Universidade de Trás-os-Montes e Alto Douro

Effect of dehydration conditions in grapes of Sagrantino variety dried in climatic chambers for the production of Sagrantino Passito wine

Master Dissertation in Agronomic Engineering

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“The contents presented in the present work are the sole responsibility of the author.”
Abstract

Sagrantino Passito from Montefalco is an Italian sweet wine with denomination of controlled and guaranteed origin, made from grapes of the Sagrantino variety, partially dehydrated, produced in the region of Montefalco.

The large influence of the atmospheric conditions in the traditional drying process enhanced the finding of alternatives to this step, trying to recreate this process artificially, in order to reduce the risks adjacent to the traditional method.

The Grapes of Sagrantino variety were dried under different conditions and, during the drying process, their effects in the must, skins and seeds were analyzed.

The reducing sugars, pH, total acidity, malic acid and gluconic acid were analyzed in the must, as well as the phenolic compounds in skins and seeds.

It has been observed that higher temperatures lead to a greater increase in the rate of dehydration and a faster accumulation of reducing sugars. These, however, suffer degradation during this process, together with the results of malic acid, total acidity and pH contents, showing that a respiratory process occurs. Also during this process of dehydration, fungal contamination occurs mainly at higher temperatures, as can be concluded by the values of gluconic acid.

In general, the concentration of phenolic compounds decreased, especially in the skins, leading to the hypothesis of an occurrence of degradation reactions as well as the dissolution of these compounds in the pulp.
**Resumo**

Sagrantino Passito de Montefalco é um vinho doce italiano com Denominação de Origem Controlada e Garantida, feito com uvas da variedade Sagrantino, parcialmente desidratadas, produzidas na região de Montefalco.

A grande influência das condições atmosféricas no processo de desidratação tradicional levou a encontrar alternativas nesta etapa, tentando recrear este processo artificialmente, de forma a diminuir os riscos adjacentes ao método tradicional.

Uvas da variedade Sagrantino foram desidratadas em diferentes condições, e durante esta desidratação foi analisado o seu efeito no mosto películas e grainhas.

Foram analisados no mosto os açúcares redutores, pH, acidez total, ácido málico e ácido glucónico. Também foram analisados os compostos fenólicos das películas e grainhas.

Observou-se que temperaturas superiores levam a velocidades superiores de desidratação e a um mais rápido acumulo de açúcares redutores. Estes, contudo, sofrem degradação durante este processo, juntamente com os resultados do ácido málico, acidez total e pH, demonstrando a ocorrência de processos respiratórios. Também durante este processo de desidratação, ocorre contaminação das uvas por fungos, principalmente com temperaturas superiores, tal como se pode concluir com os valores do ácido glucónico.

De modo geral, a concentração dos compostos fenólicos diminui, principalmente nas películas, pondo em hipótese a ocorrência de reacções de degradação bem como a dissolução destes compostos na polpa.
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1. Introduction

Sagrantino Passito from Montefalco is an Italian sweet wine made from grapes of the Sagrantino variety, partially dehydrated, produced in the region of Montefalco. It is a wine with denomination of controlled and guaranteed origin, in Italian DOCG Denominazione di Origine Controllata Garantita, since 1992 and by Ministry Decree.

Traditionally, in the production of Sagrantino Passito, the grapes were dried outdoors, which implies a huge influence of atmospheric conditions such as ambient temperature and relative humidity (Ribéreau-Gayon et al., 2006a; Serratosa et al., 2008a).

Research has been made in recent years with the aim of creating alternatives at this stage of processing, in order to reduce the influence of these factors such as the use of controlled environment chambers, bearing always in mind the organoleptic characteristics of the wine and its tipicity (Bellincontro et al., 2006; Esmaiili et al., 2007; Ramming, 2009; Serratosa et al., 2008a; Squadrito, 2007).

Therefore, this work has as main objective to study the best controlled conditions of grapes dehydration for the production of Sagrantino Passito wine, comparing the characteristics of grapes dried in different temperature and humidity conditions as well as the study of the characteristics of produced wines.

2. Characterization of the Montefalco region

The wine region of Montefalco is located in Umbria - Italy and covers the territory of Montefalco and part of the territories of Bevagna, Gualdo Cattaneo, Castel Ritaldi and Giano dell’Umbria as shown in Figure 1.
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2.1. Historical Context

The vitiviniculture in the territory of Montefalco is not at all a recent activity, as it isn’t around the Mediterranean, and particularly Italy. However the first documents that somehow evidenced this agricultural practice in the region in question date back to the eleventh century only, a century in which the vines were the subject of donations, sales and concessions contracts for sharecropping, leading to believe, that even at that time the vineyard cultivation and the wine had an important role in the development of the local economy (Buccioli and Luneia, 2007; Mattoni, 20??).

Despite the high importance of the wine trade in this region, being considered an art in the fifteenth century, it is important not to forget the role played by religion, especially in countries like Italy where the power of the clergy was often sovereign. There are references that show a strong link between the wine and the clergy and its importance in religious rituals (Buccioli and Luneia, 2007). There are numerous documents that testify the participation of the clergy in the development of this sector, because at that time much of the land belonged to the Church (Spera, 2008).

Although the wine DOCG Sagrantino of Montefalco was only recognized in 1992, its description in this region is longstanding as was previously described (Spera, 2008).
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However, it is only on that date that the Production Regulation for Sagrantino of Montefalco DOCG - DM 05.11.1992 G.U. 269 - 14.11.1992 arises.

This Ministerial Decree specified the designation of origin Montefalco Sagrantino dry and passito wine, determines the geographical area of origin of grapes for the production of this wine and all agricultural and oenological practices permitted and/or required for the wine to be recognized. Also included in this Decree were the chemical, physical and organoleptic characteristics that the wine must comply to be placed on the market.

It is up to 3A Parco Tecnologico Agroalimentare dell’ Umbria soc. cons a r.l., the controlling entity, to ensure that the entire decree is respected through a control methodology that covers the entire production chain, so that there are no wines produced with other varieties, defective or even with characteristics that are not covered by the regulation, placed on the market.

2.2. Sector Diagnosis

Besides its tradition as a wine producer, Italy is one of the biggest wine producers among the European countries. According to ISTAT, in 2011 Italy produced 42 million hectoliters of wine battling with France for the title of largest European producer. However, there has been a tendency for a controlled reduction of quantity of wine produced, resulting in an increase of wine quality and consequently increase in the product value.

At national level, the regions with greater importance, regarding the production of wine are Veneto, Emilia-Romagna, Puglia and Sicily, making up 60% of total Italian production, according to ISTAT.

According to the same source, in 2011 Umbria, which includes the Montefalco region, represented 2% of national production with only 860 000 hectoliters, and only 6% of this value corresponds to Montefalco Sagrantino DOCG and 11% to DOC Montefalco.

According to the Consorzio Tutela Vini Montefalco, the annual production of Montefalco Sagrantino DOCG is about 1,000,000 bottles with an export share of around 45%,
mainly to Germany and USA. Regarding the Montefalco DOC reaches approximately 2,000,000 bottles annually with the same export figures.

As can be seen in Figure 2 and Figure 3, in the last decade the area of the variety of vine Sagrantino has exceeded the double; however, there was a decrease in the production due to the adoption of measures to control production in order to improve the quality of the product. According to consorzio, in 2012, the maximum yield of grapes per hectare reduced from 80t/ha to 70t/ha in the production of Montefalco Sagrantino DOCG.

![Figure 2](image2.png)

**Figure 2** Trend of Montefalco DO wine production, in potential number of bottles, subdivided in typology of wine. Data provided by Consorzio Tutela Vini Montefalco.

![Figure 3](image3.png)

**Figure 3** Evolution in the area of vineyards with Sagrantino variety in hectares. Adapted from Partenza, (2009).
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In 2006, based on data from "Catasto Viticolo della Regione Umbria", the vineyard area was 1.552 ha in Montefalco with a predominance of Sagrantino with 763.58 ha, equivalent to 49% of the total area, and Sangiovese with 401.32 ha, equivalent to 26% of the total area (Palliotti et al., 2007a).

According to the same source, and referring to white varieties, despite occupying only 14% of the total vineyard area, the most used for the production of DOC Montefalco white wine is Grechetto with about 115 ha, equivalent to 7%, and Trebbiano Toscano with 60 ha, equivalent to 4% of the total area.

2.3. Climate Characterization

The climatic conditions of a particular region are, certainly, the factors that influence the plant productivity the most. Among these, the air temperature and the hydric availability are the most significant, because they will influence all physiological processes of the plant and finally the grape maturation. While the temperature is directly dependent on solar radiation, water availability is directly dependent on natural precipitation and water retention capacity of the soil (Palliotti et al., 2007a).

It is thus of great importance to have an understanding, even if not a deep one, of the climatic characteristics of the region under study.

The climate of Montefalco and the surrounding hills is of Continental type. Based on the average reference (period between 1961 and 1990), the average temperature of the coldest month, January is 3.8°C and the hottest month, July, is 25.3°C according to Production Regulation for Sagrantino of Montefalco DOCG - DM 05.11.1992 G.U. 269 - 14.11.1992.

