DIPARTIMENTO PER LA INNOVAZIONE NEI SISTEMI BIOLOGICI, AGROALIMENTARI E FORESTALI

Corso di Dottorato di Ricerca in

BIOTECNOLOGIE DEGLI ALIMENTI - XXVI Ciclo

Metabolic and structural effects of dehydration and ozone postharvest treatments on wine grape

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Tesi di dottorato di:

Dott.ssa Federica De Sanctis

Coordinatore del corso

Prof. Marco Esti

Firma ……..

Tutore

Prof. Fabio Mencarelli

Firma ……

Co-tutore

Dott. Rinaldo Botondi

Firma……

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Summary

Abstract- English version

This PhD thesis is aimed to study metabolic, biochemical and structural changes on white wine grape, red wine grape and raisins using ozone post-harvest treatments without addiction of sulfites.
In wine production, sulfur dioxide is currently used for its positive effects on wine stabilization but it's even known its negative effects on grape and human health(Bush, 1986).
Ozone was approved in 2001 by FDA (Food and Drug Administration) as GRAS (generally recognized as safe) for food sanitation and it started to be used in many food industry as natural alternative to chemicals disinfection process. The use of ozone in the food industry represent an important step forward towards technological innovations.
To evaluate the effect of ozone on wine grape was studied first to what happen when O_3 is applied on white and red wine grape and then on the modification caused by its application during postharvest dehydration.
Grape clusters variety Sauvignon Blanc (Castello de "La Sala" winery,Orvieto, Italy), Cabernet Sauvingon (Fresno State University, California) and Pignola (Cantine Di Villa, Valtellina, Italy) were carefully hand harvested, selected and placed in a single layer into perforated crates (60 x 40 x 15 cm) until 6 kilograms Kg (±500 g) each, and divided into thermohygrometric controlled rooms (12 m^3) at 10 (±1)°C and UR 70%.
The O_3 exposure effects on Cabernet S. was studied on three different stages of grape ripening, 21,3°BRIX (unripe stage), 23,6°BRIX (ripe stage), 26°BRIX (overripe stage) and each one was treated with 300, 800 and 1300 ppb of ozone.
Sauvignon blanc sampling was performed at the beginning and then after 4, 8 and 16 hours of ozone treatment (European pending patent 12704901.3-1357 PC Engineering srl, Uggiate Trevano, Italy).
Pignola grapes, after a primary sampling, was subsequently exposed to three different treatments:
O_3 for 18 hours followed by dehydration; O_3 for 18 hours followed by dehydration with 0.5g/h of O_3 for 4 hours every day; and not treated grapes (CK), dehydrated until 20% and 35% of weight loss. Sampling was performed at the beginning and then at 20 and 35% weight loss(wl).
Color parameters, weight loss, total SSC, CO_2 production and TA remained quite constant right after ozone treatment. On the contrary, important differences have been found on phenolic content.
Some fraction as cinnamates and flavonols resulted to be positively affected by the treatment whereas catechins and epicatechin are resulted to be more susceptible.
Anthocyanins content are resulted to be less sensitive to the treatments, indeed, an increase was shown on unripe grape exposed to medium ozone concentration, while others samples remain quite stable after the exposure.
Glycosilated volatiles organic compounds (VOCs) increased significantly in Sauvignon blanc treated samples expecially benzyl alcol, methoxyeugenol, Coniferol 2, 7-OH a-terpineol and Isogeraniol, while free VOCs, whom are perceptible directly from the berry, as expected after ozone treatment fallen.
High O_3 concentration for long term intensified the oxidant strength of ozone on carotenoids content especially when it's used during postharvest dehydration.
Cell wall enzymes activity increased on ozonated grapes especially during drying and cell wall composition is modified by the exposure that caused a release of neutral sugars and a decrease of cellulose and uronic acid.

Abstract- Italian version

Lo scopo principale di questa tesi di dottorato è stato quello di studiare i cambiamenti metabolici, biochimici e strutturali di uve da vino bianche, rosse e disidratate, causati dall'utilizzo di ozono come trattamento post raccolta, senza addizioni di solfiti.

Nei processi di vinificazione l'anidrite solforosa è correntemente utilizzata per i suoi effetti positivi sulla stabilizzazione del vino ma nel contempo sono anche conosciuti i suoi effetti negativi sull'uva e sulla salute umana (Bush, 1986).

L'ozono, approvato e riconosciuto come sicuro (GRAS) per la sanificazione dei prodotti alimentari dalla Food and Drug Administation nel 2001 ha iniziato ad essere utilizzato in molte industrie alimentari come valida alternativa ai classici processi di disinfezione chimica. Il suo utilizzo nell'industria alimentare rappresenta un'importante passo in avanti nelle tecnologie innovative.

