Kvaliteta stolnog grožđa cultivar Italia uzgojenog ispod plastičnog pokrova u cilju odgode berbe

Marasović, Iva

Sveučilište u Zagrebu
Prehrambeno- biotehnološki fakultet
Preddiplomski studij Prehrambena tehnologija

Iva Marasović
6532/PT

Quality characteristics of table grape cv. Italia, as affected by plastic covering to delay harvest

Mentor: prof. Laura de Palma, prof. dr. sc. Branka Levaj

Academic year: 2014-2015

NAPOMENA

Ovaj rad izrađen je na fakultetu Agronomije, prehrambene tehnologije i okoliša, sveučilšta Foggia, Italija. Praktični i teorijski dio ovoga rada odrađen je u sklopu Erasmus programa.

DOKUMENTACIJSKA KARTICA

Završni rad

Sveučilište u Zagrebu

Prehrambeno-biotehnološki fakultet

Preddiplomski studij Prehrambena tehnologija

Zavod za prehrambeno- tehnološko inženjerstvo

Laboratorij za procese konzerviranja i prerade voća i povrća

KVALITETA STOLNOG GROŽĐA CULTIVAR *Italia* UZGOJENOG ISPOD PLASTIČNOG POKROVA U CILJU ODGODE BERBE

Iva Marasović 6532 /PT

Sažetak: Provedeno je ispitivanje stolnog grožđa cv. *Italia* proizvedenog ispod dva tipa plastičnog pokrova koji se koriste za odgađanje berbe. Jedan tip plastičnog pokrova bio je eksperimantalni, a drugi komercijalni koji već ima široku uporabu, a razlikuju se po transmisiji i radiomatrijskom spektru. Glavni cilj rada bilo je usporediti završnih promjene glavnih parametara koji opisuju vanjske i unutarnje karakteristike grožđa. Provedeni testovi su: fizička i mehanička svojstva grozda i bobice, glavni sadržaj soka bobice, ukupan sadržaj fenolnih komponenti u pokožici i pulpi, antioksidativna aktivnost pokožice. Rezultati su pokazali da se fizička i mehanička svojstva grožđa jako malo i zanemarivo razlikuju, dok ukupan sadržaj fenola, ponajviše polifenola i flavonoida je veći kod grožđa uzgojenog ispod eksperimentalnog pokrova zbog njegove transmisije većeg spektra ultraljubičastog zračenja što dovodi do povećanja fenolnog sadržaja.

Ključne riječi: stolno grožđe, plastični pokrov, kvaliteta grožđa, ukupni polifenoli

Rad sadrži: 29 stranica, 4 slike, 7 tablica, 31 literaturni navod

Jezik izvornika: engleski

Rad je u tiskanom i elektroničkom (pdf format) obliku pohranjen u: Knjižnica Prehrambeno-biotehnološkog fakulteta, Kačićeva 23, Zagreb

Mentor: *prof. dr. sc. Branka Levaj*

Rad predan: rujan, 2015.

BASIC DOCUMENTATION CARD

Final work

University of Foggia
Faculty of Agricultre, Food and Environment
Undergraduate studies Food Technology
Department of Agriculture
Laboratory of Arboriculture, Section Biology and Physology

QUALITY CHARACTERISTICS OF TABLEGRAPE cv. *Italia* CULTIVATED UNDER PLASTIC COVER IN ORDER TO DELAY HARVEST

Iva Marasović 6532/PT

Abstract: In this thesis is included examination conducted on table grapes cv. Italia produced under two types of plastic cover used with the purpose to delay harvest day. One plastic cover was experimental, the other was a commercial type presently widely diffused; they differed in transmissivity to the radiometric spectrum. The main aim of work was to compare final changes regarding the main parameters that describe external and internal features of grape quality. Tests that conducted in this work were run to obtain indication about: physical and mechanical traits of cluster and berry; basic berry juice composition; total content of main skin and pulp phenol compounds; skin antioxidant activity. Results indicated that grape physical and mechanical traits and basic berry juice composition changed a very small extent. The parameters most influenced were the berry phenol contents, especially polyphenols and flavonoids: they were higher in grapes produced under the experimental film, likely due to its higher transmissivity to the ultraviolet wavelength range that is known to stimulate phenol biosynthesis.

Keywords: table grape, plastic cover, quality charecteristics, total phenol content
Thesis contains: 29 pages, 4 figures, 7 tables, 31 references
Original in: English
Final work in printed and electronic (pdf format**) version is deposited in:** Library of the Faculty of Food Technology and Biotechnology, Kačićeva 23, Zagreb
Mentor: *PhD Professor Laura de Palma*
Thesis delivered: September, 2015.

TABLE OF CONTENTS

1. INTRODUCTION

It is known that, in last decades, there is an increasing demand for fresh products of fruit and vegetables during the whole year. Table grape is one of most popular fruit, famous because of its taste and benefits on human health, and now-days it can be found in the shelf of supermarket all the time of year. One of the ways used in agriculture to provide extra-seasonal products is to select new varieties or to cultivate plant under plastic cover which protect it from meteorological adversities but also improve quality traits of product.

The usage of plastic covering is growing, whether to advance or to delay ripening period, so new materials are testing in search for more convenient results.
Thus, several grape traits were examined to get any evidence of influence of this cover respect to commonly used. All the phenol compounds are known to exert many positive effects on human health. White grape normally contains less total phenols then black grape, but, any case, they are enough to suggest that white grape may be consumed as a source of anti-cancer and antioxidant substances (Cantos *et al*., 2002).

The present experimental work was conducted to verify if a new plastic sheet cover (used for delay of harvest day) could improve cv Italia grape features. The experiment was run, in Italy, at a big private farm sited into Foggia area (Apulia region). Traits that were examined are: cluster appearance, berry physical and mechanical characteristics, basic composition of berry juice, skin and pulp phenol content, as well antioxidant activity on skin.

