia Maturazione Ragionata") technique to produce wines with a more agreeable taste but still characterized by the organoleptic peculiarities of this local variety. With this approach, grapes are allowed to over-ripe in the field following detachment of clusters by cutting the bearing cane at different positions and/or in correspondence of different ripening stages, according to enological objectives (Cargnello, 1992; Spera et al., 1994; Cargnello et al., 1995).
Aim of the present work was that of comparing the effect of different dehydration rates on evolution of some biochemical and molecular parameters of Corvina grapes, and the DMR technique application on grape and wine quality of Raboso Piave for a better characterization of specific "territories".

Materials and Methods

After harvest (early October), Corvina grapes have been maintained at two different temperature regimes: constant temperature of 20°C (rapid dehydration, RD) and fluctuating temperature between day and night (natural conditions, slow dehydration, SD). At different intervals, clusters have been weighed and loss of water rate determined. Selected berries (representative of the whole sample) have

been collected and immediately stored at -20°C or, separately for pulp and skin, frozen in liquid nitrogen and stored -80°C. Total sugar (Total Sugar Test, Merck) and glucose (Glucose test, Merck) content, and tartaric (Revue Francaise d'Oenologie 78/79, 70) and malic (Malic Acid Test, Merck) acid concentrations have been determined. Total phenols and anthocyanin of skins have been determined through spectrophotometric methods (280 and 520 nm). Total RNA from berry skins has been extracted according to Bais et al. (2000). Northern blot analysis has been carried out as described by Tonutti et al. (1997) using the Phenylalanine Ammonia Lyase (PAL), Chalcone Synthase (CHS), UDP-Glucose:flavonoid 3-O-glucosyltransferase (UFGT), and Stilbene Synthase (StSy) molecular probes (Tornielli, 1998).
As far as trials on Raboso Piave are concerned, three different samples (grapes and wines) have been considered and compared: grapes harvested at mid October (Control), grapes harvested and dehydrated in protected areas (with no control of environmental parameters) for 2.5 months (FT sample), grapes over-ripened and dehydrated through DMR technique for one month (DMR sample). Vinifications have been carried out immediately after harvest for control grapes and at the end of the dehydration processes for FT and DMR samples.
Chemical and sensory analyses of grapes and wines have been performed as described by Cargnello (1993) and Spera et al. (1994).

Results and Discussion
The different temperature conditions maintained during the post-harvest phase of Corvina grapes strongly affected the dehydration rate (Fig. 1): in fact, about 10% of water loss was reached after 8 and 21 days and about 40% of water loss after 40 and 65 days for rapid (RD) and slow (SD) dehydration, respectively. Total sugar increased in both samples with no significant differences when compared in terms of same values of water loss (data not shown). Malic and tartaric acid contents slightly increased throughout the dehydration period with no significant differences between samples (data not shown). Skin total phenol content showed a decreasing trend more pronounced in SD up to 20% of water loss and in RD from 20 to 40% of dehydration (Fig.2). Anthocyanin content of skin remained almost constant in RD samples, whereas a marked reduction has been observed in SD samples up to 20% of water loss (Fig. 2). A recovery of this parameter was observed in SD skins in correspondence of 40% of dehydration.
A more detailed analysis has been carried out concerning expression of genes involved in the polyphenol biosynthetic pathway. Northern analyses pointed out a different expression profile between SD and RD samples concerning PAL (Fig. 3): in fact, in SD samples PAL mRNA markedly accumulated starting from 14% of water loss and reached the maximum level in correspondence of the last sampling date. In RD samples an increase of specific transcripts has been detected only at the end of the experimental period (42% of water loss). No significant differences in terms of hybridization signals have been observed between SD and RD samples for CHS and UFGT transcript accumulation (Fig. 3): a basal expression level of these two genes has been detected in both samples throughout the withering period.
Stilbene Synthase genes are involved in the biosynthetic pathway of stilbenic compounds including resveratrol. The dehydration rate markedly affects the expression of StSy being specific transcript accumulation induced by the slow dehydration process starting from 7% of water loss with strong hybridization signals occurring throughout withering. Differently from SD, RD samples showed an increase of StSy mRNA only when berries lost about 17% of their weight and never displayed intensities of hybridization signals comparable to those of SD samples (Fig. 3).
Concerning trials with cv Raboso Piave, an increase of sugars and pH, and a decrease of tartaric acid has been detected in both DMR and FT grape samples. Malic acid significantly increased in DMR and the titratable acidity significantly decreased in FT (Tab. 1). Wine analyses indicated that both FT and DMR strongly induced an increase of alcohol content (particularly in FT wine), dry extract, titratable acidity, glycerol, total phenols, and anthocyanins: these last two groups of biomolecules were particularly abundant in DMR samples (tab. 1). The sensorial analysis clearly demonstrated that wine obtained following DMR reached the highest score in terms of taste, colour, aromatic traits and overall evaluation and indicated that tannins of DMR wine were smoother and more evolved in comparison to the control and FT wines (tab. 2). A panel test carried out using the same descriptors on both grapes