Per valutare gli effetti dell'ozono sulle uve da vino sono state studiate le modificazioni a carico di uve da vino bianche e rosse ed in seguito gli effetti su uve poste in disidratazione post raccolta.

Grappoli di uva varietà Sauvignon Blanc (Castello de "La Sala",Orvieto, Italia), Cabernet Sauvingon (Fresno State University, California) e Pignola (Cantine Di Villa, Valtellina, Italia) sono stati accuratamente raccolti a mano, selezionati e posti su singolo strato in cassette perforate(60 x 40 x 15 cm) di circa 6 kg (±500 g) ognuna, e divise in celle termo igrometriche ($12m^3$) a 10 (±1)°C e UR 70%. Gli effetti dell'esposizione all'ozono su uve Cabernet S. sono stati studiati su tre differenti gradi di maturazione delle uve, 21,3°BRIX (immature), 23,6°BRIX (mature), 26°BRIX (surmature) utilizzando 300, 800 and 1300 ppb di ozono.

Il campionamento del Sauvignon blanc è avvenuto dopo 4, 8 e 16 ore di trattamento con ozono (European pending patent 12704901.3-1357 PC Engineering srl, Uggiate Trevano, Italy).

Le uve Pignola, in seguito ad un campionamento iniziale, sono state esposte a tre differenti trattamenti: O_3 per 18h seguito da disidratazione, O_3 per 18h seguito da disidratazione con 0,5 g/h di O_3 per 4h al di e uve di controllo disidratate fino al 20% e 35% di calo peso. Il campionamento è stato effettuato all'inizio e al 20 e 35% di calo peso (wl).

Colore, calo peso, contenuto in solidi solubili, produzione di CO_2 e acidità sono rimasti piuttosto costanti in seguito al trattamento con ozono. Al contrario, sono state trovate importanti differenze sul contenuto fenolico. Alcune frazioni come cinnamati e flavonoli sono stati positivamente influenzati dai trattamenti mentre catechine e epicatechine sono risultate essere le più suscettibili.

Gli antociani sono risultati essere poco influenzati dai trattamenti, infatti, un incremento è stato riscontrato in uve immature esposte a medie concentrazioni di ozono mentre gli altri campioni sono rimasti piuttosto stabili in seguito all'esposizione.

I composti organici volatili glicosilati (VOCs) sono incrementati significativamente nei campioni trattati di Sauvignon blanc, specialmente l'alcol benzilico, il metossieugenolo, il coniferolo 2, 7-OH a-terpineolo e l'isogeraniolo mentre i VOCs liberi, i quali sono percettibili direttamente nelle bacche, come atteso, in seguito all'esposizione con ozono sono diminuiti drasticamente.

Alte concentrazioni di O_3 per lunghi tempi ne intensificano il potere ossidante sui carotenoidi specialmente quando l'esposizione avviene durante la disidratazione post raccolta.

Un incremento dell'attività degli enzimi di parete è stato riscontrato su campioni esposti al gas specialmente durante la disidratazione mentre la parete cellulare è risultata modificata dal trattamento causando un rilascio di zuccheri neutri e un decremento del contenuto in cellulosa e acido uronico.

Introduction

In wine production, sulfur dioxide is currently used for its positive effects on wine stabilization but it's even known its negative effects on grape and human health(Bush, 1986).
Recognized from US EPA (Environmental Protection Agency) as a safe agent for food contact, ozone was approved in 2001 by FDA (Food and Drug Administration) as GRAS (generally recognized as safe) for food sanitation.
It is fungistatic, effective to control decay, although it's dose dependent, and high concentrations (above 5000 ppm h^{-1}) can be phytotoxic. Many cold storage facilities in California have installed equipment that generates a constant low dose of ozone (100 ppb/day and 300 ppb/night cycle) allowing for the reduction of the spread of gray mold and to prolong the storage of grapes for several weeks (Smilanick et al., 2010).
The risk of injury to table grapes from ozone has not been completely evaluated. There are no reports indicating that ozone harms grape berries themselves; when injuries have been reported, the rachis was harmed. Constant low concentrations of ozone (0.3 ppm) caused no harm to the rachis (Palou et al., 2002; Smilanick et al., 2010), while rachis injuries developed in some tests after treatments of 30 min with very high concentrations (5000 ppm) of ozone (Mlikota Gabler et al., 2010).
Ozone reacts with apoplast constituents to form reactive oxygen species (ROS), including hydroxyl radicals (OH•), superoxide anions (O2–) and hydrogen peroxide (H2O2) (Mehlhorn et al., 1990).
The first reaction in detoxification of superoxide anion is its conversion (a dismutation reaction) to H_2O_2 by the enzyme superoxide dismutase. Hydrogen is further reduced to H_2O by catalases in peroxisomes and by ascorbate peroxidase in the chloroplasts and cytosols. The ultimate scavenging of H_2O_2 involves at least two antioxidants, ascorbate and reduced glutathione (Srivastava, 1999). Unfortunately, the inability of ascorbate to totally block O_3 molecules has been confirmed by Jakob and Heber in 1998 using fluorescence technique on spinach leaves.
Postharvest ozone treatment enhances synthesis of resveratrol and other bioactive phenolics in grapes (González- Barrio et al., 2006; Artés-Hernández et al., 2007; Cayuela et al., 2009), confirming earlier work on this subject (Sarig et al., 1996). Biosynthesis of phenilpropanoids, flavonoids and phytoalexins is elicited by ozone treatment, causing accumulation of different