The average annual precipitation is about 700mm, distributed by 89 days with a relative minimum in summer, being July the least rainy month, and a maximum in autumn especially in the month of November. Snow occurs, on average, seven times per year and there are 40 icy days. In soils characterized by low water retention capacity, in the driest months hydric stress can occur as reported in Production Regulation for Sagrantino of Montefalco DOCG - DM 05.11.1992 G.U. 269 - 14.11.1992 and Palliotti et al. (2007a).
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2.4. Pedologic Characterization

Regarding the soil characteristics of the Montefalco region, it is affected by various types of geological formations, as follows: alluvium clay, turbidites, sand and conglomerates, despite no significant qualitative differences of grapes and wine from these different formations have been seen (Calandra and Leccese, 2007; Palliotti et al., 2007c).

In their research on the environment description of the Montefalco region, Calandra and Leccese (2007) observed that the layer thickness explored by the roots decreases progressively, being higher in alluvial soils which are 150 cm thick, followed by clay sandy soils, and finally the turbidites and conglomerates with a thickness of 70 cm. The apparent density increases as the soil deepens (with values between 1.35 and 1.65).

Regarding the chemical characteristics, and according to the same author, the amount of CaCO3 is very high, with high values of active calcium, whereas the presence of carbonates interfere with the soil pH, making it alkaline; values between 7.8 to 8.2 were observed in this region. The content of organic matter is present in considerable amounts with values between 1.5% and 2.2%, and the cation exchange capacity has values between 14.3 and 31.5 meq/100 g.

It is important to bear in mind that the chemical characteristics are strongly related with the geological origin, with erosion and rejuvenation as well, but not least, with the current and previous agronomic management (Calandra and Leccese, 2007).

3. Sagrantino Variety

Nowadays, one varietal standardization can be observed in the world vitiviniculture context, in which a large part of the prestigious red wines are produced with small number of ubiquitous and international grape varieties grown in all continents, such as Sauvignon, Merlot and Pinot Noir. However, according to Palliotti et al. 2007a, the presence of a discrete number of autochthonous grape varieties, complex in a compositional standpoint and highly diversified represents a great potential which needs to be transformed into opportunity.
In the region of Umbria, the Sagrantino variety is certainly among the red varieties qualitatively valid and able to represent a precious territory such as Montefalco. The characterization of oenological products by harnessing the authenticity and typicity offered by autochthonous grape varieties such as Sagrantino determines, nowadays, the possibility for companies to generate higher added value (Palliotti et al., 2007a).

Historically speaking, as far as the Sagrantino variety is concerned, the earliest references date from the thirteenth century, but refer a different name. It is, however, in the fifteenth century that the first reference to a variety of red grape called Sagrantino can be found (Buccioli and Luneia, 2007).

The origin of this variety has no clear origin. The possibility of not being a local variety but an imported one has been considered; perhaps it was imported by one of many pilgrims, followers of St. Francis of Assisi and other congregations, which spread everywhere looking for a life of penitence and redemption (Spera, 2008).

In the region of Montefalco, as we could see before, beyond Sagrantino, there is another important variety with a large tradition, the Sangiovese, but with the difference that this last one occupies near 70000 ha in Italy, corresponding to 10% of the national vineyard area (Palliotti et al., 2007a).

3.1. Ampelographic Characterization

A single grape variety may present numerous synonyms, and the same name or similar names are often used for genetically different varieties. Therefore Ampelography - derived from the Greek words *ampelos* (“vine”) and *graphe* (“description”) is used - it is the science concerning the description of vine species and cultivated varieties, through complete botanical description.

For a description of the Sagrantino variety, Bruni (1962) used a clone grown in the Agriculture Foundation vineyard, University of Perugia, located in Casalina, in the territory of Deruta, and obtained the following ampelographic characterization:
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Bud of 10-15 cm

Apex: a fan, cottony, whitish green color, often with hem carminate, of medium size.

Apex leaves (from the 1st to 3rd): cottony on the 1st two pages, the second on the upper and fluffy cotton wool on the bottom, green-white, often with hem carminate, especially the first one in the gutter.

Basal leaves (from the 4th onwards): glabrous above, downy on the bottom, in green, three-lobed, revolute at the edges.

Axis of bud: arachnoid end, green in color, slightly bronzed, slightly curved.

Bud at blooming

Apex: a fan, cottony, whitish green color, often with carmine edge, small.

Apex leaves: the 1st cottony on both two pages, the second on the upper page, and cottony on the bottom page, whitish green color, often with carmine edge, angled eaves the 1st, the 2nd expanse.

Basal leaves: glabrous on the upper page, woolly on the bottom, green color, with revolute edges, orbicular, petiole sinus open V.

Axis of bud: arachnoid at the end, green or slightly bronzed, curved.

Herbaceous shoot: round cross-section, smooth contour of green color with bronze shades and streaks, glabrous and slightly arachnoid towards the end.

Tendrils: intermittent distribution, bi-trifffids of green color.

Inflorescence: Medium-sized, cylindrical or cylindrical-conical, semi-tightened bunches and flowers and green peduncle.

Flower: floral button regular in shape, medium or nearly small; green corolla, with regular opening; open flower: hermaphrodite, stamens slightly enlarged; auto fertile.
Leaf (Figure 4 a) and b)): Medium-sized, orbicular, three-lobed or rarely with five lobes; petiole sinus U-shaped, with closed edges and overlapped, very deep; upper lateral sinuses, elliptical, semi-closed or open in V shallow or just hinted. Green and vesicular or bullous top page; lanuginous lower page. Corrugated limbo; revolute lobes with obtuse or right angles. Main veins on the lower side in green color, bristly. Regular teeth, in one or two series, medium-sized or nearly large with narrow base and slightly convex margins.

Figure 4 a) Top page of a Sagrantino variety leaf; b) Lower page of a Sagrantino variety leaf (Palliotti et al., 2007a).

Petiole: Medium length and thick, rounded section, glabrous, green, often slightly tainted pink.

Autumn coloring of the leaves show yellowish nuances and intense red crimson stains.

Bunch at industrial maturity (Figure 5): Medium size or nearly small, cylindrical or cylindrical-conical, winged, semi-sparse for light leaking, rather regular in appearance and shape; rachis slightly faded green or pale red-vinous; peduncle of medium length and thickness, semi-woody; pedicels of medium length, slim and green in color; cercine warty, thick and green or slightly tinted red-winey; brush thick and short, yellowish-green shaded in pale red-winey.
Berry: round or slightly sub-round, regular cross section; skin fairly or very waxy, black, medium thickness, consistent; pulp dissolved with a simple flavor, separation from the berry pedicel of medium difficulty.

Grape seed: in number of 2-3 per berry, medium-sized or nearly small, regular shape, short beak.

Woody shoot: Medium length and thickness, medium vigor, circular cross-section, smooth surface, slightly waxy; nodes of medium evidence and slightly more pronounced than the internodes, which are mid-length or nearly short and pale brown, with dense streaks, smooth and slightly marked; aperture of medium thickness; marrow medium thickness; buds
of medium size or nearly large, conical, obtuse, rather protruding cercine petiolar medium apparent.

Trunk: medium vigor and medium thickness; roots of the year with deep brown color, the main ones forming a geotropic angle of about 50°.

Phenology

Vegetative Phenomena

Germination: ordinary period.

Flowering: precocious period.

Ripening: intermediate period.

Grape maturation: IV period, likely to be kept on the plant without rotting.

Beginning of leaf color change: early period;

Leaf fall: late period.

Features and Cultivation Characters

Vigor: medium; prefers farming systems with medium expansion in vineyards, and also at great expansion vines by maple, and medium or long pruning.

Production: medium and low, irregular.

Position of the first floriferous bud: From the 4th node.

Average number of inflorescences per shoot: none or one from the 1st bud at the base, one or two from the others.

Resistance to adversity: very resistant to cold winter and spring ones; low resistance of leaves to downy mildew, normal resistance of grapes to oidium,
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...mildew and rottenness. According to observations the roots have shown resistance to phylloxera.

It is known that different varieties confer different characteristics to the wine. While some cultivars are indicated for the production of wine others, due to their characteristics, are preferentially used as table grapes, and even for the production of raisins.

The variety in question is known for its richness in phenolic compounds. The skins and seed grapes are characterized for a high amount of tannins and medium high amount of anthocyanins in the skins. Among these prevails malvidin-3-glucoside, being also important the percentages of delphinidin and petunidin that exceed 10% of the total. Also peonidin, anthocyanin di-substituted reach 10% of the total (Di Stefano et al., 2008).

According to this author, the wines produced from grapes of the Sagrantino variety, are featured by the content in polyphenols, including proanthocyanidins, so high that it significantly surpasses the great red wines traditionally meant to last long time. Nevertheless, the phenolic compounds will be described adequately later in this paper.

The agronomic practices are also a factor that must be taken into account. These reflect the needs of plants, as well as the possibility of mechanization, maximization of production yield, and even quality improvement actions.

In Montefalco, the traditional conduction system of the vine used was palmette, in which the vine grows with large vegetative expression. This system was gradually replaced by cordon spur, in which the trunk is developed horizontally on one side only, starting from a certain height.

In order to promote a better and more balanced development of the new conduction system, all vineyards were planted with a density of 4000-6000 vines per hectare, a much higher density than that used in the previous system, 1600 plants per hectare. Thus, the yield per plant decreased considerably, improving the quality and maintaining production per hectare within economically acceptable values.