2.THEORETICAL PART

2.1. IMPORTANCE AND DIFFUSION OF TABLE GRAPE

The grapevine (*Vitis vinifera* L.) is one of the oldest cultivated species in the world, and is regarded as 'queen of fruit' in many countries. It is grown for direct consume (table grape cultivars) or for wine transformation (wine grape cultivars). Table grapes are intended for fresh consumption or for drying into raisins. Moreover, grapes may be also utilized for juice production. Generally speaking, grape has high water content and thus is good for hydration. It provides a large amount of essential nutrients while containing relatively few calories. Among other compounds, in the grape there are mainly: sugars (fructose and glucose), acids (malic and tartaric acids), minerals (potassium, calcium, magnesium), vitamins (B and C)

Table 1. Nutrient value and weight of European type of white table grape (USDA, http://ndb.nal.usda.gov/ndb/foods)

NUTRIENT	AMOUNT (value per 100g)	NUTRIENT	AMOUNT (value per 100g)
Water	80.54 g	Potassium, K	191 mg
Energy	69 kcal	Calcium, Ca	10 mg
Protein	0.72 g	Magnesium, Mg	7 mg
Total lipid (fat)	0.16g	Phosphorus, P	20 mg
Carbohydrate	18.10 g	Vitamin C	3.2 mg
Fiber, total dietary	0.9 g	Niacin	0.188 mg
Sugars, total	15.48 g	Thiamin	0.69 mg

Grapes contain significant amounts of organic acids. The major organic acids in the must are tartaric, malic, and citric. Of these three acids, tartaric and malic acids account for over 90% of the total acid constituents of the juice. During ripening, the tartrate and malate content of the fruit decrease. This is accompanied by a steady increase in pH. Due to variation in buffer capacity, there is no direct relationship between titritable acidity and pH. In general, however, higher acid levels in fruit are often associated with lower pH values and vice-versa. Thus the acids of the fruit have a significant bearing on pH. They also play a significant role in taste, color, and microbial stability of the juice (Amerine and Joslyn, 1950). The biggest influence

on overall flavor and taste of table grape the biggest have total amount of sugar and organic acids.(Munoz- Robredo *et al*, 2011). Comparing these results with results conducted on table grape cv *Thompson* which is described as is the most widely planted white table grape because of its pleasant taste (UC Integrated Viticulture,2015). It can be seen that values are different but relations between two aspects influencing the flavor are similar It is one of the reasons why cv *Italia* is so commonly used.

Table 2. Comparison between two cultivars and its traits affecting the flavor

	Thopmson	***Italia***	
		CF	**EF**
Total acidity (g/L)	3,85	4,27	4,61
Total sugars (Brix°)	18,83	19,3	18,67

Furthermore, it contains polyphenols (300 mg/kg), compounds that are well-known for their roles on human's health such as the protection of heart and blood vessels and the antioxidant activity; among antioxidants represented in grape, there are also anthocyanidins (400 mg/kg) (Hemingway, Laks, 2012). Some of benefits provided by grape consumption there are: reduced risk of heart attacks and of several types of cancer (lung, mouth, prostate), reduced blood pressure, stabilization of digestion, help to reduce weight, help to maintain memory and concentration (Divković, 2010).

Because of its good taste and influence on human's health, table grapes production is widely spread over the world. The biggest producer is Asia with 59.2% of total world's production, followed ahead Europe with 17.3%, North and South America with 12.8% and finally Africa with 10%. The same order is also in world's consumption of grapes. The leading country in grape production is China with an increase of 271% in the last decade. In Europe, the main producing country is Italy (1.268 Mt), that is also the second biggest exporter in the world, after Chile. The highest year consumption of fresh grape per habitant (hgb) is in the Balcan countries: Albania (54.7% kg/hbt), Bosnia and Herzegovina (46.5% kg/hbt) and FRJ of Macedonia (44,2% kg/hbt) (OIV, 2010-2011; de Palma and Novello, 2014).

Of total world's grape production, share of table grape cultivars is only 21.5% comparing with wine cultivars (Bušić *et al*., 2002). However, in last five years, the production of table grape has showed a growing trend (41 milions of quintals between 2007 and 2011) while production of wine cultivars is decreasing (OIV, 2010-2011).

2.2. TABLE GRAPE cv ITALIA

According to the description reported in the on-line Italian National Catalog of Grapevine, *Italia* cultivar has a big bunch about 20 cm and 500 g heavy, properly loose, having a large seeded berry with a pleasant skin color (yellow-alabaster or golden), fleshy and juicy pulp and a delicate muscat flavor (Fig. 1). The bud-break occurs in the first part of April, the flowering in second part of June and the ripening from middle to late September (http://catalogoviti.politicheagricole.it/scheda.php?codice=514). This variety was obtained, in Italy, by professor Alberto Pirovano in 1911. It is considered easy to grow. Its first advantage is the excellent productivity; moreover it is suitable for protected cultivation under plastic film to delay harvest. Furthermore, the grape keeps its properties during conservation and transport, even on long distance. On the other hand, bad a drawback of this cultivar is that, in order to obtain big bunches and berries, it often needs bunch thinning and, especially berry thinning to eliminate shot berries deriving from to non fertilized ovaries. Because of the great quality of its grape, Italia is the table grape most diffused in Italy, and, moreover, it is very well appreciated around the world (Angelini, 2010; Coombe and Dry, 1992).

Presently, Italia occupies about 40% of the Italian surface cultivated to table grape grapevine (Novello and de Palma, 2014). According to trials carried out in the Apulia region, Italia starts to be harvested from mid August and reaches notable carpolgical traits (Fig. 2), such as berry weight from about 9 up to 10-12 g and bunch weight from about 440 up to 1060 g (Novello *et al*., 1999a; Novello *et al*., 1999b).

Fig. 1 – Bunches of cv Italia from the on-line Italian National Catalog of Grapevine Varieties.

Fig. 2 – Bunch of cv Italia from a commercial vineyard of the Apulia region (Southern Italy, harvest 2014).

2.3. INTRODUCTION OF AGRICULTURE PLASTIC COVERING IN EUROPE AND ITALY

Fruit crop protected cultivation started in 16th century to protect from unfavorable environmental conditions the exotic plants introduced in Europe. In 17th century the first glasshouses were used to grow citrus, pineapple and grapevine in the unfavorable climate of Central Europe. The beginnings were hard because of lack of knowledge about some plant biological needs, such as that for an adequate chilling exposure. Until the 20th century, the use of this technique was too expensive comparing with cultivation on open air. In 1948, it started the usage of plastic materials in agriculture, first in USA with covering small greenhouses with cellophane, then in Japan with covering them with polyvynilchloride (PVC). In last few decades of past century, after large amount of trials on plastic covering were carried out and the usage of this technique was improved in the modern agriculture: from 1950 to 2004 the total world's production of plastic has grown from 1.3 million tons to 225 million of tons. Presently, total global consumption in agriculture per year is 6.5 millions. History of plastic covering in Italy started in the '50s with experimental work on table grapevine and peach in order to stimulate early ripening. After Italian scientists accomplished success in visage of cluster and taste of berry, first vineyards appeared in Central and Southern Europe for in commercial purposes. Furthermore, field of examination was extended to covering to delay harvest. Today, both of these technique are used in Italian agriculture, especially, in cultivation of table grape (Scarascia Mignozza, 1999; Novello and de Palma, 2008; Scarascia-Mugnozza *et al*., 2011).