types of secondary metabolites as lignin and phenols (Saleem et al., 2001; Caban´e et al., 2004). Production of peroxidase, catalase, superoxide dismutase as well as polyamide and glutathione increased after ozone exposure (Srivastava 1999, Sharma and Davis, 1997).

In the last decades ozone (O_3) has been studied for its effects on fruit rheological properties.

Cell wall represents the first defense line against ozone. The plant cell wall is a highly organized composite of cellulose, water, cross-linking glycans (often called hemicelluloses), pectins, structural proteins and aromatic substances (Carpita and McCann, 2000). Of these constituents, the aromatic compounds, lignin and proteins are most vulnerable to oxidative modification (Wiese and Pell, 2003). Differences in cell wall morphology and composition could explain the varietal differences observed in the easiness of anthocyanin extraction from skin to must during winemaking (Romero-Cascales et al., 2005).

Despite grape pulp tissue represent the 75% of fresh weight of berries, CW from grape skin tissue is threefold higher than in the pulp (Vidal et al., 2001), ascribing the differences on the wall thickness or cell volume of pulp and skin tissue.

As sugar, pH, color and phenolic composition change during grape berries development, differences in cell wall composition have been reported. The amount of cell wall isolated (gram of fresh weight) decreased steadily throughout development. The CW protein content increased during ripening (Nunan et al.,1998) while cellulose and xyloglucan content did not significantly change. An increase in water-soluble polysaccharides has been reported in grape as well as for other fruit (Gross and Wallner, 1979; Ahmed and Labavitch,1980).

Plant exposure to O_3 could induced the activity of cell wall-associated enzymes that could then modify the cell wall compounds.

In tomato, Aguayo et al. (2006) analyzed the effect of cyclic exposures to ozone (4 μL/L for 30 min every 3 h) in minimally processed fruit, in which softening delay and high levels of sugars and organic acids was promoted.

In wine process the addition of sulfites is a great advantage in term of wine stabilization but it is toxic for human being. So alternatives to the use of sulfites in wine process are continuously under research. Recently it has been proposed the Purovino® technology (EU pending patent 12704901.3-1357) to produce wine without addition of sulfites.

In this PhD research I present the results of different experimental studies conducted using ozone as postharvest treatment on different types of grape even during grape dehydration to evaluate its effect on metabolites formation and texture changes.

Ozone

General aspects and chemical properties

Ozone is an important constituent of the atmosphere, although present in trace amounts (Iriti and Faoro 2003). Actually, two different pools of O3 exists, the beneficial and the detrimental one. In the stratosphere (the higher atmosphere, ranging approximately from 15 to 40 km in altitude), the ozone layer absorbs the harmful UV-B and UV-C radiations, thus saving the living organisms (Dutsch 1978; Kerr and McElroy 1993). In the past decades, emission of ozone-depleting chemicals led to the reduction of the ozone shield against UV radiation, worsening its harmful effects on animals and plants (Platt and Honninger 2003). Otherwise, in the troposphere (the lower part of the atmosphere, approximately from the earth surface to 10–12km in altitude), that is to say the layer where the climatic conditions originate, and temperature decreases with elevation, ozone is regarded as a pollutant (Logan 1985).

In nature, ozone is formed by UV irradiation (185nm) from the sun and during lightening discharge (Suslow 1998).