Therefore, from the technical point of view, these new vineyards should be considered highly efficient and able to ensure high quality and constant production over time.
4. **Overripening**

Overripening, in Italian *Appassimento*, is a practice used in the production of Sagrantino passito wine and its use is the only difference observed when compared to the diagram of dry wine production. Physiologically speaking overripening is the natural extension of maturity, and in case of grapes, involves a dehydration process that promotes the increase of sugar concentration, obtaining sweet wines with residual sugars or dry wines with special aromas, depending on the level of dehydration.

The maturation of vascular tissues of grape peduncle progressively isolate from the rest of the plant. As a result, the volume of grapes decreases, since the losses by evaporation are no longer compensated by the plant (Ribéreau-Gayon et al., 2006a).

Overripening is also characterized by an increase of fermentative metabolism and activity of alcohol dehydrogenase. With a water loss of about 0.5%, the activity of cell wall enzymes increases, and the increase of water lost by dehydration accelerates the process of respiration and ethylene production, along with the loss of volatile compounds (Ribéreau-Gayon et al., 2006a; Costantini et al., 2006).

An initial stress occurs when the grapes lose 10 - 15% of their weight, leading to an alteration in the metabolism from aerobic metabolism to anaerobic metabolism. This alteration can be explained by the modification in the architecture of the cell, leading to a change in the membrane functionality, thus reducing the gas diffusion when there is the greatest need of oxygen by the cells, due to the increase of respiratory metabolism which appears after water stress (Costantini et al., 2006).

According to the same source, the measurement of respiration metabolism and the production of ethanol in off-vine overripening revealed a partial conversion of glucose and malic acid in ethanol and CO$_2$ by pyruvic acid pathway. Changes in the metabolism from aerobic to anaerobic lead to the production of ethanol, CO$_2$ and byproducts of fermentation. During the dehydration process, the concentration of ethanol increases until water loss reaches a certain level, being lost by oxidation and esterification.

The overripening of grapes can be made on vine, as is the case of the production of Sauterne, Tokay and Ice Wine. The berries shrivel gradually, losing their composition in
water. They produce, naturally, concentrated musts rich in sugars and aromatic compounds. The acidity does not increase in the same proportions and may even decrease due to oxidation of malic acid. Another biochemical phenomenon that occurs is the deterioration of the skin cells, leading to the development of *Botrytis cinerea* (Ribéreau-Gayon et al., 2006a; Rolle et al., 2009).

In certain regions, the overripening can be performed after the harvest and limited to a simple sun exposure, for a variable period of time. The bunches are turned regularly and covered at night, so that they can be protected from the dew.

This method of sun drying rather than concentrating grape sugar, increases the aromas of the must, which tend to have high concentrations of terpene alcohols (Ribéreau-Gayon et al., 2006a).

This natural drying method results in high losses by rot being contaminated clusters eliminated regularly, being this way an operation difficult to control. Grapes are also susceptible to insect attack, intense solar radiation and eventual climate change, such as, occurrence of rain (Ribéreau-Gayon et al., 2006a; Serratosa et al., 2008a).

Since the beginning of the twentieth century it has been attempted to replace this natural process by an adapted technology. The principles for an artificial overripening are simple. The equipment must be based on the circulation of hot dry air over the grapes that are placed in small boxes inside a heated compartment. The ventilation system circulates 2500 - 5000 m$^3$ dry air (less than 15% relative humidity) per hour, with temperatures varying between 25 and 35 C° (Ribéreau-Gayon et al., 2006a).

If we consider that there were no reactions involving phenolic compounds, the increase of their concentration during dehydration of grapes would be expected. However, some phenols may be present in different reactions such as non-enzymatic browning and/or reactions of autoxidation and enzymatic oxidation, involving the oxidase and peroxidase enzymes, leading to a decrease of their concentrations. Likewise some derivatives of flavan-3-ols with a high molecular weight can originate phenolic compounds of lower molecular weight by hydrolysis. (Serratosa et al., 2008a).
Serratosa et al. (2008a) when studied the grapes dehydration of variety Pedro Ximenez in chambers with controlled temperature (40 and 50 ° C) observed that the gallic acid content increased in concentrations higher than what should be expected just through the dehydration of grapes (2.33 to 7.24 mg/L at 40 ºC and to 10.2 mg/L at 50ºC). Esters of hydroxycinnamic acids also increased but in smaller proportions than those observed in reducing sugars, suggesting their involvement in some kind of degradation reactions. The content of catechin and epicatechin, monomers of flavan-3-ols, increased during dehydration but in a smaller scale than would be expected, indicating that they participate in oxidation and condensation reactions, being more evident at higher temperatures. On the other hand, the differences observed in oligomeric flavan-3-ols not show large influence of temperature; however, its content increased in small quantities showing its involvement in degradation reactions.

5. Phenolic Compounds

The phenolic compounds are present in grapes as well as in all plants, being represented, in nature, by numerous compounds with diverse chemical structures, sharing between them the fact of having, at least, one benzene ring.

One of the most notable features of ripening is the rapid accumulation of phenolic compounds, which give, especially to red wines, its oenological importance. These phenolic pigments are secondary products of sugar catabolism and their biosynthetic pathways are present and partially active right from the beginning of grape development and the concentrations of these compounds increase during this period, whereas high and balanced levels are essential to produce red wines with high quality and suitable to aging (Pérez-Magariño and González-San José, 2006; Ribéreau-Gayon et al., 2006a).

Polyphenols are dissolved in pulp cell vacuoles: adsorbed or attached to the polysaccharides of fibrovascular vessels and free in the cellular vascular juice of the skins. In the skins they can also be found bonded to the cell wall polysaccharides and to proteins of membrane vacuoles. In the seeds, the polyphenols are mainly located in the outer tissues.

Xu et al. (2010) observed that the tegument contains the major part of phenolic compounds, obtaining for Cabernet Sauvignon 87% of the total phenolic compounds in the tegument.
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It is already known that these compounds are the source of color and large part of flavor in red wine, as well as its body and structure, being therefore of utmost importance to know its nature and composition in order to adjust, eventually, the winemaking diagram (Pérez-Magariño and González-San José, 2006).

One of the possible classifications of polyphenols in grapes and wines is its division into flavonoids and non-flavonoids (Figure 4). Flavonoids are molecules characterized by a particular 15-carbon structure formed by two polyphenolic rings with a tetrahydropyran heterocycle between them. Flavonoids can be classified depending on the oxidation degree of the heterocycle. The most common flavonoids in grapes and wines are flavonols, flavan-3-ols and anthocyanins. Non-flavonoids are polyphenols with a different kind of structure: phenolic acids, stilbenes and hydrolysable tannins. The latter originate mainly in the wood of barrels, so they can be found in wine and can naturally be found in grapes only in very small quantities.

Figure 7 Classification diagram of phenolic compounds adapted from Cheynier et al. (2000).
5.1. Non-flavonoids

5.1.1. Phenolic Acids

Phenolic acids are organic acids made of an aromatic ring directly or indirectly bound to a carboxylic group, and the benzoic and cinnamic acids are two types of phenolic acids naturally occurring in the pulp and in the skin of grape berries. Their concentration in grapes and wine are in the order of 100 – 200 mg/l in red wines and 10 – 20 mg/l in white wines (Fogarty, 2010; Ribéreau-Gayon et al., 2006b).

Phenolic acids are colorless in diluted alcoholic solution, but may become yellow due to oxidation. From the organoleptic point of view, these compounds have no particular taste or flavor. However, they are precursors of volatile phenols produced by yeasts of genus Brettanomyces and some bacteria. It is the case of ethyl phenols and ethyl guaiacols found in red wine and that give to the wine aroma defects. Also, when the wines are aged in new oak barrels, the toast of the wood leads to breakage of lignin and to the formation of compounds of the same family, with roasted, smoke and burned aromas: guaiacol, methyl guaiacol, propyl guaiacol, allyl guaiacol, syringol and methyl syringol (Jackson, 2008; Ribéreau-Gayon et al., 2006b).

Seven benzoic acids were identified (Figure 5) of which two are present in trace amounts: salicylic acid (ortho-dihydroxybenzoic acid) and gentisic acid (2',5'-dihydroxybenzoic acid). The various acids differ in the substitution of their benzene ring, being present in grapes, as glycosidic combinations, which are released by acid hydrolysis, and esters (ellagic and gallic tannins), obtained by alkaline hydrolysis. (Ribéreau-Gayon et al., 2006b).
Several cinnamic acids are present in grapes and wine (Figure 5). They have been identified in small quantities in the free form, although they occur principally as esters with tartaric acid, but may also be associated with sugars, various alcohols, or other organic acids. Common examples are caftaric, coutaric, and fertaric acids – the tartaric acid esters of caffeic, p-coumaric, and ferulic acids, respectively. In the presence of pectin methyl esterase, the esters break down to their monomers. The esters also slowly hydrolyze during fermentation. The most common non-flavonoid in grapes, caftaric acid is one of the primary substrates for polyphenol oxidase. It often plays an important role in oxidative browning of must. In small amounts, the oxidized derivatives of caftaric and coutaric acids may lend much of the straw yellow–gold coloration of white wines. Although equally present in red wines, the abundance of anthocyanins and procyanidins masks the presence of oxidized grape non-flavonoids (Fogarty, 2010; Jackson, 2008; Ribéreau-Gayon et al., 2006b).