2.4. COVERING TABLE GRAPE VINEYARD

The techniques used in Italy to advance table grape ripening or to delay its harvest have been specified by Novello and de Palma (2008). The two types of techniques differ as concerns the period of covering, the choice of varieties, the vineyard management and suitable plastic material, and are referred by the authors as "early covering" and "late covering". They are very used in Apulia and Sicily, the leading regions for table production in Italy.

The **"early-covering"** consists in cover the vineyard before bud-break, in order to obtain the warming of the indoor air that, in turn, induces a precocious bud-break and, as a consequence, an earlier grape ripening. This technique uses a "closed" type of protecting structure that covers the top and lateral belts of the vineyard with a transparent plastic films (Fig. 3) that should have some particular properties, that is, to be able to allow the penetration of a large

amount of solar radiation while should block the out-coming of the infrared radiation emitted from all the solid bodies inside the cover.

However, if the indoor air temperature reaches 30-35 □C during blooming and berry growth, these processes are negatively effected giving poor ovule fertilization and many shot berries. This problem may be mitigated or avoided by winding up the lateral plastic sheets or by eliminating them.

The advance of grape ripening ranges from 10 to 40 days. The earlier the time of covering (end of winter), the earlier the time of harvest. The advance depends, as well, on the genotype and outdoor environmental conditions. As for the genotype, the early-maturing grapes are the most suitable, since their natural tendency is exalted. In Apulian environmental conditions, the seedless varieties improve the berry quality.

For delaying the grape harvest the **"late covering"** technique is used. In this case, the aim of plastic film is to protect plants from meteoric agents, thus this method utilize an "open" type of protecting structure, that covers only the top of vineyard with a plastic film (Fig. 4). In this case, any warming property are not required to the plastic film and should be avoided.

The cover is set up veraison, that is the phase in which the berry starts to become softer and the skin starts to change the color from green to the final one. During veraison, moreover, the juice turns sweeter and the skin becomes thinner, thus the berry is more sensitive to fungal attacks, especially in case of rainy summer. By putting the plastic sheet above the crop, the berry sanitary status is preserved and, as a consequence, the grapes may stay on-vine longer and the harvest period is extended. Under best conditions (low rainy season) harvest can be delayed by 3 months after the normal date in open air.

However, the success of table grape cultivation demands certain vigilance. To maintain the grape freshness, it is important to take care about berry hydration and turgidity by using a proper water supply. As well, it should be carried out periodical cluster cleaning to ensure the control of fungal diseases and reducing inoculation sources. This technique is especially suitable for cultivars having a late-ripening period, in order to exalt the natural tendency, and by a large berry, pruinose and strength skin, that help to preserve the berry hydration status. Presently, the "late covering" is widely used to improve the berry traits.

Fig. 3 – "Closed" plastic structure for "early covering" table grape vineyard (Apulia region, Southern Italy).

Fig. 4 – "Open" plastic structure for "late covering" table grape vineyard (Apulia region, Southern Italy).

2.5. PLASTIC FILMS

The use of plastic materials in agriculture has been widely illustrated by Scarascia Mugnozza and coll. (2011).

Use of plastic materials in agriculture is expanding extensively because it contributes to higher quality and quantity, for example: yield increase, earlier or later harvest, reduction of herbicide and pesticide use, etc. Plastic materials in agriculture are used for greenhouses and tunnel covering, mulching, vineyard covering, nets, irrigation tubes and pipes.

The main traditional agricultural polymers are: polyethylene (PE), polypropylene (PP), polyvinylchloride (PVC), ethylenvinylacetate (EVA). According to the use, plastic materials are added with additives.

For the plastic films, that is, thin, flexible and transparent plastic laminated, some characteristics are required; they may be summarized as follows: low cost, high transparency and durability, low thickness (0.12-0.20 mm) useful to limit the internal shading, lightness (~ 170g/m^2), wide strip workability (22-14 m), strength, dust prevention, anti-droplet effect, resistance to mechanical forces and to photo-thermal-oxidation. Polyethylene has most of these traits, others may be improved by adding some chemical compounds to the basic plastic polymer, hence, polyethylene is a thermoplastic material most used for plastic films. From polyolefin family, two main forms are obtained: low density PE (LDPE) and high density (HDPE). PE materials are characterized low cost, good strength, high transparency to solar radiation, but also by low thermal effect. It is used in greenhouses, low tunnels and mulching (LDPE) but also irrigation pipes, nets and pesticide cans (HDPE).

To support the plant growth and productivity, the spectroradiometric properties of the plastic films, that is, their transmittance, absorptance and reflectance in the different wavelength range of radiometric spectrum, are very important because they influence the internal light environment and microclimate and, as a consequence, the harvest time, the fruit quality and the grape yield. Among the radiometric properties, a special importance may have the transmittance to the photosynthetically active radiation (PAR, 400-700 nm) that is fundamental for the leaf photosynthetic activity and hence the organic matter production, the transmittance to the infrared radiation (IR), that is fundamental for the warming, and the transmittance to the ultraviolet radiation (UV), that enhances the polyphenol biosynthesis (Novello and de Palma, 2008).

3.MATERIALS AND METHODS

The activity has been carried out at the Laboratory of Arboriculture of the Department Science of Agriculture, Food and Environment (SAFE) of the University of Foggia, Italy, within an Erasmus study program.

It has been focused on the analysis of the carpological and chemical features of the Italia table grape produced, with the "late covering technique", using two types of plastic covers having different radiometric properties. They were:

- a thin plastic film (80 μm) made by low density polyethylene (LDPE): we named this first treatments as "commercial film" **(CF)**;
- a thick plastic film (200 μm) made by a mix of low density polyethylene and high density polyethylene (LDPE+HDPE): we named this second treatment as "experimental film" **(EF)**.

In both row plastic materials there were also additives apt to protect the films from photoxidation caused by UV rays and to reduce the droplet of condensation water. The additives differed between the two films, especially as for those having the anti-UV effect and IR_{long} retention effect, respectively. In fact, the transmissivity into these wavelength ranges was the most changing between the two types of plastic cover: the experimental film was much more permeable to UV radiation, allowing 436% more UV-B and 111% more UV-A to pass through, and 35% less permeable to IR_{long} radiation, that is to the thermal radiation emitted from soil, plant ad all the solid body under the covered structure.

Table 3 – Total transmissivity of the commercial film and the experimental film used for the "late covering" trial.