and wines confirmed the positive effect induced by DMR and, at a lesser extent, by FT techniques on parameters directly linked to polyphenol content, metabolism and chemistry (tab. 3).

Conclusions

Results presented in this paper demonstrate that grape withering and the manipulation of the ripening process in detached clusters represent important tools for characterizing specific “territories” producing wines with peculiar and unique traits. These wines are the result of a strict interaction among environmental parameters, genetic factors, and technical approaches affecting the evolution of some ripening processes and aimed to obtain berries improved for specific parameters. As previously reported, grape dehydration techniques may be used to reach different enological objectives and the final results markedly vary in relation to the technique applied: data on cv Raboso Piave clearly demonstrate the positive effect of the DMR technique in enhancing several qualitative and sensory parameters, in particular those linked to polyphenols. The quality improvement of this wine typical of the “Terra della Valle del Piave” has been, in general, more pronounced with the DMR than with the FT technique, indicating that specific trials are needed to identify the best protocol to be applied. This is confirmed also by the experiments performed on cv Corvina in which the rapid and slow rates of dehydration differently affected the evolution of some parameters as polyphenol and anthocyanin contents of the skin. According to the results concerning these bio-molecules, the application of a rapid initial dehydration (up to about 15-20% of weight loss) followed by a slow withering process might be suggested. Also considering the expression data of StSy gene(s), slow rates of dehydration, particularly in an advanced stage of the withering process, stimulate the activation of the resveratrol biosynthetic pathway thus enhancing nutritional values of the wine.

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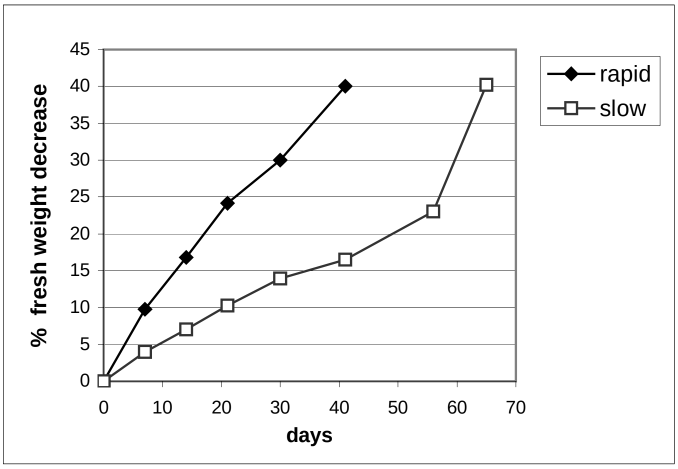

Figure 1. Grape (cv Corvina) fresh weight decrease following rapid and slow dehydration treatments.