### 5.1.2. Stilbenes

Stilbenes are a subclass of phenolic compounds naturally occurring in various families of plants, but grapes and wine are considered the most important dietary sources of these substances. Stilbenes can be biosynthesized by grapevines as a defense response to stress, such as microbial infection and UV irradiation, and they are transferred during the
winemaking process into the must and wine. Due to their antioxidative, anticarcinogenic and antimitotic potency, stilbenes are considered to play a central role in the human diet (Buiarelli et al., 2007; Rentzsch et al., 2009).

5.1.3. **Hydrolyzable tannins**

By definition, tannins are compounds able to form stable bonds with proteins and other vegetal polymers such as polysaccharides, being in a chemical perspective, large phenolic molecules derive from the polymerization of monomeric units containing phenolic groups. A tannin polymer must reach a certain dimension to form a stable complex with proteins, anyway, if the polymer is too large it cannot reach the active site of proteins and, thus, there is no complex formation (Ribéreau-Gayon et al., 2006b).

In wine there are two classes of tannins: hydrolyzable and condensed. The latter belong to the group of flavonoid polyphenols and will be approached later in this work.

Concerning hydrolyzable tannins, they occur only in very small quantities in grapes and often cannot be found in their composition, being obtained from the oak of the wooden barrels used for wine fermentation, conservation or even in the ageing. The use of commercial products composed by hydrolyzable tannins as is an authorized oenological practice in order to facilitate the precipitation of excessive protein compounds and assist in clarification processes. Other uses have been described, such as the improvement of the wine body because of its antioxidant properties, the elimination of aromatic and gustative defects attributed to reduction phenomena, color stabilization and flavor improvement (Lempereur et al., 2002; Vallejo et al., 2000).

Hydrolyzable tannins can be classified into gallotannins and ellagitannins, depending on weather they release gallic acid or ellagic acid by acid hydrolysis, also containing a glucose molecule. Despite ellagic acid in wine being only originated either from wooden containers or from the addiction of enological tannins, on the other hand, gallic acid also originates from skins and seeds (Ribéreau-Gayon et al., 2006b).
5.2. Flavonoids

Flavonoids are phenolic compounds that share the same phenylbenzopyran chemical structure. The general structure includes a C15 (C6-C3-C6) skeleton joined to a chroman ring (benzopyran moiety). The heterocyclic benzopyran ring is known as the C ring, the fused aromatic ring as the A ring, and the phenyl constituent as the B ring, as can be seen in Figure 5.

![Figure 9 Basic structure of flavonoids.](image)

5.2.1. Anthocyanins

Anthocyanins are important pigments responsible for the red, violet and blue colours mainly located in the grape skins, with the exception of the teinturier varieties that also contain anthocyanins in the pulp; large amounts in the leafs at the end of growing period – Autumn can also be found. The anthocyanins identified in grape skins and wines from *Vitis vinifera* are the 3-O-monoglucosides and the 3-O-acylated monoglucosides of five main anthocyanidins – delphinidin, cyanidin, petunidin, peonidin and malvidin – which differ from each other by the number and position of the hydroxyl and methoxyl groups located in the B-ring of the molecule. Acylation can occur at the C-6 position of the glucose molecule by esterification with acetic, p-coumaric and caffeic acids, Their chemical structures can be seen in Figure 7 (Fogarty, 2010; Monagas and Bartolomé, 2009; Ribéreau-Gayon et al., 2006b).
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Notwithstanding Ribéreau-Gayon et al. (2006b) described the existence of monoglucoside anthocyanins and acylated monoglucoside anthocyanins only in *Vitis vinifera* grapes and wines, the use of modern and more sensitive analytical techniques has allowed the confirmation of the occurrence of anthocyanidin-3,5-diglucosides in *V. vinifera* grape skin extracts and wines. Recently, the presence of 3,7-diglucosides has also been proposed (Monagas and Bartolomé, 2009).

The color of the anthocyanidins depends on the kind and number of substituents and on the solvent’s pH. The substituents give different chemical properties to each anthocyanidin: malvidin is more sensible to thermal degradation as the methyl substituents activate the B ring, making the aliphatic chain easier to break down to a chalcone, while cyanidin is more resistant to high temperatures. On the other hand, the methyl substituents of malvidin protect it from oxydation, while cyanidin is more easily oxidized (Monagas et al., 2005).

As said before, these molecules are mainly located in the skin cells with the concentration gradient from the inside to the outside of the grape berry. They are found in solution in cellule vacuoles with other polyphenols, sometimes affecting their color by copigmentation reactions, lending a violet color and an increase in intensity to wine (Ribéreau-Gayon et al., 2006b).

These factors explain the differences that can be observed in different red wine colors. All grape varieties have the same basic structures of anthocyanidins, but there are some
variations in their composition. In fact, between the five main anthocyanins, the malvidin is the dominant molecule in all grape varieties, with percentages which go from less than 50% in Sangiovese variety to 90% in the Grenache variety (Ribéreau-Gayon et al., 2006b).

Often, climatic factors such as sun exposure, may influence the rate of complexation phenomena where anthocyanins are involved, causing an acceleration in the evolution of the wine color (Palliotti et al., 2007b; Palliotti et al., 2007c; Ribéreau-Gayon et al., 2006a).

Anthocyanins change the color depending on the pH: in acid solutions they are red and they lose color as pH rises up to pH 3.5; at pH 4 they are blue and they eventually become yellow in an alkaline environment.

Free anthocyanins undergo many reactions during wine ageing. They form stable red complexes with condensed tannins. If the anthocyanin and the tannin are linked by an ethanal bridge, the resulting complex is particularly stable. The formation of these molecules prevents the complete decoloration of red wine due to the complete loss of free anthocyanin. This is caused by chemical degradation or by other causes such as the decolorating action of SO2 and precipitation (Fogarty, 2010).

5.2.2. **Flavonols**

Flavonols are yellow pigments, also responsible for the bitterness of wines and are present in both white and red berry grapes as well as in a large number of other fruit and flowers and play a protective role against UV radiations. They can be found in grape skins and in leaves, being some of them also detected in pulp, but none in the seeds (Pereira et al., 2006; Rodríguez Montealegre et al., 2006; Terrier et al., 2009).

There are three major flavonols in grapes and wines, distinguished by the substituents of the B-ring: 3-4’-dihydroxy-flavone (kaempferol), 3,3’-4- trihydroxy-flavone (quercitin) and 3,3’,4’,5’-tetrahydroxy-flavone (myricetin) as can also be seen in figure 7. Small quantities of isorhamnetin may be found as well. The proportion of flavonols is cultivar dependent, and myricetin is totally missing in white berry grapes. The concentration of flavonols in red wines reaches a few hundred mg/l, while in white wines it ranges from 1 to 3 mg/l. Glycoside flavonols are rapidly hydrolyzed during the wine making process, so they are
found in wine only as aglycones. The chemical structures of the main flavonols can be seen below in Figure 8 (Mattivi et al., 2006; Monagas et al., 2005)

\[
\begin{array}{c|c|c}
\text{Flavonols} & R'_3 & R'_5 \\
\hline
\text{Kaempferol} & H & H \\
\text{Quercetin} & OH & H \\
\text{Myricetin} & OH & OH \\
\text{Isorhamnetin} & OCH_3 & H \\
\end{array}
\]

**Figure 11** Chemical structures of the main flavonols (Melero, 2009).

They also act as a cofactor in the copigmentation of several fruits and flowers. Full light exposed grapes have higher levels of flavonols. The concentration of flavonols in the grapes also depends on the variety, on the thickness of the skin, on the dimension of the berries and on the skin/berry ratio (Fogarty, 2010; Monagas et al., 2005; Ribéreau-Gayon et al., 2006b; Terrier et al., 2009).

### 5.2.3. Flavan-3-ols, Proanthocyanidins and Condensed Tannins

Grape flavanols, more accurately called flavan-3-ols as they are hydroxylated in the 3rd position, are found as monomers but also as oligomers and polymers. They are located mainly in grape seeds, but small amounts of monomers and dimers have also been detected in the pulp (Bourzeix et al., 1986; Terrier et al., 2009).

Proanthocyanidins and condensed tannins are formed by the polymerisation of flavan-3-ols monomers. The oligomers and polymers of flavan-3-ol form stable bonds with proteins and polysaccharides, including the proteins in the mouth, causing wine astringency. The stability of these complexes depends on the tannin dimension and on the number of free phenolic groups. Monomeric flavan-3-ols are too small to form stable complexes with proteins, thus they cannot be considered condensed tannins by definition (Dixon et al., 2005; Monagas et al., 2003; Ribéreau-Gayon et al., 2006b).
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Monomeric flavan-3-ols represent only a small part of these, as most of them are in polymerized form. The major flavan-3-ol monomers in grapes are (Figure 9) (+)-catechin and its isomer, (−)-epicatechin, and, to a lesser extent, the gallic ester of (−)-epicatechin, (−)-epicatechin 3-gallate. Gallocatechin has also been reported in *Vitis vinifera* and catechin 3-gallate and gallocatechin 3-gallate have been detected in some non-*V. vinifera* varieties (del Álamo et al., 2004; Souquet et al., 1996; Sun et al., 1998a; Terrier et al., 2009; Yilmaz and Toledo, 2003).