Type of plastic cover	Wavelenght range (nm)				
	UV-B (280-320)	**UV-A** (320-380)	**PAR** (400-700)	**IR_{short}** (700-2500)	**IR_{long}** (7500-12500)
Commercial Film	15.1	35.2	74.1	79.1	75.8
Experimental Film	81.0	74.3	72.0	72.5	49.4

The grapes were produced by "Fratelli Laporta" farm, localized at Cerignola (Foggia province). The "late covering" treatments started on 19th August 2014: at one of the farm vineyard plot, two adjacent groups of 4 rows were selected to be covered with CF and EF, respectively (Fig. 5); the grapes were harvested on 9th October 2014.

In this period, 5 berry samplings were periodically performed before harvest; moreover, they were used to analyze the evolution of sugar and acid concentration.

At farm harvest, entire bunches were sampled in order to analyze several parameters useful to assess the grape quality.

Fig. 5 – Late covering of cv Italia table grape vineyard at F.lli La Porta farm (Apulia region, Italy): adjacent rows covered with "commercial film" and "experimental film".

3.1. SAMPLING

Berry samples consisted, for each treatment, of 3 replicates, each having at least 50 berries. Berries were randomly cut (with their pedicel) from the up, bottom and lateral portions of clusters along the central row of each treatment (3-6 berries per cluster). The first sampling

was done the same day of the covering set-up, the others four sapling were scheduled every 8-10 days.

At harvest, 10 mature bunches, free from serious biotic or abiotic damages, were randomly collected from each treatments.

Samples were rapidly brought to the laboratory to be analyzed.

On the whole, carpological, physical, mechanical and chemical parameters were analyzed by using instruments and procedure described as follows. A test of antioxidant activity was also performed.

3.2 CARPOLOGICAL, PHYSICAL AND MECHANICAL PARAMETERS

First, 5 clusters per each repetition were weighted. The next step was to separate all berries from the rachis and weight them, separately. Of each repetition, 10 representative berries were taken from different cluster portion to examine:

- the detachment force, by pulling the berry until detaching it from its peduncle by using a device specially designed to accommodate the berries. The device was a dynamometer (Somfy Tec, Carpano et Pons, France) expressing the force in grams;
- berry calibration, that is the measurement, by digital caliper, of longitudinal and transversal diameters of berry, stated in millimeters as unit;
- skin firmness, by using a bench digital penetrometer with a 2 mm diameter probe (Turoni, Forlì, Italy);
- skin color, by using a colorimeter (Chroma Meter Cr-400 Minolta, Minolta, Osaka, Japan).

3.3 CHEMICAL PARAMETERS OF BERRY JUICE

Ten berries for each repetition were crashed to get a juice which is centrifuged (speed: 4500 rpm, time:10 minutes, temperature: 5 °C) to separate liquid compounds from solid ones. Liquid part of juice was used for analyzing technological parameters: □Brix, pH, total acidity, other chemical compounds.

3.3.1 Total soluble solids (TSS)

The refractometer measures the deviation that a ray of light undergoes the effect of dissolved compounds in the juice with optical activity (precisely sugars, acids, salts, etc.). The measurement of the deviation (refractive index) gives the value of the residual optical (RO) or

refractive, corresponding to percentage of total soluble substances, expressed in °Brix (Guce, 1990).

Procedure: it was used the model of refractometer Atago WM-7 which, as every instrument, needs to be checked for optical accuracy, or in other words calibrated before the usage. Calibrations is made by putting standard solution (distillated water) in cell for sample (area of optical lens) and pressing ZERO key. After this step, the water must be removed and the instrument is ready for readings. The grape juice, that had been extracted by manual pressure, was centrifuged; the limpid part of the juice was thoroughly mixed and a small sample of the juice was placed upon the prism-cell for samples, pressed START key, after which it could be read the TSS value in °Brix on display (immediately, to avoid evaporation). The working temperature is 20 □C which is set automatically by temperature sensor of the instrument. For precise results, the refractometer shall be washed and dried after each use (Guce, 1990).

3.3.2. pH

Real acidity, or pH, refers to concentration of hydrogen ions. The pH value of grape juice depends on amount of acids present in it and intensity of their dissociation. The pH is determined by pHmeter equipped with glass electrode.

Procedure: as pHmeter, is was used an automatic titralyser calibrated with two solutions (pH=4 and pH=7) on temperature of 20 □C. After that step, in a beaker it was put approximately 16-17 ml of the earlier prepared juice. The electrode was immersed in content of the beaker, after on display could be seen a value of pH (Guce, 1990).

3.3.3 Total acidity (TA)

Total acidity gives information about the acids contented in must (mostly tartaric, malic and citric acids) which is titrated with alkaline solutions until the pH of 7. It is expressed meq/l or g/l of tartaric acid (or sulphuric acid).

Procedure: in a beaker, containing a prepared solution (10 ml of must with 40ml of water) it was immersed the electrode of the pH meter to read the value of pH during titration with NaOH (N/10). The measurement is over after display shows value 7 of pH. Total acidity is expressed by multiplying the milliliters of used NaOH by 0.75 (that is, the equivalent weigh of tartaric acid divided by the juice milliliters used and by the NaOH normality) and final result is expressed as g/l of tartaric acid (Guce, 1990).

3.3.4. Total nitrogen, malic and tartaric acids

Amount of total nitrogen, malic and tartaric acids was measured on instrument 'Miura One - Biogamma'. This instrument works on automatic principle, that after putting reagent and samples in specific place on the instrument, by enzymatic reaction and spectrophotometric analyzes, gives information of amount (concentration) of determinate compounds.

Procedure: being an automatic instrument, it demands only few actions to do. Prepared must of each repetition is put in place for samples. In the place for reagents, it was put the specific reagent for determining total nitrogen, malic and tartaric acids, respectively. After this, on computer is made a work list of samples, set start and the instrument begins to work.

3.4. PHENOL COMPOUNDS

Of phenol substances, on this samples of table grape was examined: Hydrosi-cynnamil-tartaric acids (HCTA) in the juice of pulp, total polyphenols, flavonoids, flavans and proanthocyanids in the skin.

Preparation of samples: first, in beaker should be prepared approx. 2.000 mg of sodium disulfite, after cutting berries of each repetition and separating pulp, skin and seed, their pulp and juice are poured in the beaker. Thus, the sodium disulfite prevents compounds (AICT) from oxidation. Skins are wiped, weighed and put in a solution of ethyl chloride (EtCl) for phenol extraction, prepared by using : 70 ethanol : 30 water distilled : 1 chloride acid (37%). The skin were put in 25 ml of EtCl solution and were left for 24 hours in dark. The pulp and their juice were transferred from beaker into falcons and centrifugated for 10 minutes to separate solid part from liquid which was used to detect AICT.