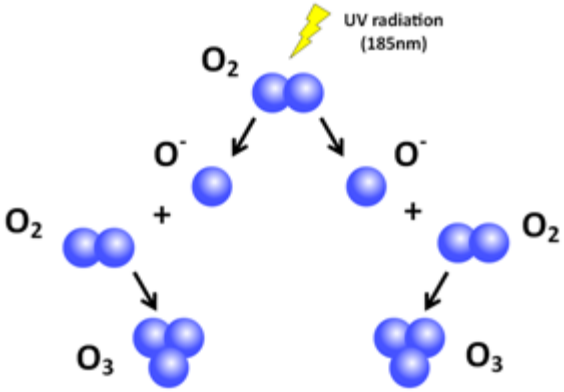


Fig. 1.1Ozone formation in nature

Commercially, UV-based generators pass ambient air (20% O2) across an UV light source, typically less than 210nm.

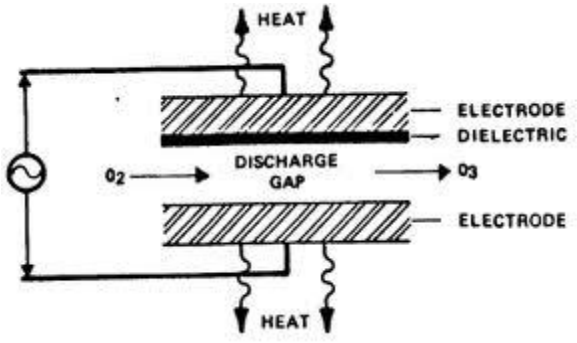


Fig.1.2 Ozone generator

These systems have a lower cost but also have a more limited output than corona discharge systems. Corona discharge generators pass dry O2 enriched air across a high electric voltage (>5,000 V) or corona; similar to a spark plug. Excess O3 not dispersed in water must be captured and destroyed to prevent corrosion and personal injury. One method of destruction is by UV light at a longer wavelength, 254nm, combined with the use of a catalytic agent.

In the 1990s, member utilities of the Electric Power Research Institute (EPRI) encouraged EPRI to conduct research into new technologies that enhance food safety. One of the technologies studied was the use of aqueous and gaseous ozone as a contact antimicrobial agent. Through the efforts of EPRI and other interested parties, ozone was recognized and allowed as an antimicrobial food additive by the US Food and Drug Administration (FDA) in 2001. The chemical ozone (CAS Number 10027-15-6) has been recognized for more than 100 years and has been used extensively in water purification and other sanitizer and fumigant functions in several countries since the early 1900s. In response to a petition filed with the FDA by the American Bottled Water Association (now the International Bottled Water Association), ozone was classified GRAS for use in bottled water in 1982.

The FDA reaffirmed this GRAS classification in 1995.

In order to win ozone clearance for use in food processing, EPRI in 1996 convened an Expert Panel of independent food scientists to evaluate the history and safety of ozone use in food processing.

An Expert Panel Report, "Evaluation of the History and Safety of Ozone in Processing Foods for Human Consumption" (TRStudies 108026, volumes 1-3) was published in 1997 by EPRI. Based on its critical evaluation of available information, the panel concluded that: "The available information supports the safety of ozone when used as a food sanitizer or disinfectant, and further that the

available information supports a Generally Recognized as Safe (GRAS) classification of ozone as a sanitizer or disinfectant for foods when used at levels and by methods of application consistent with Good Manufacturing Practices". (Sopher C.D., Graham D.M., Rice R.G., Strasser J.H..2002)

Brief History of Ozone Use for Water and Food Products

- 1906 – Ozone used to provide safe drinking water in Nice, France
- 1910 – First use of ozone in a German meat packing plant
- 1918 – Ozone used to sanitize swimming pools in the United States

• 1936 – Ozone used to treat shellfish in France
• 1942 – Ozone used in egg storage rooms and in cheese storage facilities in the United States
• 1972 – Ozone used to purify process water in Germany
• 1977 – Ozone used to reduce Salmonella in shell eggs in Russia
• 1982 – Ozone declared GRAS (Generally Recognized as Safe) for bottled water in the United States – Reaffirmed Gras in 1995
• 1997 – Expert Panel convened by EPRI declared ozone GRAS in food processing in the United States
• 2000 – Food Additive Petition filed with the FDA, August 15, 2000
• 2001 – FDA recognizes ozone as a secondary direct food additive. (Federal Register, Vol. 66, no. 123, Tuesday, June 26, 2000. Rules and Regulations)
• 2001 – FSIS determines the use of ozone on meat and poultry products is

Ozone, a molecule consisting of three oxygen atoms, was first discovered in the 1830s by the German scientist Christian Schönbein (Guzel-Seydima et al. 2001) . It was first used commercially in 1907 in municipal watersupply treatment in Nice and in 1910 in St. Petersburg (Kogelschatz, 1988).
Major physical properties of pure ozone were given in Table 1. Ozone is the second most powerful common oxidizing agent.