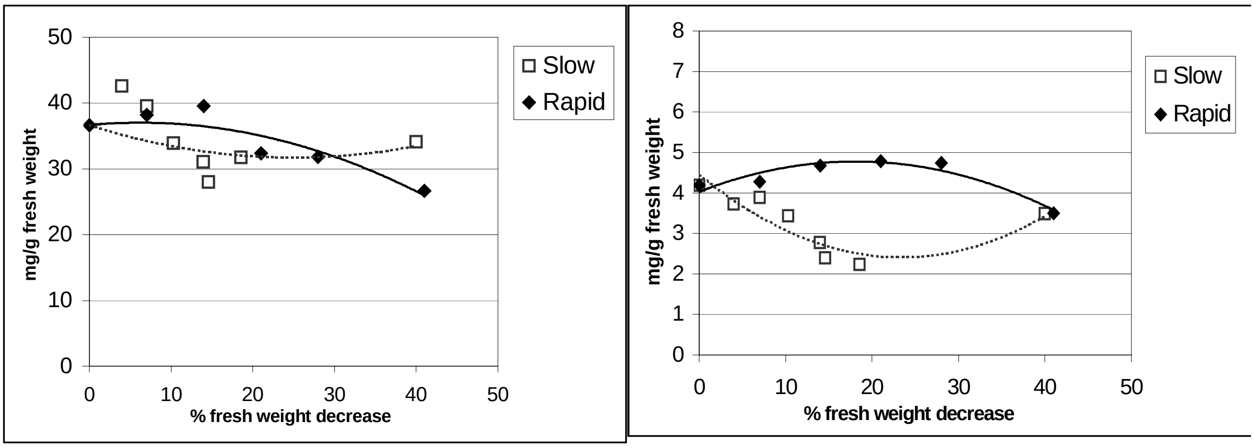

Figure 2. Berry skin total polyphenol (left panel) and anthocyanin (right panel) contents following rapid and slow dehydration of grapes (cv Corvina).

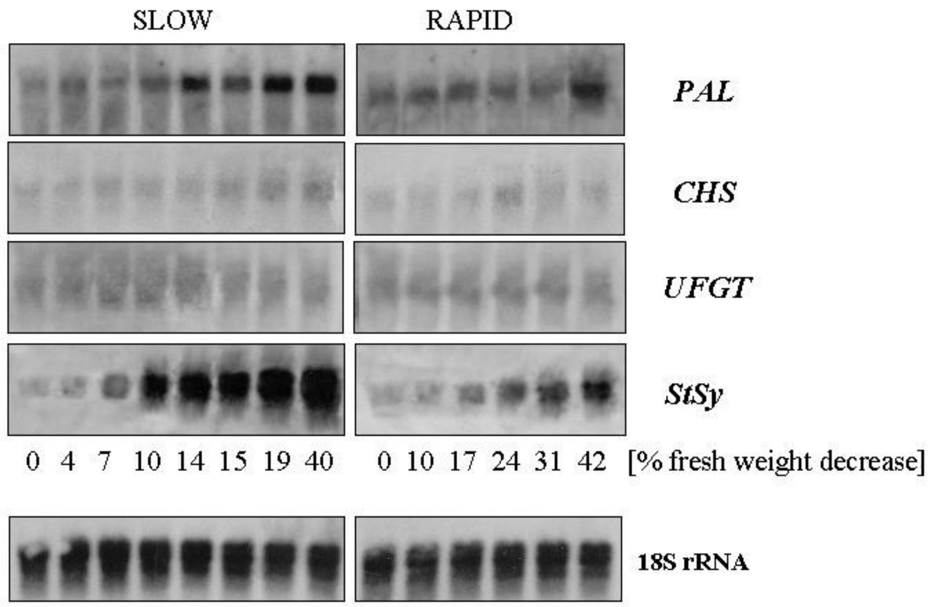

Figure 3. Northern analysis of genes involved in polyphenol biosynthesis (PAL, CHS, UFGT, StSy) during slow and rapid dehydration of grape (cv Corvina). Hybridization of 18S rRNA represents the control of the equal amount of total RNA analyzed.