3.4.1. HYDROSSI-CINNAMYL TARTARIC ACIDS (HCTA)

After that centrifugation separated pulp from juice, from the falcon, with an automatic pipette, it was taken 1 ml of juice, put in beaker and diluted 10 times with sulphuric acid. HCTA compounds were determined with spectrophotometer on wavelength of 325 nm, calibrated with sulphuric acid. Amount of HCTA was expressed in mg/l (Di Stefano and Cravero, 1991).
Formula: A_{325}*100/0,9 = mg/l

3.4.2 TOTAL POLYPHENOLS

Analysis starts with putting 0.1 ml of extract (skins in EtCl) in volumetric flask of 20 ml. In that volumetric flask it should be also added approximately 5 ml of water and with automatic pipette 1 ml of Folin-Ciocalteu reagent. After 3-4 minutes, in the flask is added $NaCO_3$ (10%) and filled with water till the mark. The content is agitated heavily and left in dark place for 90

minutes. After adding Folin-Ciocalteu reagent, it started oxidation of phenol compounds, causing blue color of solution which can be determinate in visible light. More dark solution, more phenolic compounds are presented in sample. The value of length depends about prepared concentration of extract in the solution. In this analysis it was used wavelength of 750 nm on spectrophotometer calibrated with a blank (solution is prepared the same as extract solution but without adding the extract) (Di Stefano and Cravero, 1991).

Formula : (+) Catechine $\mathrm{mg/L} = \frac{186{,}5 * A\ (750nm)}{V\ (extract)}$

total polyphenols= $\mathrm{mg/kg} = \frac{+\ Catechine * V(EtCl)}{weight\ (10\ berries)}$

A- absorbance (wave length 750nm)

V(extract)- volumen of extract (0,1 ml)

V(EtCl)- volumen of ethyl chloride- solution for preparation of skin extract

3.4.3. TOTAL FLAVONOIDS

Extracts of skins were diluted with ethyl chloride. The spectrophotometer was calibrated with media for dilution EtCl or water and then flavonoids were read on spectrum of wavelength 230-400 nm. Using the "UV-probe" program, after a reading the solutions in spectrophotometer, on display was shown a curve with peak. That peak was sign of flavonoid presence in the sample and its wavelength should be calculated (wavelength of top of peak diminished with bottom of peak) to get information of flavonoid concentration (Di Stefano and Cravero, 1991).

Formula: total flavonoids $mg/L = A_{230} * 82{,}4 * d$

A- absorbance (wave lenght 230-400nm)

d- diluition (100 times)

3.4.4. TOTAL PROANTHOCYANIDS

Proanthocyanids are composed of dimmers and polymers, which heated in presence of strong mineral acids and oxygen are transformed into cyanidine and catechin and epicatechin that give a red coloration (reaction of Bate-Smith). This transformation allows colorimetric determination of these compounds, regardless of their degree of polymerization (Usseglio-Tommaset, 1995).

Procedure: in flat bottom flask (incased with aluminum film) it was added 0.2 ml of extract and 12.3 ml of ethanol(95%). The flask was then placed into a container with ice,

added 12.5 ml of concentrated HCl (contains 300 mg/L $FeSO_4$ x $7H_2O$) and than the content of flask was mixed. The ice is important because it stops the activation. The flask was put on a water bath (heated on 90 □C) while in its top hole was situated a reflux colon (in which water circulated). After 50 minutes the reaction was over, but to stop it totally, flask should be put into ice. The solution should be agitate for minute and read at spectrophotometer on range of wavelength from 380 to 700 nm. Calibration was made without solutions. Using the program, this reading is also giving curve with peak which determines presence of proanthocyanids in extract.

Formula: (+) Catechine $mg/L = \frac{\Delta Atop - \Delta Abottom * 1162,5}{V(extract)}$

Proanthocynidins $mg/kg = \frac{+ Catechina * V\ (EtCl)}{weight\ (10\ berries)}$

A_{top}- absorbance at top of the peak

A_{bottom}- absorbance at the bottom of the peak

V(extract)- volumen of extract (0,2 ml)

V(EtCl)- volumen of ethyl chloride- solution for preparation of skin extract

3.4.5. TOTAL FLAVANS

This analysis gives an index degree of polymerization of tannins. When polymerization of more monomers occurs, the number of free positions on 6 and 8 decreases, increase with number of polymerized molecules. The vanillin, an aromatic aldehyed, reacts in acid environment with positions 6 and 8, giving compounds of red color as in the reaction of tannins with vanillin. The intensity of red colorization lowers, as higher is degree of polymerization because the few places for attack are free (Usseglio-Tommaset, 1995).

Procedure: before starting, the extract has to be diluted 10 times with methanol (absolute) . Then, in tubes covered with aluminum film (reaction requires dark) was added 0.5 diluted extract but one of tubes is sample in which was added 3 ml of vanillin (4% of vanillin in absolute methanol) while in the other, which was used as blank, it was added 3 ml of methanol. Both of tube were put into ice while it was added 1.5 ml of concetrated HCl. By removing tubes from ice, the reaction started. The reaction finished after 15 minutes, solutions were put into cuvettes and the amount of flavans was read at spectrophotometer on wavelength of 500 nm.

Formula: (+) Catechine $mg/L = A_{500} * 290,8 * d$

Flavans $mg/kg = \frac{+\ Cathecine*V\ (EtCl)}{weight\ (10\ berries)}$

A_{500}- absorbance at wavelength 500nm

d- dilution (10times)

V(EtCl)- volumen of ethyl chloride- solution for preparation of skin extract

3.5 ANTIOXIDANT ACTIVITY

Antioxidant activity was evaluated on the skin extracts. The ABTS method was used. It is based on discoloration that occurs when the radical cation $ABTS^+$ is reduced in ABTS (2,2-azinobis-3-ethylbenzothiazoline-6-sulfuric acid) in presence of compounds that have antioxidant activity (Rivero-Pérez *et al*., 2008).

Preparation of reagent for antioxidant activity test.

a) Potassium persulfate (PP): in volumetric flask of 10 ml it was added 0.3780 grams of potassium persulfate and filled with water till mark.

b) ABTS: in volumetric flask of 5 ml it was added 0.0192 grams of ABTS and filled with water till mark. The 88 µl of PP was added in solution of ABTS. The flask was wrapped with aluminum film (because reaction must develope in the dark) and left in dark for 14-16 hours (Re *et al*., 1999).

Extract: the prepared solutions of extract were diluted 50 times with bi-distilled water.

Procedure of method: in a volumetric flask of 100 ml, wrapped with aluminum film, 88 ml of ethanol and 1 ml of prepared solution ABTS were put, and the flask was agitated for couple minutes. Spectrophotometer was set on zero with ethanol on wavelength of 734 nm. The next step was to put solution and read absorption which should be 0.7±0.02. Depending on showed value it must be added ethanol and solution of ABTS to set the required value. After setting the right value of solution of ABTS, in cuvettes 2 ml of that solution were put and the value of blank was read. In the same cuvettes it was added 0.2 ml of diluted sample, stirred and left in dark for 15 minutes to activate the reaction. After that time, it was read value of the sample (Pellegrini, *et al.*,2003).