<table>
<thead>
<tr>
<th>Flavanol monomers</th>
<th>R</th>
<th>R₁</th>
<th>R₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-catechin</td>
<td>H</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>(−)-epicatechin</td>
<td>H</td>
<td>H</td>
<td>OH</td>
</tr>
<tr>
<td>(−)-epicatechin 3-gallate</td>
<td>H</td>
<td>H</td>
<td>O-G</td>
</tr>
<tr>
<td>(+)-gallocatechin</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>(−)-epigallocatechin</td>
<td>OH</td>
<td>H</td>
<td>OH</td>
</tr>
<tr>
<td>(+)-gallocatechin 3-gallate</td>
<td>OH</td>
<td>OH</td>
<td>O-G</td>
</tr>
<tr>
<td>(−)-epigallocatechin 3-gallate</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
</tr>
</tbody>
</table>

*Figure 12* Chemical structures of the of the flavan-3-ols monomers (Terrier et al., 2009).

Flavan-3-ol oligomers and polymers are also called, as said before, condensed tannins or proanthocyanidins. Oligomeric strictly refer to dimer and trimer polymerizations of flavan-3-ol. The term tannin refers to their capacity to interact or react with proteins and precipitate them out, capacity that was mentioned on the hydrolysable tannins subject. When heated under acidic conditions, these molecules release red anthocyanidin pigments, hence the term proanthocyanidins, more specifically, procyanidins. They produce cyanidin from catechin and epicatechin, while prodelphinidins produce delphinidin from gallocatechin and epigallocatechin (Monagas et al., 2005; Ribéreau-Gayon et al., 2006b)

The proanthocyanidins may be beneficial or detrimental to the quality of the wine according to their chemical properties as they play an important role in many aspects, namely
the occurrence of turbidity in wines and protein interactions, oxidation and browning, color and aging stability of wines (Sun et al., 1998b).

They have a high structural diversity due to the large number of hydroxyl groups, and their position in aromatic nucleus and their concentration in wine ranges from 100 mg/l in white wine to 4,000 mg/l in red wines. It varies depending on the grape cultivar, farming practices and the season (Ribéreau-Gayon et al., 2006a).

The concentration of flavan-3-ols in red wines varies according to the variety and further to the used vinification methods. Specially in red wines, these compounds are dissolved in the wine during the fermentation, thus the fermentation occurs with the solid parts of grapes (skins and seeds). The values are between 1 and 4 g/l (Ribéreau-Gayon et al., 2006b).

The increase of flavan-3-ols in grapes is fast in the beginning of grape development, being, however, followed by a slow accumulation during maturation. This biosynthesis may therefore be less active than the increase in volume of grape berry. (Ribéreau-Gayon et al., 2006a).

At veraison, the concentration of condensed tannins in skins is already high sometimes higher than half the concentration observed in ripening (Ribéreau-Gayon et al., 2006a).

As regards the oligomeric and polymeric proanthocyanidins in grape seeds it was observed that they are essentially made of (+)-catechin, and (−)-epicatechin 3-gallate molecules linked by C4-C8 or C4-C6, being (−)-epicatechin the one with higher proportion. These condensed tannins derived from polymerization of flavan-3-ol units, reach maximum concentration in grape seeds before the veraison, decreasing then to low and relatively stable values when at ripening. (Ferrer-Gallego et al., 2012; Ribéreau-Gayon et al., 2006a; Souquet et al., 1996)

It was demonstrated that the concentration and location of condensed tannins either in skins and grape seeds differ substantially among cultivars with fundamental consequences in the kinetics of extraction, in the possibility of adequately stabilize color and in the possibility of varying the final product. (Mattivi, 2006).
6. **Objectives**

Therefore, objective of this work was the development of a system for controlled drying of the grapes used for the production of Sagrantino sweet wine and the identification of optimal operating parameters in order to recreate, in a controlled environment, climatic conditions which, in the past have distinguished the best vintages, minimizing risk situations.

There were carried out on the Sagrantino grape variety, the tests comparing different conditions of the process, in climatic chambers for drying checked by analyzing the effect of different variables on the most important metabolites of grapes.
Chapter 2 Materials and Methods

1. Materials and experimental procedure

The work reported in this paper was integrated on a project carried out by the Dipartimento di Scienze Economico-Estimative e degli Alimenti from University of Perugia, which consisted in the optimization and standardization of the drying process of grapes in controlled environment for the production of Sagrantino Passito wine and the valuation of enological potentiality of sweet wines produced by Grechetto G5 grapes.

The research was conducted during 2010 and 2011 seasons and only a part of all research carried out will be reported in this dissertation.

Each season (2010 e 2011) the grapes were harvested and submitted to an off-vine overripening followed by vinification and aging. In the first year all these steps were held at the Centro Regionale Servizi per la Vitivinicolture in Orvieto - Italy, and in the second year, only the vinification and stabilization steps were held in this place, having the overripening carried out at the University of Perugia.

The grapes used for this research were grapes of the Sagrantino variety, and were provided by the Terre de la Custodia Company located in Gualdo Cattaneo, Perugia, Italy, being distributed on plastic boxes with circa 8 kg each. There, climactic chambers Sunrise SU1500V Angelantoni Industrie SpA, Italy were used for the artificial overripening, which was controlled by Winkratos software, varying, this way, the conditions of temperature and relative humidity from chamber to chamber.

In the first year of this study four different drying conditions were analyzed as reported by Grieco (2010), the study being only replicated in the second year in chambers which have shown the most interesting results with the following characteristics:

Chamber 1 (C1): The temperature (T) and relative humidity (H) varied according to a cycle that reproduced the environmental conditions of a typical climate of dehydration (reference year 2005) which T and H were extrapolated from data provided by the Parque Tecnológico Agro-alimentar of Umbria relating to the Montefalco climate. T consisted of 12-
hour cycles with 12 °C and 24 °C and H also with 12-hour cycles of 85 and 60%. Every four days, the maximum and minimum temperatures decreased 0.5 degrees.

**Chamber 2 (C2):** The T and H are constant at 15°C and 50% respectively, simulating the controlled dehydration conditions adopted in many wineries that produce wines from dehydrated grapes.

A representative sample of grapes was collected before the start and at the end of the dehydration process and weekly during the dehydration on each chamber until the amount of sugar reaches 33%. Each sample was split into two; one for chemical and physical analysis and the other was frozen for extraction of skins and seeds, after which the polyphenolic composition was analyzed.

When sugar concentration reached about 33%, as said before, the grapes of each chamber were fermented separately but following the same vinification diagram which consisted in fermentation with *Saccharomyces cerevisiae bayanus* (EC Lalvin 1118, Lallemand) and maceration for 10 days.

2. **Phenolic compound extraction from grape skins**

The skins were carefully removed from the frozen grapes, making sure that they were free of pulp. To 10g of skin 100 ml of ethanol 80% vol. were added, being then triturated with a Ultra-Turrax T50 (Janke&Kunkel, Ika-Labortechnik, Germany) for two minutes and centrifuged in a centrifuge, model Universal 32Hettich, for 10 minutes at 9000 rpm.

The supernatant was transferred to a flask and the entire process was repeated from the solid phase four more times and described thoroughly beforehand. The supernatants were gathered in the same flask. At the end, the solvent of the solution was removed through a rotary evaporator (LABO-ROTA SEM-320, Resona Technics, Gossau, Switzerland) at 27°C under N2 atmosphere.

To the obtained extract was led to a volume of 100 ml with distilled water and then frozen for future analysis.
3. Phenolic compound extraction from grape seeds

In a bottle 10g of grape seeds were mixed with 200 ml of acetone 80% vol. and the internal atmosphere was saturated in N2; the bottle was then closed and covered with tin foil to prevent the light contact.

The bottle was placed in agitation on an orbital shaker model Lab-Therm LT-V Labor Schüttler Shaker Rüttler, for 48 hours at room temperature.

The seed-free solution was transferred to a flask and the solvent removed through a rotavapor model rotary evaporator (LABO-ROTA SEM-320, Resona Technics, Gossau, Switzerland) at 27°C under N2 atmosphere.

To the obtained extract was led to a volume of 100 ml with distilled water and then frozen for future analysis.

4. Analytical Methods

Some of the main physico-chemical parameters of grapes were analyzed: reducing sugars, pH and total acidity, following the community methods described in the list and description of methods of analysis referred to in the first paragraph of Article 120g of Council Regulation (EC) No 1234/2007 (published in accordance with Article 15(2) of Commission Regulation (EC) No 606/2009 of 10 July 2009).

The quantification of malic acid and gluconic acid, being the last one only analyzed at the beginning and end of the dehydration process, was carried on with an enzyme test kit from Steroglass Srl brand.

Concerning the phenolic composition of grape skins and seed extracts, they were analyzed according to colorimetric methods and using a spectrophotometer model Cary 100 UV-Vis Varian.

The total phenolic compounds were measured with Folin-Ciocalteu reagent according to the official method of analysis in the list and description of methods of analysis referred to in the first paragraph of Article 120g of Council Regulation (EC) No 1234/2007 (published in
accordance with Article 15(2) of Commission Regulation (EC) No 606/2009 of 10 July 2009) and expressed as mg/l of gallic acid.

The measurement of total anthocyanins was based on the method described by Ribéreau-Gayon and Stonestreet (1965) and calculated based on a standard curve prepared with malvidin. This procedure is based on the capacity of anthocyanins have to change color according to pH and the capacity to react with bisulfite ions originating uncolored products.