GRAPE ANALYSIS	**Control**	**FT**	**DMR**
Sugars (°Babo)	18.1 c*	22.5 a	19.7 b
Titratable acidity (g/L)	14.7 a	12.5 b	14.3 a
pH	2.92 c	3.08 a	2.98 b
Tartaric acid (g/L)	9.41 a	8.01 c	8.45 b
Malic acid (g/L)	7.25 b	7.78 b	7.88 a
WINE ANALYSIS			
Density (g/mL)	0.9942	1.0045	0.9949
Alcohol (%vol)	10.8	13.1	11.4
Sugars (g/L)	0.0	0.0	0.0
Dry extract (g/L)	29.2	32.6	38.0
pH	2.95	2.95	2.95
Total titratable acidity (g/L)	6.7	8.5	9.5
Volatile acidity (g/L)	0.20	0.21	0.22
Tartaric acid (g/L)	3.08	3.98	3.29
Malic acid (g/L)	0.26	3.99	3.89
Lactic acid (g/L)	1.8	0.22	0.12
Ashes (g/L)	2.87	3.05	3.99
Total alkalinity (meq/L)	24.0	26.5	28.5
Total SO_2 (mg/L)	70	75	72
Free SO_2 (mg/L)	10	12	11
Total phenols (mg/L)	2972	3138	3744
Total anthocyanins (mg/L)	147	343	514
Glycerol (mg/L)	6.08	7.15	7.99

Table 1. Major compound analyses of Control, FT and DMR grapes and wines of Raboso Piave. (*) Same letters in a row indicate values not different for $p \leq 0.05$.

WINE ANALYSIS	Control	FT	DMR
Evaluation:			
Overall impression	78 c*	84 b	93 a
Colour	73 c	84 b	96 a
Odor	76 c	85 b	91 a
Taste	71 c	86 b	95 a
Typicalness for consumers:			
Traditional local	92 a	70 b	95 a
Traditional international	60 c	90 b	95 a
Richness	81 b	89 a	87 a
Acidity	77 c	88 b	94 a
Aroma:			
Varietal character	72 c	88 b	93 a
Intensity	81 c	86 b	91 a
Elegance	80 c	87 b	93 a
Balance	72 c	85 b	92 a
Overall impression	73 c	86 b	95 a
Tannin evaluation:			
Intensity	83 a	82 a	81 a
Roughness	76 c	88 b	86 a
Harshness	74 c	86 b	93 a
Overall impression	72 c	87 b	95 a

Table 2. Sensory evaluation of Control, FT and DMR Raboso Piave wines. (*)Same letters in a row indicate values not different for p≤0.05.

GRAPE ANALYSIS	Control	FT	DMR
"Strength"	50 c*	76 b	91 a
Phenol maturity	30 c	65 b	90 a
Harshness	45 c	65 b	86 a
"acidity quality"	48 c	73 b	90 a
Drying sensation	40 c	85 b	90 a
Roughness	43 c	81 b	92 a
Intensity	90 a	90 a	90 a
Roundness	45 c	82 b	90 a
"bitterness quality"	50 a	50 a	50 a
Overall quality	50 c	81 b	95 a
WINE ANALYSIS			
"Strength"	51 c	78 b	96 a
Phenol maturity	30 c	72 b	90 a
Harshness	48 c	68 b	93 a
"Acidity quality"	49 c	75 b	96 a
Drying sensation	40 c	86 b	92 a
Roughness	50 c	82 b	95 a
Intensity	90 a	90 a	90 a
Roundness	45 c	82 b	90 a
"Bitterness quality"	50 a	50 a	50 a
Overall quality	50 c	82 b	95 a

Table 3. Polyphenol sensory evaluation of Control, FT and DMR grapes and wines of Raboso Piave. (*)Same letters in a row indicate values not different for p≤0.05.