Formula: inhibition (%) = $1 - \frac{A(s)}{A(b)}$

A (s) = absorbance of sample

A (b) = absorbance of blank.

3.6. STATISTICAL ANALYSIS

All data were tested for the analysis of variance (ANOVA) by using the "ASSISTAT 7.7 beta" free software.

4. RESULTS

The TSS and TA aanalysis performed on berry samples when the "late covering" started, showed that grapes of vines assigned to the two treatments had very close values: TSS 12.9-12.4 °Brix, TA 8.8-8.9 gL^{-1} for CF and EF, respectively (Fig. 6). These two parameters are the most used to evaluate the grape quality, and their evolution is known as the major factor characterizing berry ripening (Palu *et al.*, 2010). Going on in the season, very EF showed a very slight tendency for higher SST and lower AT.

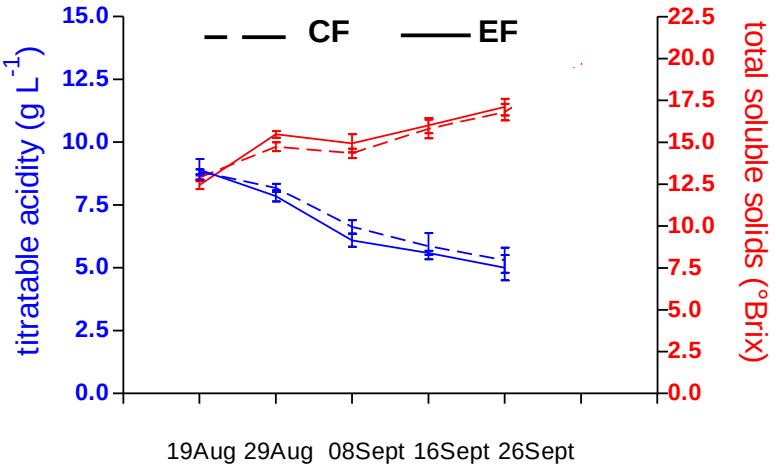

Fig. 6 – Evolution of total soluble solids and titratable acidity in berry juice of table grape cv Italia, during the "late covering", according to two types of cover plastic films (bars represent the standard errors)**.**

At farm harvest, CF and EF grapes looked very similar (fig 7).

Fig. 7 – Grapes of cv Italia produced by "late covering" technique by using two types of plastic cover.

Carpological traits were similar between treatments (Tab. 2); their principal values (cluster weight ~660 g, berry weight 9.2-10.5 g) exceeded the lower limit of cv Italia range (Novello *et al*., 1999a; Novello *et al*., 1999b).

Differences between treatments were small (mostly from 3 to 5%) and not significant but for berry width, that was significantly higher by 5% for EF grape. It is to notice that the rachis weight was 13% lower in EF grape; generally speaking, a lighter rachis means more total berry weight, but also thicker tissues that can resist better to dehydration during storage and thus respond better to the consumer demand for long-lasting of fruit freshness.

Tab. 4 – Carpological traits, at farm harvest, of table grape of cv Italia produced under "late covering" by using two types of plastic cover.

Type of plastic cover	Cluster weight (g)	Rachis weight (g)	Berry weight (g)		Berry diameter (mm)	
			average berry	representative berry	longit.	transv.
Commercial Film	670	10.59	9.23	10.55	26.98	22.78
Experimental Film	642	9.25	9.49	10.14	26.99	23.85
Statistical significans[1]	n.s.	n.s.	n.s.	n.s.	n.s.	*

[1]n.s. = not significant; * = significant at probability level ≤ 0.05; ** = significant at probability level ≤ 0.01

Also mechanical and physical traits of grapes were mostly similar between treatments and did not show any statistical difference (Tab. 3).

In particular, changes in colorimetric coordinates ranged from 1.5 to 5.3%, hence the diversities in sunlight transmission of the two plastic films did not exert relevant influence on the yellow berry skin color.

The berry removal force, was just 8% lower for EF grape; this parameters, that indexes the abscission potential, is sensitive to adverse micro-environmental factors such as heat or moisture stress: high loss of berry moisture may exalt berry shatter by stimulating ethylene production (Burger *et al*., 2005). Berry removal force is often measured using a texture analyser; in this trial we used a low-cost portable instrument, easily adoptable also by growers.

The skin resistance to rupture, that is due to the skin characteristics, was 23% higher in EF grapes; this difference seems quite relevant, but, however, it did not reached a statistical level due to a high fluctuations among mean values of each repetition. More measurements should be taken in order to verify the sensitivity of this parameters to the microenvironment under covering.

Tab. 5 – Mechanical and physical traits, at farm harvest, of cv Italia grape produced under "late covering" by using two types of plastic cover.

Type of plastic cover	Berry removal force (g)	Skin resistance (kg)	Colorimetric coordinates		
			L*	a*	b*
Commercial Film	652.83	0.26	38.57	6.88	7.94
Experimental Film	598.83	0.32	39.18	6.78	8.36
Statistical significans[1]	n.s.	n.s.	n.s.	n.s.	n.s.

[1] n.s. = non significant, * = significant at probability level ≤ 0.05, ** = significant at probability level ≤ 0.01

At farm harvest, berry juice had TSS ~19 °Brix and TA ~4.5 (Tab. 4) that are high values compared with those found in other trials with not-covered Italia harvested in the 3rd week of September (Novello *et al*., 1999a; Río Segade *et al*., 2013).

In the present trial, EF grapes had a slightly higher TSS (+3%) and lower TA (-7%) than CF grapes; differences between treatments were small and not statistically significant. This tendency could be related to the EF lower transmissivity in the IR_{long} radiation wavelength range, that is, in the "hearth" thermal emission. Hence, although the "late covering" structure was open-type, air under cover was likely a little warmer during night, stimulating maturation.

EF grapes showed relatively lower values of juice pH, tartaric and malic acid, ranging from -3 to -10%: also in this case differences were small and not statistically significant. Organic

acids are faster respired when temperatures increase (especially malic acid): hence the tendency for lower acid content seemed to confirm the hypothesis that night air temperature was slightly higher uunder cover. Ammonia plus □-amminic nitrogen content refers about nitrogen most present in berry juice and thus available as human nutrients. Values of this parameter were almost similar between treatments, just 4% higher in EF grapes.

Table 6 – Basic berry juice composition, at farm harvest, of cv Italia grape produced under "late covering" by using two types of plastic cover.