The method used for total tannin quantification is from Montedoro and Fantozzi (1974) and is based on tannin precipitation when in contact with methylcellulose.

The total flavonoid content was determined according to the procedure described by Kramling and Singleton (1969). In this method occurs a precipitation of flavonoid compounds in presence of formaldehyde, resting in solution the non-flavonoids which are quantified, using the Folin-Ciocalteu reagent like in total polyphenol analysis.

The method used for catechin quantification is from Pompei and Peri (1971) and is based on the reaction with vanillin in acid medium.

To phenolic acid procedure was used based on a double precipitation as described by Taticchi (2003). The first is obtained by precipitation with formaldehyde according to the methodology already indicated for total flavonoid quantification. Then, in the supernatant, another precipitation takes place with methylcellulose, precipitating hydrolysable tannins and leaving in this last supernatant the phenolic acids.

5. **Qualitative and quantitative analysis of the anthocyanins profile**

Analyses of anthocyanins in grape skin extracts were performed, according to Mazza et al. (1999), using a HPLC model Agilent Technologies series 1100, equipped with quaternary pump, degasser, heater columns and UV-vis photodiode array detector. Separation was achieved with an ODS column (Inertsil ODS-3, 5 μm, 150 mm x 4.6 mm; GL-Sciences, Tokyo, Japan) and the loop was of 20 μl. The qualitative and quantitative estimation of anthocyanidin was performed using standards from Extransynthèse, Genay, France, for the cyanidin, peonidin and malvidin, and from Polyphenols laboratories, Sandnes, Norway, in the
case of delphinidin and petunidin. The concentration of acylated compounds and dimers was calculated using the response factor of malvidin.

6. Extraction, fractionation and analysis of proanthocyanidins

For the analysis of proanthocyanidins the method described by Sun et al. (1998b) was followed, according to which the proanthocyanidins are fractionated on C18 Sep-Pac cartridges into three fractions with different solvents.

The phenolic compounds were measured in the three methanolic fractions with Folin-Ciocalteau reagent. In fraction I the monomeric proanthocyanidins (catechin) were present, in fraction II the oligomeric proanthocyanidins (dimers and trimers), while in fraction III all compounds characterized by a higher degree of polymerization.

Some other analyses were performed in grapes and wines but they will not be reported in this dissertation.
Chapter 3 Results and Discussion

1. Grape analyses

In both chambers, the evolution of grape weight was observed, in general, as expected. A loss of weight was observed during the drying process, being however more accentuated in Chamber 1 comparatively to Chamber 2, as can be seen in Figure 10. Comparing with values obtained in the previous year, reported by Grieco (2010), the differences between Chamber 1 and Chamber 2 observed by the last one were higher.

![Figure 13](image)

**Figure 13** Grape weight lost percentage during the drying process. (C1=Chamber 1, C2= Chamber 2). Results are given as the average value and the bars represent the standard deviation of the sample (n=4).

In fact, the conditions in Chamber 1 promoted a higher period of dehydration; about 10 days more. In chamber 2 the dehydration period was identical to the year in study which means that those conditions in Chamber 2 are somehow more standard.

Apparentely, the conditions observed in Chamber 1 promote faster dehydration in 2011, leading to the belief that higher relative humidity and temperature values and the conditions that are similar to the ones that occur in traditional *appassimento*, promote further dehydration.
Barbanti et al. (2008) observed that an increase in temperature resulted in an increased drying rate, while an increase in relative humidity caused a reduction of the drying rate. The last part concerning relative humidity was not observed in this study, perhaps because the differences between the temperatures of each chamber might be high enough to overlap the effect of relative humidity.

It is important to note that if on one hand 2010 vintage was considered normal, on the other hand, the 2011 vintage in Montefalco was not the best for this study. According to meteorological data, during the ripening of the grapes in this region there was very hot and dry weather.

The reducing sugars had a similar behavior than what would be expected, as can be seen in Table 1. During the drying process the grape weight loss is mainly due to water evaporation and the increase of grape compound concentration due to the decrease of the solvent for the same amount of solute and not by biosynthesis.

Table 1 Evolution of Reducing Sugars % (p/p) on grapes during the drying process in Chamber 1 (C1) and Chamber 2 (C2).

<table>
<thead>
<tr>
<th>Date</th>
<th>Days</th>
<th>C1</th>
<th>C2</th>
</tr>
</thead>
<tbody>
<tr>
<td>13-10-2011</td>
<td>0</td>
<td>23.44</td>
<td>23.44</td>
</tr>
<tr>
<td>20-10-2011</td>
<td>7</td>
<td>28.58</td>
<td>27.67</td>
</tr>
<tr>
<td>27-10-2011</td>
<td>14</td>
<td>30.67</td>
<td>29.38</td>
</tr>
<tr>
<td>03-11-2011</td>
<td>21</td>
<td>33.60</td>
<td>33.87</td>
</tr>
<tr>
<td>08-11-2011</td>
<td>26</td>
<td>31.58</td>
<td>30.00</td>
</tr>
</tbody>
</table>

It was observed that the increase in reducing sugars was higher in Chamber 1, with variable temperature and relative humidity, and at least 12 hours with temperatures higher than the verified in Chamber 2, with constant temperature and relative humidity. In both cases, increase in reducing sugars was proportional to the weight loss velocity.
After 21 days the concentration of reducing sugars in Chamber 1 was lower than that observed in Chamber 2, decreasing in both after 26 days with lower values in the second chamber. This decrease may eventually be explained by the malic respiration process.

Comparing the data reported by Grieco (2010) with the data reported in this work it can be seen that in 2010 the Chamber 2 increased the sugar concentration 7 days faster than in 2011 and without sugar degradation.

Also, it is important to note that the initial sugar concentration in 2010 was much higher than in 2011 leading to the belief that the ripening process stopped and premature dehydration of the grapes occurred.

The total acidity, pH and malic values are directly dependent on this respiratory mechanism, since the degradation of malic acid is implicated, decreasing the total acidity and in turn the increase of pH; the pH value is inversely proportional to the total acidity, in other words, this value increases with decreasing value of total acidity.

In Table 2, both in Chamber 1 and in Chamber 2 an initial tendency of concentration increase could be observed, such as reducing sugars, perhaps due to weight loss, being the subsequent decrease likely due to malic respiration. These results were also verified by other authors (Bellincontro et al., 2004; Rolle et al., 2009).

<table>
<thead>
<tr>
<th>Date</th>
<th>Days</th>
<th>Total Acidity</th>
<th>pH</th>
<th>Malic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C1</td>
<td>C2</td>
<td>C1</td>
</tr>
<tr>
<td>13-10-2011</td>
<td>0</td>
<td>7,12</td>
<td>7,12</td>
<td>3,45</td>
</tr>
<tr>
<td>20-10-2011</td>
<td>7</td>
<td>8,40</td>
<td>7,91</td>
<td>3,62</td>
</tr>
<tr>
<td>27-10-2011</td>
<td>14</td>
<td>7,35</td>
<td>7,04</td>
<td>3,59</td>
</tr>
<tr>
<td>03-11-2011</td>
<td>21</td>
<td>6,41</td>
<td>8,51</td>
<td>3,58</td>
</tr>
<tr>
<td>08-11-2011</td>
<td>26</td>
<td>6,50</td>
<td>7,09</td>
<td>3,63</td>
</tr>
</tbody>
</table>
Chapter 3 Results and Discussion

If we observe the initial and final values of total acidity, malic acid and pH in chamber 1 we can see that, as expected, the total acidity and malic acid decreased and pH increased. The same happened to the Chamber 2 but in smaller proportions.

However, fluctuations in the intermediate values can be observed; they may be due to respiration metabolism, weight loss effect, or even to measurement and sampling errors. Rolle et al. (2009) has also found in his work that some parameters showed high variability, explaining that different grape berries often have different levels of dehydration.

The evolution of these compounds was observed by Grieco, (2010) that, in general, obtained similar results. Note that the initial values reported in this work and by Grieco (2010) were different, thus being the final values different as well. However it was observed in 2010 higher degradation on malic acid, in other words, higher respiration metabolism.

Despite the grapes used in both researches were form the same variety an vineyard, they were harvested in different years, being the environmental conditions and the concentrations of all compounds different. Palliotti et al. (2007c) observed that the concentration of sugars, total acidity and pH are influenced by year, calling it year factor.

In an overview, and analyzing both the data of weight loss and the values of reducing sugars, total acidity, malic acid and pH in an integrated way, it may be assumed that the conditions of Chamber 1 promote a faster accumulation of sugars with a higher occurrence of respiration reactions and at the end, grapes are much more dehydrated. From a technical standpoint, these differences in weight between the grapes from both chambers at the end of the dehydration, may not be advantageous, since, on a larger scale, they reflect large losses of must and, consequently, of wine.

Also the gluconic acid was analyzed, since it is indicative of microbiological activity. As mentioned earlier, a major problem of dehydration is to ensure the absence of microbial activity mainly B. cinerea. These microorganisms find the ideal conditions for their development during dehydration, degrading grape sugars and conferring often defects to musts and wines.

The obtained results, Table 3, indicate that the grapes were already contaminated before the drying process. Also it can be seen that there was possibly higher microbial activity
in Chamber 1 when compared to Chamber 2, which means that the conditions of Chamber 1 are potentially more conducive to fungal contamination.