Fluorine	3.06
Ozone	2.07
Permanganate	1.67
Chlorine dioxide	1.50
Hypochlorus acid	1.49
Chlorine gas	1.36

Table 1.1 Oxidizing agents and their oxidation potential (mV) (Manley and Niegowski,1967)

Ozone is formed in the stratosphere, in photochemical smog and by UV sterilization lamps, high voltage electric arcs, and gamma radiation plants (Mustafa, 1990). At room temperature, ozone decomposes rapidly and, thus, does not accumulate substantially without continual ozone generation (Peleg, 1976; Milleret al., 1978). At room temperature, ozone is a nearly colorless gas. Ozone has a pungent, characteristic odor described as similar to ‘‘fresh air after a thunderstorm’’ (Coke, 1993). It is readily detectable at 0.01–0.05 ppm level (Milleret al.,

1978; Mustafa, 1990; Mehlman & Borek, 1987). It is found in low concentration in nature. Ozone has a longer half-life in the gaseous state than in aqueous solution (Rice, 1986). Ozone in pure water rather quickly degrades to oxygen, and even more rapidly in impure solutions (Hill and Rice, 1982). Ozone solubility in water is 13 times that of oxygen at 0–30C and it is progressively more soluble in colder water (Rice, 1986). Ozone decomposition is faster at higher temperatures (Rice et al., 1981).

Temperature (°C)	Solubility(liter ozone/liter water)
0	0.640
15	0.456
27	0.270
40	0.112
60	0.000

Table1.2Temperature and solubility relationship of ozone in water (Rice et al.,1981)

Ozone is a blue gas at ordinary temperature, but at concentrations at which it is normally produced the color is not noticeable. At 112C, ozone condenses to a dark blue liquid. Liquid ozone is easily exploded if greater than 20% ozone to oxygen mixtures occur.

Explosions may be detonated by electrical sparks or by sudden changes in temperature or pressure. However, in practical usage explosions of ozone are extremely rare.

The three atoms of oxygen in the ozone molecule are arranged at an obtuse angle whereby a central oxygen atom is attached to two equidistant oxygen atoms; the included angle is approximately 116490 and the bond length is 1.278A (Oehlschlaeger, 1978).

Although in low concentrations it is not an extremely toxic gas, at high concentration ozone may be fatal to humans. After1–2 h exposure to ozone (0.65 ppm) dogs exhibited rapid breathing whereas long-term (4–6 weeks) ozone exposure (0.2 ppm) to young rats exhibited lung distension (Barlett, Faulkner, & Cook, 1974). It was found that 0.2 ppm and higher concentrations of ozone can cause varying degrees of damage to the respiratory tract, depending on exposure length (Schwartz, Dungworth, Tarkington, & Tyler, 1976) bronchi (Castleman, Dungworth, & Tyler, 1973), and alveoli.

Ozone is almost insoluble in water (0.00003g/100mL at 20°C and effective dispersal is essential for antimicrobial activity (Suslow, 1998) ANCHE QUESTO.. Ozone's disinfectant activity is inefficient at a water pH from 6 to 8.5. Ozone is highly corrosive to equipment and lethal to humans with prolonged exposure at concentrations above 4 ppm. Ozone is readily detectable by human smell at 0.01 to 0.04 ppm. OSHA limits of exposure specify a 0.1ppm threshold for continuous exposure during an 8-hr period and 0.3ppm for a 15-min period. At 1 ppm ozone has a pungent disagreeable odor and is irritating to eyes and throat.

Purity and pH of water greatly affect the rate of ozone solubilization.

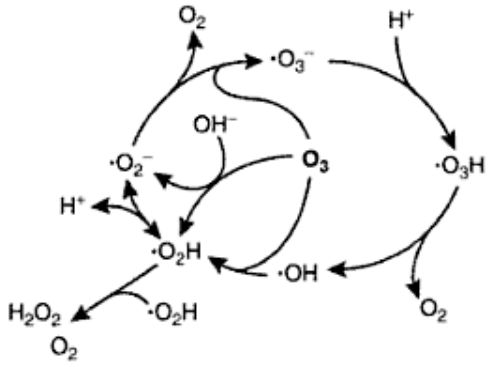


Fig.1.3 Decomposition cycle of O3 in water. (J. N. B. Bell,Michael Treshow, 2002)

J-G Kim (1998) bubbled gaseous ozone (1mM) into double distilled, deionized or tap (from two sources) water. Ozone gas dissolved faster in deionized and distilled water than in tap water. Higher maximum ozone concentration was also obtained in the water from the former two sources. The pH values, measured before ozonation, were 5.6 and 5.9 for deionized and distilled water, respectively, and 8.23 and 8.39 for tap water from the two sources. The high pH of tap water may have destabilized ozone, and thus the apparent rate of solubilization decreased. In addition, tap water may contain organic matter that consumes ozone.