Type of plastic cover	TSS (°Brix)	T.A. (g L^{-1})	pH	Tartaric acid (g L^{-1})	L-malic (g L^{-1})	Ammonia + a-amminic Nitrogen (g L^{-1})
Commercial Film	18.67	4.61	3.43	5.34	2.11	205.33
Experimental Film	19.30	4.27	3.42	5.18	1.89	246.67
Statistical significans[1]	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

[1] n.s. = non significant, * = significant at probability level ≤ 0.05, ** = significant at probability level ≤ 0.01

TSS/TA ratio is considered as a good indicator of grape ripeness (Jayasena and Cameron, 2008; Fahmi *et al*., 2012), and thus it is very used as grape maturity index.

In this trial, it reached the value of 40 for CE and 45 for CF grapes (Fig. 8): the difference was not significant, confirming that the grape maturity had to be considered very close for the grapes produced using the two types of plastic films.

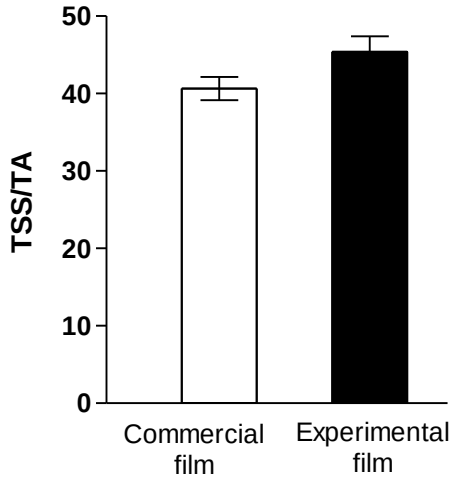

Fig. 8 TSS/TA ratio, at farm harvest, of table grape cv Italia produced under "late covering" by using two types of plastic films

(n.s. = not significant at probability level ≤ 0.05; bars represent the standard errors)

All phenol compounds in the skin or the pulp were higher in EF grape (Tab. 5). This variations ranged from +16% for HCTA to about +40% for skin otal polyphenols and flavonoids that reached a statistical level of the differences. The phenol synthesis is well-known to increase with the increase of UV radition (Arakawa *et al*., 1985), hence the higher transmittance of the experimental film to the UV radiation is likely responsible for this results. The amounts of phenol compounds were similar to those found in trials run with not-covered Italia (Río Segade *et al*., 2013), except for total flavonoids that, in the present trial, were considerably lower; it should be verified if the "late covering" technique and the consequent long stay of grapes on-vine affect in a negative way the skin flavonoid content.

Table 7 – Indices of berry skin and pulp phenol content, at farm harvest, in table grape cv Italia produced under "late covering" using two types of plastic films.

Type of plastic cover	Skin total polyphenols[a] (mg kg^{-1} grape)	Skin total flavonoids[a] (mg kg^{-1} grape)	Skin proanthoc.[b] (mg kg^{-1} grape)	Skin flavan catechins[a] (mg kg^{-1} grape)	Pulp HCTA[c] (mg L^{-1})
Comm. Film	204.94	180.80	303.76	177.90	92.85
Exper. Film	288.11	255.86	406.22	226.55	107.59
Statistical sign.[1]	*	*	n.s.	n.s.	n.s.

[1]n.s. = not significant; * = significant at probability level ≤ 0.05; ** = significant at probability level ≤ 0.01

[a]as (+)catechin; [b]as cyanidin chloride; [c]as caffeic acid

No difference was found between treatments as for the skin antioxidant activity (Fig. 7). It is known that antioxidant activity is often well correlated with total polyphephenol content of grape juice and of berry extracted in methanol (Burin *et al*., 2010; Balick *et al*., 2008); it is possible that the different extracting reagent (chloride ethanol) that we used to obtain a complete phenol extraction was not a suitable to match with the analytical procedure that we apply to evaluate the antioxidant activity.

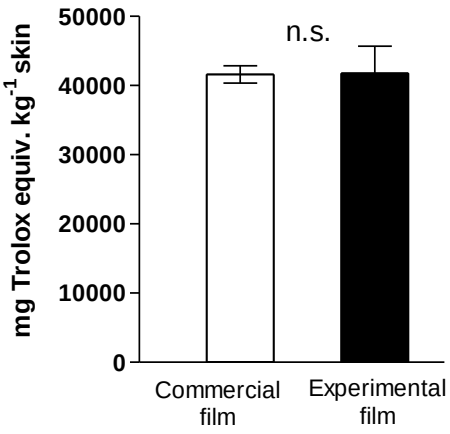

Fig. 7 – Antioxidant activity detected, at farm harvest, in skin extracts of table grape cv Italia produced under "late covering" technique using two types of plastic films.
(n.s. = not significant at probability level ≤ 0.05; bars represent the standard errors)**.**

5. CONCLUSIONS

The experimental film showed a slight tendency to delay berry ripening less than the commercial film did: this tendency was shown by the TSS and TA evolution during the covering period and by the TSS/TA ratio at harvest. However, this effect was light, thus no negative consequences was noticed on the grape stay on-vine.

The experimental film was not able to improve either the grape skin color or the carpological traits, except for the berry transversal diameter, that was enlarged in the EF grape although at a low extent: if this effect could be more exalted by this new cover, it might give a positive answer to the consumer demand for more attractive berries. The new film showed also a tendency to improve the skin resistance, making berries more suitable for manipulation, transport and storage; nevertheless, the resistance to chewing should be also tested, since consumers do not like too hard berry skin.

Finally, the new plastic sheet cover showed an aptitude to enhance the grape phenol content, especially as for polyphenols and flavonoids, likely thanks to its higher transmissivity to the UV wavelength range.

To summarise, the experimental film showed some positive influence on grape nutritional quality, but it did not prove to impact in a relevant way on the parameters that are presently most important for the grape price (such as bunch and berry weight, berry diameters, skin colour). However, this type of film might result interesting for growers if, in a next future, a higher commercial value will be attributed to grapes that prove to be more healthy for the consumers.