Table 3 Evolution of Gluconic Acid during the drying process in Chamber 1 (C1) and Chamber 2 (C2).

<table>
<thead>
<tr>
<th>Date</th>
<th>Days</th>
<th>C1</th>
<th>C2</th>
</tr>
</thead>
<tbody>
<tr>
<td>13-10-2011</td>
<td>0</td>
<td>0.025</td>
<td>0.025</td>
</tr>
<tr>
<td>08-11-2011</td>
<td>26</td>
<td>0.54</td>
<td>0.14</td>
</tr>
</tbody>
</table>

2. **Grape skin phenolic compounds**

In general, Phenolic compounds from skins, which are characterized by the values of total polyphenols, have decreased during the dehydration process, as can be seen in Table 4, showing evidence of degradation reactions during the drying process. Comparing the values of total polyphenols between chambers a most significant decrease in Chamber 2 has occurred. Also worth highlighting was the increase in the Chamber 1 on day 7, possibly due to the weight loss of grapes that overlapped the downward trend. Rolle et al. (2009) observed too that the drying process induces the decrease of polyphenol content of berry skins.

The concentration of phenolic acids had an opposite tendency comparing to that observed for total polyphenols, increasing during dehydration, and resulting in the end of dehydration, in higher values in Chamber 1. However this increase was not observed in the same proportions as the reducing sugars, suggesting their involvement in some type of reaction, leading to a reduction in their concentrations, which was observed by Serratosa et al. (2008a) as well.

As can be seen in Table 4, flavonoids, catechins, tannins and anthocyanins followed the same trend of total polyphenols, by decreasing. Comparing the two chambers under study it was noted that the values of Chamber 2 at the end of grape dehydration were higher in most analyzed compounds other than anthocyanins, which were higher in Chamber 1. They may have been dissolved in the pulp in larger quantities, or undergone degradation reactions on higher intensity, obtaining thereby those values.
Table 4 Evolution of Total polyphenols, Phenolic Acids, Flavonoids, Catechins, Tannins and Anthocyanins in the grape skins from Chamber 1 (C1) and Chamber 2 (C2) according to drying period (days). Results are given as the average value ± standard deviation (n=2) and in mg/g of dried skin.

<table>
<thead>
<tr>
<th>Days</th>
<th>Total polyphenols</th>
<th>Phenolic Acids</th>
<th>Flavonoids</th>
<th>Catechins</th>
<th>Tannins</th>
<th>Anthocyanins</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7,85 ± 0.47</td>
<td>0.19 ± 0.01</td>
<td>7.64 ± 0.00</td>
<td>7.24 ± 1.40</td>
<td>3.94 ± 0.02</td>
<td>3.80 ± 0.04</td>
</tr>
<tr>
<td>7</td>
<td>9.28 ± 7.38</td>
<td>0.21 ± 0.09</td>
<td>8.81 ± 0.06</td>
<td>4.29 ± 0.39</td>
<td>7.75 ± 0.18</td>
<td>2.56 ± 0.06</td>
</tr>
<tr>
<td>C1</td>
<td>4.90 ± 0.07</td>
<td>0.23 ± 0.00</td>
<td>4.54 ± 0.12</td>
<td>5.01 ± 0.13</td>
<td>3.12 ± 0.02</td>
<td>2.90 ± 0.04</td>
</tr>
<tr>
<td>21</td>
<td>4.01 ± 0.30</td>
<td>0.25 ± 0.04</td>
<td>3.67 ± 0.02</td>
<td>4.81 ± 0.30</td>
<td>2.71 ± 0.20</td>
<td>2.61 ± 0.03</td>
</tr>
<tr>
<td>26</td>
<td>3.49 ± 0.01</td>
<td>0.17 ± 0.01</td>
<td>3.28 ± 0.02</td>
<td>2.12 ± 0.85</td>
<td>2.74 ± 0.01</td>
<td>2.28 ± 0.07</td>
</tr>
<tr>
<td></td>
<td>7.85 ± 0.47</td>
<td>0.19 ± 0.01</td>
<td>7.64 ± 0.00</td>
<td>7.24 ± 1.40</td>
<td>3.94 ± 0.02</td>
<td>3.80 ± 0.04</td>
</tr>
<tr>
<td>7</td>
<td>7.78 ± 0.22</td>
<td>0.13 ± 0.16</td>
<td>7.67 ± 0.00</td>
<td>8.15 ± 0.01</td>
<td>5.64 ± 0.05</td>
<td>4.11 ± 0.90</td>
</tr>
<tr>
<td>C2</td>
<td>6.17 ± 0.04</td>
<td>0.12 ± 0.08</td>
<td>6.13 ± 0.00</td>
<td>3.57 ± 0.70</td>
<td>4.74 ± 0.14</td>
<td>3.21 ± 0.15</td>
</tr>
<tr>
<td>21</td>
<td>4.47 ± 0.34</td>
<td>0.48 ± 0.26</td>
<td>4.12 ± 0.02</td>
<td>2.87 ± 0.07</td>
<td>3.21 ± 0.08</td>
<td>2.66 ± 0.06</td>
</tr>
<tr>
<td>26</td>
<td>4.99 ± 0.28</td>
<td>0.09 ± 0.09</td>
<td>4.82 ± 0.07</td>
<td>3.49 ± 0.42</td>
<td>0.12 ± 0.12</td>
<td>1.67 ± 0.01</td>
</tr>
</tbody>
</table>

Some phenolic compounds, as previously mentioned, participate in different kinds of reactions, including nonenzymatic browning, autoxidation and enzymatic oxidation reactions involving polyphenol oxidases or peroxidases reducing all their concentrations (Macheix et al., 1991; Serratosa et al., 2008a).

Besides the different reactions in which they may have been involved, anthocyanins and other phenolic compounds might have been dissolved in the pulp. Marquez et al. (2012) suggest in their work that chamber-drying must cause the epidermal layers in the grapes to break up, which releases the anthocyanins into the pulp.

According to the qualitative profile of anthocyanidins shown in Table 5, it was not possible to draw any conclusion concerning their behavior during the dehydration process. The observed fluctuations are likely to be due to sampling error or to the fact that different grape berries may have different degrees of dehydration.

However there was a reduction of various compounds during dehydration, putting on the hypothesis of their involvement in condensation reactions or their transfer to the pulp.
### Table 5 Evolution of Anthocyanin profile of grape skins from Chamber 1 (C1) and Chamber 2 (C2) according to drying period (days). Results are given as the average value ± standard deviation (n=2).

<table>
<thead>
<tr>
<th>Chamber</th>
<th>Date</th>
<th>Days</th>
<th>Delphinidin</th>
<th>Cyanidin</th>
<th>Petunidin</th>
<th>Peonidin</th>
<th>Malvidin</th>
<th>Anthocyanins acylated + dimeric</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>13-10-2011</td>
<td>0</td>
<td>0.8 ± 0.003</td>
<td>0.1 ± 0</td>
<td>0.8 ± 0.001</td>
<td>0.1 ± 0</td>
<td>1.7 ± 0</td>
<td>1 ± 0.009</td>
</tr>
<tr>
<td></td>
<td>20-10-2011</td>
<td>7</td>
<td>0.4 ± 0.0005</td>
<td>0.02 ± 0.00001</td>
<td>0.5 ± 0.0001</td>
<td>0.1 ± 0.00003</td>
<td>1 ± 0.00001</td>
<td>0.5 ± 0.0008</td>
</tr>
<tr>
<td></td>
<td>27-10-2011</td>
<td>14</td>
<td>1 ± 0.0007</td>
<td>0.1 ± 0.0003</td>
<td>1 ± 0.0005</td>
<td>0.2 ± 0.0004</td>
<td>2 ± 0.002</td>
<td>0.6 ± 0.006</td>
</tr>
<tr>
<td></td>
<td>03-11-2011</td>
<td>21</td>
<td>0.36 ± 0.0014</td>
<td>0.02 ± 0.0002</td>
<td>0.39 ± 0.0009</td>
<td>0.06 ± 0.0001</td>
<td>0.82 ± 0.0021</td>
<td>0.29 ± 0.0012</td>
</tr>
<tr>
<td></td>
<td>08-11-2011</td>
<td>26</td>
<td>0.5 ± 0.0007</td>
<td>0.03 ± 0</td>
<td>0.5 ± 0.0017</td>
<td>0.1 ± 0.0001</td>
<td>1 ± 0.0014</td>
<td>0.2 ± 0.0015</td>
</tr>
<tr>
<td>C2</td>
<td>13-10-2011</td>
<td>0</td>
<td>0.8 ± 0.003</td>
<td>0.1 ± 0</td>
<td>0.8 ± 0.001</td>
<td>0.1 ± 0</td>
<td>1.7 ± 0</td>
<td>1 ± 0.009</td>
</tr>
<tr>
<td></td>
<td>20-10-2011</td>
<td>7</td>
<td>0.7 ± 0.002</td>
<td>0 ± 0.0002</td>
<td>0.6 ± 0.001</td>
<td>0.1 ± 0</td>
<td>1.2 ± 0.001</td>
<td>0.6 ± 0.003</td>
</tr>
<tr>
<td></td>
<td>27-10-2011</td>
<td>14</td>
<td>0.9 ± 0.002</td>
<td>0.1 ± 0.0003</td>
<td>0.9 ± 0.001</td>
<td>0.3 ± 0.0004</td>
<td>2.3 ± 0.003</td>
<td>0.6 ± 0.005</td>
</tr>
<tr>
<td></td>
<td>03-11-2011</td>
<td>21</td>
<td>0.3 ± 0.001</td>
<td>0.03 ± 0.0001</td>
<td>0.32 ± 0.001</td>
<td>0.06 ± 0.0002</td>
<td>0.65 ± 0.001</td>
<td>0.29 ± 0.002</td>
</tr>
<tr>
<td></td>
<td>08-11-2011</td>
<td>26</td>
<td>0.4 ± 0.0001</td>
<td>0.02 ± 0.0003</td>
<td>0.5 ± 0.0012</td>
<td>0.1 ± 0.0001</td>
<td>1.3 ± 0.0005</td>
<td>0.5 ± 0.0008</td>
</tr>
</tbody>
</table>
As far as the proanthocyanidin is concerned, these have been reduced during dehydration, as can be seen in Table 6. However, after analyzing each fraction, some differences between the chambers were observed.