Presence of minerals in water may also catalyze ozone decomposition (Hoigné and Bader 1985). Therefore, solubility of ozone increases when purity of water increases.

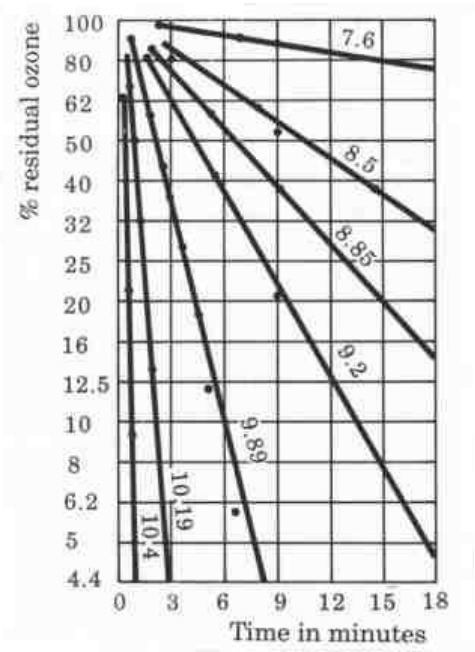


Fig.1.4 Effect of pH on ozone decay (T = 15 °C)(Lenntech)

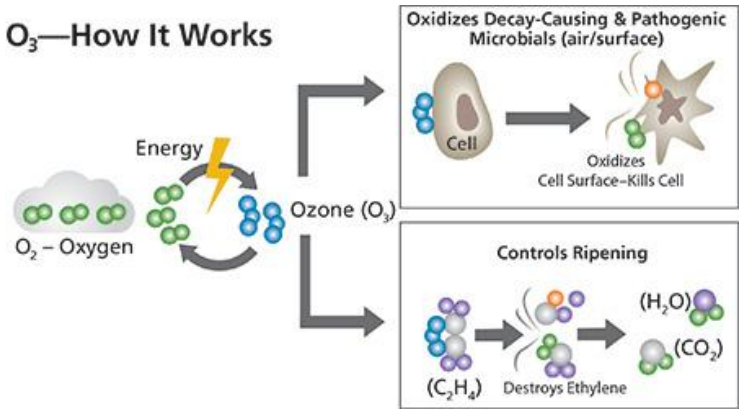


Fig.1.5 Mechanism of O3 action (www.purfresh.com)

Ozone and microorganisms

Ozone is 1.5 stronger than chlorine (Xu, 1999) and 3000 times higher than hypochlorous acid (HOCL). The O_3 contact time is generally 4-5 times less than chlorine (Khadre et al., 2001).

One of the most important aspect of the ozone application is the absence of residuals and byproducts as trihalomethane (like chloroform) or Hydrocarbon that usually occurs when chlorine is applied.

Several studies recognize the importance of air humidity for the antiseptic action of ozone, finding that its action on bacteria in a dry air treatment is considerably lower respect to a high humidity treatment.

Attribute	**Hypochlorite**	**Ozone**
Microbial potency	Kills plant pathogens and microbial saprophytes effectively. Some humanpathogenic, spore-forming protozoa resistant. Maximum allowable rates under regulatory control	Kills plant pathogens and microbial saprophytes effectively, including spore-forming protozoa. Maximum rate limited by ozone solubility, difficult to exceed about 10 µg/ml
Cost	Chemical cost low. Repeated delivery required, sometimes pH and concentration controller systems needed, minor maintenance and energy costs, chlorine storage issues	Variable: no chemical cost, but high initial capital cost for generator, usually needs filtration system when water re-used some are complex, modest maintenance and energy costs
Influence of pH	Efficacy diminishes as pH increases, above pH 8, pH	Potency not influenced very much by