6.REFERENCES

1. Amerine, M. A. and M. A. Joslyn. (1950). Table wines: the technology of their production, University of California Press
2. Angelini R. (2010) L'uva da Tavola, Bayer CropScience s.r.l., Italy, pp 400
3. Arakawa O., Hori Y., Ogata R. (1985) Relative effectiveness and interaction of ultraviolet-B, red, and blue light in anthocyanin synthesis of apple fruit, *Physiol. Plant.* **64**,323-327.
4. Balík J, Kyseláková M., Vrchotová N., Tříska J., Kumšta M., Veverka J., Híc P., Totušek J., Lefnerová D. (2008) Relations between Polyphenols Content and Antioxidant Activity in Vine Grapes and Leaves, *Czech J. Food Sci*., **26**, Special Issue: S25–S32.
5. Burger D.A., Jacobs G., Huysamer M., Taylor M.A. (2005) Berry Abscission in *Vitis vinifera* L. cv. Waltham Cross: Changes in Abscission-related Factors during Berry Development, *S. Afr. J. Enol. Vitic.*, **26(2**), 71-74.
6. Burin V.M., Falcão L.D., Gonzaga L.V., Fett R., Jean Pierre Rosier J.P., Marilde Terezinha Bordignon-Luiz M.T. (2010) Colour, phenolic content and antioxidant activity of grape juice, *iênc. Tecnol. Aliment*., Campinas, **30(4):** 1027-1032.
7. Bušić Z., Kirigjija I., Puljić F., Rakušić D., Tomić M. (2002) Proizvodnja stolnog grožđa, Proizvodnja u svijetu i u nas, HZPSS, Croatia, pp 7
8. Cantos E., Espín J. C., Tomás-Barberán, F.A. (2002) Varietal differences among the polyphenol profiles of seven table grape cultivars studied by LC-DAD-MS-MS. *Journal of Agricultural and Food Chemistry*, **50(20):** 5691-5696.
9. Cirami R.M., Cameran I.J., Hedberg P.R. (1992) Special Cultural Methods for Tablegrapes,in coltura del pesco, *Riv. Frutticoltura* **50(2)**:48–56.
10. Coombe B.G. and Dry P.R. (1992) Special Cultural Methods for Tablegrape, in: Viticulture. Volume 2 Practices, Winetitles, Adelaide (Australia), pp 297
11. Di Stefano, Cravero M.C. (1991) Metodi per lo studio dei polifenoli dell'uva, *Rivista di Viticoltura e - Enologia*, **40**, pp 37-45

12. Division of Agriculture and Natural Resources, University of California, 2015, http://iv.ucdavis.edu/, pristupljeno 31.08. 2015.
13. Eynard I., Dalmasso G. (1990) Viticoltura moderna, Hoepli, Itay, pp 10-15.
14. Fahmi A.I., Nagaty M.A., El-Shehawi A.M. (2012) Fruit quality of Taif grape (*Vitis vinifera* L.) cultivars, *Journal of American Science*, **8(5):** 590-599.
15. GUCE n. L 272 del 3 ottobre 1990. Regolamento (CEE) N. 2676/90 della Commissione, del 17 settembre 1990, che determina i metodi di analisi comunitari da utilizzare nel settore del vino
16. Hemingway R. W., Laks P. E. (2012) Grape Phenols, in: Plant Polyphenols: Synthesis, Properties and Significance, edt: Springer Science and Business Media, USA, pp: 86
17. International Organisation of Vine and Wine (OIV) (2010/ 2011) Paris (France) pp 14,15,19
18. Jayasena and Cameron (2008) °Brix/acid ratio as a predictor of consumer acceptability of crimson seedless table grape, *Journal of Food Quality*, **31**, 736–750.
19. Maria Cefola, Bernardo Pace, Donato Buttaro, Pietro Santamaria and Francesco Serio (2011) Postharvest evaluation of soilless-grown table grape during storage in modified atmosphere, *Journal of the Science of Food and Agriculture*, **91(12):** pp 2153-2159
20. Munoz- Robredo P., Robledo P.Manriquez D., Molina R., Defilippi B. G. (2011) Characterization of sugar and organic acid in commercial varieties of table grapes, *Chilean Journal of Agricultural Research*, **70**, 452-458
21. Nicoletta Pellegrini, Mauro Serafini, Barbara Colombi, Daniele Del Rio, Sara Salvatore, Marta Bianchi and Furio Brighenti (2003) Total Antioxidant Capacity of Plant Foods, Beverages and Oils Consumed in Italy Assessed by Three Different In Vitro Assays, *Journal of Nutrition*, **133**, pp:2812–2819
22. Novello V., de Palma L. (2008) Growing Grapes under Cover, in: Proc. ISHS on grape production and processing, edt. P. G. Adsule et al., pp.353-357
23. Novello V., de Palma L., 2014. Local and most used table grape varieties in Italy. In: 2nd International Symposium of Oenoviti International Network: Exploitation of Autochthonous and more common vine varieties. Geisenheim, 3-5 November 2014 (pp. 87-91).Vigne et Vin Publication Internationales (ISBN 2-915883-13-0).

24. Novello V., de Palma L., Tamborra P., Montemurro P. (1999) Variazioni del contenuto in linalolo in *Vitis vinifera* L. cv'Italia' in relazione al sistema di gestione del suolo, *Quad. Vitic. Enol. Univ. Torino*, **23**, 271-278.
25. Novello V., de Palma L**.**, Tarricone L. (1999). Ecophysiological and agronomical characteristics of leading (Italia' and 'Red Globe) and emerging (Dalmasso crosses' Emilia' and'Franca) table grapes, Explotation of Autochtonous and more common vine varieties, Proc. XI GESCO Meeting, France, pp. 704-710.
26. Re R., Pellegrini N., Proteggente A., Pannala A., Yang M. and Rice-Evans C. (1999) Antioxidant activity applying an improved abts radical Cation decolorization assay, *Free Radical Biology & Medicine*, Vol. **26**: 1231–1237
27. Río Segade S.,Giocosa S., de Palma L., Novello V., Torchio F., Gerbi V., Rolle L. (2013) Effect of the cluster heterogeneity on mechanical properties, chromatic indices and chemical composition of Italia table grape berries (Vitis vinifera L.) sorted by flotation, *International Journal of Food Science and Technology*, **48**: 103-113.
28. Rivero-Pérez D.M., González-Sanjosé L.M., Ortega-Herás M., Muńiz P. (2008) Antioxidant potential of single-variety red wines aged in the barrel and in the bottle, *Journal of Agricultural and Food Chemistry* **111**: 957-964) Spain
29. Scarascia Mugnozza G., Sica C., Russo G. (2011) Plastic Materials in European Agriculture: Actual Use and Perspectives, Journal of Agricultural Engineering, **3**:15-20
30. United States Department of Agriculture, National Nutrient Database for Standard Reference Release 27, http://ndb.nal.usda.gov/ndb/foods, pristupljeno 28.08. 2015.
31. Usseglio-Tomasset (1995) Equilibro degli antociani in soluzione, in: Chimica enologica, edt: A&B Brescia, Italy, pp:73-75
32. Vaše zdravlje (2010) Raznobojno, sočno i zdravo, Divković Slađana, Croatia http://www.vasezdravlje.com/izdanje/clanak/2033/, pristupljeno 20. siječnja 2015.