<table>
<thead>
<tr>
<th>Campione</th>
<th>Fraction 1 (mg/L)</th>
<th>Fraction 2 (mg/L)</th>
<th>Fraction 3 (mg/L)</th>
<th>Total (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(13/10/11)</td>
<td>12.92</td>
<td>25.91</td>
<td>300.25</td>
<td>339.08</td>
</tr>
<tr>
<td>C1 (08/11/11)</td>
<td>7.60</td>
<td>28.33</td>
<td>211.93</td>
<td>247.87</td>
</tr>
<tr>
<td>C2 (08/11/11)</td>
<td>11.94</td>
<td>35.48</td>
<td>246.15</td>
<td>293.57</td>
</tr>
</tbody>
</table>

In Chamber 1 there was a decrease of fraction I, corresponding to monomer proanthocyanidin, an increase in fraction II which comprises oligomeric proanthocyanidins, and a decrease in fraction III equivalent to proanthocyanidins characterized by a higher degree of polymerization.

In Chamber 2, fraction I declined but in smaller proportions, the fraction II increased in proportions higher than those observed in the Chamber 1 and fraction III decreased more than in Chamber 1.

Apart from the hypothesis that these compounds are dissolved in the pulp, monomeric proanthocyanidins may have their concentration reduced by polymerization or degradation reactions.

The increase of fraction II, in addition to the effect of weight loss during dehydration, may be due to polymerization of fraction I and/or degradation of fraction III. Serratosa et al. (2008b) observed in their work that these compounds must have unavoidably suffered degradation reactions leading, in some cases, to a complete degradation of some flavan-3-ols.

Proanthocyanidins from fraction III may have suffered degradation reactions, originating the oligomeric proantocyanidins, fact that would be justified by the increase of fraction II. According to Dallas et al. (2003) and Serratosa et al. (2008a) some flavan-3-ol high molecular weight derivatives can be hydrolyzed to phenolic compounds of lower molecular weights, increasing the contents in the latter.
3. Grape seed phenolic compounds

Looking at the results of phenolic compound analyses from grape seeds presented in Table 7, a decrease in total polyphenols both in Chamber 1 and Chamber 2 could be observed, suggesting degradation reactions in them. It is important to bear in mind that the influence of the drying process is not as great in seeds as in the grape skins because the former are in the center of the grape berry, not being expected a concentration increase due to weight loss of grapes.

Table 7 Evolution of Total polyphenols, Phenolic Acids, Flavonoids, Catechins and Tannins in the grape seeds from Chamber 1 (C1) and Chamber 3 (C3) according to drying period (days). Results are given as the average value ± standard deviation (n=2).

<table>
<thead>
<tr>
<th>Days</th>
<th>Total phenylools</th>
<th>Phenolic Acids</th>
<th>Flavonoids</th>
<th>Catechins</th>
<th>Tannins</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>39.70 ± 0.04</td>
<td>0.16 ± 0.02</td>
<td>39.64 ± 0.01</td>
<td>110.13 ± 2.52</td>
<td>12.91 ± 0.19</td>
</tr>
<tr>
<td>7</td>
<td>36.21 ± 1.14</td>
<td>0.12 ± 0.00</td>
<td>36.12 ± 0.05</td>
<td>72.37 ± 2.16</td>
<td>8.76 ± 0.53</td>
</tr>
<tr>
<td>14</td>
<td>27.30 ± 0.79</td>
<td>0.26 ± 0.05</td>
<td>26.92 ± 0.13</td>
<td>58.24 ± 0.83</td>
<td>7.57 ± 0.55</td>
</tr>
<tr>
<td>21</td>
<td>28.42 ± 0.70</td>
<td>0.27 ± 0.05</td>
<td>28.02 ± 0.14</td>
<td>61.09 ± 0.87</td>
<td>8.24 ± 0.07</td>
</tr>
<tr>
<td>26</td>
<td>33.42 ± 0.23</td>
<td>0.05 ± 0.01</td>
<td>33.21 ± 0.02</td>
<td>67.37 ± 2.20</td>
<td>13.72 ± 0.75</td>
</tr>
<tr>
<td>C2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>39.70 ± 0.04</td>
<td>0.16 ± 0.02</td>
<td>39.64 ± 0.01</td>
<td>110.13 ± 2.52</td>
<td>12.91 ± 0.19</td>
</tr>
<tr>
<td>7</td>
<td>31.51 ± 0.58</td>
<td>0.16 ± 0.14</td>
<td>31.44 ± 0.04</td>
<td>72.23 ± 4.66</td>
<td>11.97 ± 0.53</td>
</tr>
<tr>
<td>14</td>
<td>30.67 ± 0.29</td>
<td>0.73 ± 0.23</td>
<td>30.35 ± 0.16</td>
<td>71.49 ± 0.39</td>
<td>8.24 ± 1.00</td>
</tr>
<tr>
<td>21</td>
<td>24.04 ± 0.71</td>
<td>0.08 ± 0.02</td>
<td>23.84 ± 0.04</td>
<td>72.78 ± 2.79</td>
<td>7.23 ± 0.64</td>
</tr>
<tr>
<td>26</td>
<td>32.70 ± 0.86</td>
<td>0.23 ± 0.05</td>
<td>32.64 ± 0.03</td>
<td>31.64 ± 0.01</td>
<td>16.21 ± 0.81</td>
</tr>
</tbody>
</table>

Phenolic acids showed very high values compared with those observed by Grieco (2010). These decreased in Chamber 1 and increased in Chamber 2.

The results regarding flavonoids show a reduction in them, setting in case, as in the total polyphenols, the occurrence of degradation reactions. On the other hand Frangipane et al. (2012) found that flavonoids maintained a final value similar to the one obtained before dehydration. This author justifies that this evolution is probably due to the grape drying technology used, and the artificial dehydration, in fact, it ensures a protection of these compounds.
Chapter 3 Results and Discussion

Catechins decreased and tannins increased, so then we may suppose that the first, catechins, were polymerized originating oligomeric and polymeric proanthocyanidins, which have tannin properties. This behavior was also observed by Rolle et al. (2009)

The oscillations observed in these results may be related to the fact that different grape berries may exhibit different degrees of dehydration or related to sampling errors.

In general, the phenolic composition in 2010, data reported by in Grieco (2010), was much higher than in 2011, evidencing as well, a stop in the ripening process.
Chapter 4 Conclusions

Based on the obtained results, the following main conclusions can be drawn:

- High temperatures promote an increase of dehydration speed with faster accumulation of reducing sugars.
- The conditions of the Chamber 1 originate drier grapes, which may reduce the production yield.
- Notoriously the degradation of malic acid and sugars by respiration was observed, with a decrease of total acidity and an increase of pH, being hypothetically the conditions of Chamber 1 the most conducive to the occurrence of these reactions.
- The higher values of temperature and relative humidity can lead to greater contamination by *B.cinerea*. It seems that their inclusion is quite important since in large proportions, they can destroy the integrity of the films.
- The phenolic compounds of the skins suffered, in general, a decrease, leading to assume their involvement in various types of reactions, with more evidence in Chamber 1. The hypothesis that they might be dissolved in the pulp was considered.
- Fraction III of proanthocyanidins might have been degraded during the drying process and fraction II, synthesized. Chamber 2 results in greater amounts of tannins.
- The phenolic compounds from grape seeds have experienced degradation maybe due to degradation reactions. Yet there are clues of polymerization of catechins in tannins in both chambers.

The environmental conditions in 2011 were not the best for this study.

This work should be supplemented with a more detailed study in particular, with a greater number of samples in order to confirm these results statistically.

The effect of temperature and relative humidity variations was not very light, therefore it would be interesting to study them separately.
Chapter 4 Conclusions

As well, it would be interesting to complement this study with the analysis of phenolic compounds from the pulp of grapes, since there is the possibility that many compounds were dissolved in it during the dehydration process.
Chapter 5 Bibliographic References


Chapter 5 Bibliographic References


Chapter 5 Bibliographic References


Mattoni N. (20??) Il Caso Sagrantino in Umbria, Facoltà di Agraria, Dipartimento di Arboricoltura e Protezione delle Piante, Università degli Studi di Perugia, Perugia.


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