	adjustment may be needed. Chlorine gas released at very low pH (4 or less)	pH, but ozone decomposition increases at high pH
Disinfection byproducts	Some regulatory concern, tri-halo compounds, particularly chloroform, of some human safety concern	Less regulatory concern, small increase in aldehydes, ketones, alcohols, and carboxylic acids created from organics, BrO_3^- from OBr^-
Worker safety issues	Chloroamines can form and produce an irritating vapor, chlorine gas systems require on-site safety measures, OSHA (TWA) limit for chlorine gas: 1 µg/ml	Off-gas ozone from solutions an irritant and must be managed. MnO_2 ozone destruction efficient and long-lived. OSHA (TWA) limit for ozone gas: 0.1 µg/ml
Persistence in water	Persists hours in clean water, reduced persistence to minutes in dirty water	Persists minutes in clean water, reduced persistence to seconds in dirty water
Use rates	Limited by regulation to 25 to 600 µg/ml, depending on application	Not limited by regulation, but Henry's law limits theoretical maximum ozone in water to about 30 µg/ml at 20°C (68°F). Most ozone systems produce 5 µg/ml or less.
Use in warm water	Increases potency, some increase in vapors	Not practical, rapidly accelerates ozone decomposition, increases offgassing, decreases ozone solubility
Influence on product quality	Little risk of injury at recommended rates. Some injury possible above 50 µg/ml on tree fruits. Off-flavors on some products at high rates	In brief water applications, risk of product injury low. Stem, calyx, and leaf tissue more sensitive than fruits. Risk of injury needs more evaluation
Impact on water quality	Minor negative impact: water salt concentration increases somewhat, may interfere with fermentation used to reduce Biological Oxygen Demand, some pesticides inactivated, discharge water dechlorination may be required.	Mostly positive impact: does not increase salt in water, many pesticides decomposed, Biological /Chemical Oxygen demand may be reduced, flocculation and biodegradability of many organic compounds enhanced, precipitates iron, removes color, odors
Corrosiveness	High, particularly iron and mild steel damaged	Higher, particularly rubber, some plastics, yellow metals, aluminum, iron, zinc, and mild steel corroded

Table 1.3 Comparison of various aspects of hypochlorite and ozone use in water. (Smilanick et al., 1999)

Sarig et al. (1996) found ozone concentrations fell rapidly upon contact with organic matter and the amount which reacted with grape berries and the microflora on their surface was about 0.1 mg/g, when supplied at a rate of 8 mg/ min for 20 min. The number of colony forming units (cfu) of fungi, yeasts and bacteria naturally present on the berry surface was considerably reduced by a 20 min exposure to ozone. Positive effect of ozone on gray mold

nesting on ‘Thompson Seedless’ table grapes was found by Palou et al., (2002): 0.3 ppm ozone on table grapes stored for 7 weeks at 5 °C completely inhibited the gray mold.

O3 use in citrus storage rooms is now common in California to retard the production of conidia on decaying fruit infected with Penicillium digitatum or Penicillium italicum (Palou et al., 2001). These authors observed that, although spread of decay by the growth of aerial mycelia was effectively inhibited by $0.3 \mu L L^{-1}$ O_3, conidia on berries in this atmosphere could germinate and infect the fruit, which indicated higher concentrations of O3 gas were needed to inactivate conidia.

Early work by Spalding (1966, 1968) showed that ozone did not significantly retard the growth of both *M. fructicola* and *R. stolonifer* on artificially inoculated peaches until a concentration of at least 0.5 ppm was used; ozone at this concentration and higher inhibited the fungal surface growth. In contrast, Ridley and Sims (1967) found reductions in decay incidence and severity on peaches inoculated with *M. fructicola* or *Rhizopus* sp. and stored under 0.5 ppm ozone. High atmospheric ozone levels (0.1 ppm) during the growing season increased postharvest weight loss in plums, but did not affect internal fruit quality or the incidence of internal breakdown (Crisosto et al., 1993). Ozone at 0.3 ppm for two 6 h exposure periods significantly inhibited in vitro sporulation and germination of *B. cinerea* (Krause and Weidensaul, 1977).

Treatment with 5000 ppm h^{-1} ozone in a commercial chamber of organically grown ‘Autumn Seedless’ and ‘Black Seedless’ table grape bunches reduced gray mold incidence from natural inoculum by about 50% after 6 weeks storage at 0 ◦C and on ‘Redglobe’ grapes decay reduction was 65% (Mlikota Gabler et al., 2010). Many cold storage facilities in California have installed equipment that generates a constant low dose of ozone (100 ppb day and 300 ppb night cycle) and it reduces the spread of gray mold and prolongs the storage of grapes for several weeks (Smilanick et al., 2010)

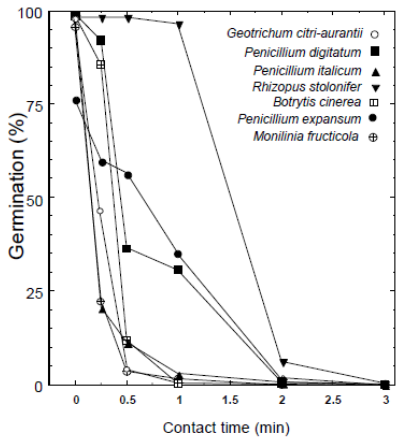


Fig. 1.6 Germination of spores of various postharvest pathogenic fungi after exposure to 1.5 µg/ml ozone in water at 16.5°C (62°F) and pH 6.4. (Smilanick et al. 1999)

A number of commercial fruit juice processors in the USA have started to employ ozone to meet the recent FDA mandatory 5 log reduction of the most resistant pathogens in their finished products (Cullen et al., 2009)

In winery, ozone has been generally accepted and documented to be effective for barrel cleaning and sanitation, tank cleaning and sanitation, clean-in-place systems, and for general surface sanitation (Hampson, 2000) but apparently no research has been reported on the use of ozone on wine grape to produce wine without addition of sulfites.

Ozone is a strong, broad-spectrum antimicrobial agent that is active against bacteria, fungi, viruses, protozoa, and bacterial and fungal spores. Strain of the microorganism, age of the culture, density of the treated population, presence of ozone-demanding medium components, method of applying ozone (that is, gas bubbles, or uniform aqueous solution), accuracy of ozone measuring procedures and devices, and method of measuring antimicrobial efficacy are some of the confounding factors that make comparison among different studies unfeasible.

Kim and Yousef in 2000 estimated nz (number of ozone molecules sufficient to inactivate a single bacterial cell) for *Leuconostoc mesenteroides* at 109. Finch and others (1988) found that 3 × 108 molecules of ozone were used to inactivate each cell of *E. coli*.

The rate of microbial inactivation {D(log10 CFU/mL)/(D time)} is calculated using the linear plot or the steepest slope on the survivor curve. The negative reciprocal of this inactivation rate, known as decimal reduction time or D-value, is a useful term in comparing resistance to ozone of different microorganisms or of the same microorganism under different conditions (Khadre et al., 2001).

Usually heat and other physical factors are applied constantly during the course of the treatment, while ozone can be applied in a single dose at the beginning of the treatment or using low concentration throughout the treatment. Ozone reacts with microorganisms rapidly, and a nonlethal threshold concentration is reached quickly in a batch treatment.

Inactivation of bacteria by ozone is a complex process because ozone attacks numerous cellular constituents including proteins, unsaturated lipids and respiratory enzymes in cell membranes, peptidoglycans in cell envelopes, enzymes and nucleic acids in the cytoplasm, and proteins and peptidoglycans in spore coats and virus capsids. Some authors concluded that molecular ozone is the main inactivator of microorganisms, while others emphasize the antimicrobial activity of the reactive by-products of ozone decomposition such as ~OH, ~O2

–, and HO~3 (Chang 1971; Harakeh and Butler 1985; Glaze and Kang 1989; Bablon and others 1991b; Hunt and Marinas 1997).

Organism	Concentration	Exposition time
E. Coli, Legionella, Mycobacterium fecal, Streptococcus	0,23-2,2ppm	< 20 min
Poliovirus type-1, uman Rotevirus, Enteric virus	0,2-4,1ppm	< 20 min
A. Niger, Penicillum, Cladosporium	2ppm	60 min
Candida Parapsilosis, Candida Tropicalis	0,02-0,26ppm	< 1,67 min
Acarus Siro, Tyrophagus Casei, Tyrophagus Putrescientiae	1,5-2ppm	30 min

Table 1.4 Inactivation of bacteria, virus, fungi, mould and bugs after ozonization (Edelstein et al., 1982; Joret et al., 1982; Farooq and Akhlaque,1983; Harakeh and Butle, 1985; Kawamuram et al. 1986)

Ozone and Cell membranes. Ozone may oxidize various components of cell envelope including polyunsaturated fatty acids, membrane-bound enzymes, glycoproteins and glycolipids leading to leakage of cell contents and eventually causing lysis (Scott and Lesher 1963; Murray and others 1965). When the double bonds of unsaturated lipids and the sulfhydryl groups of enzymes are oxidized by ozone, disruption of normal cellular activity including cell permeability and rapid death ensues. Komanapalli and Lau (1996) found that short-term exposures of *E. coli* K-12 to ozone gas compromised the membrane permeability but did not affect viability, which progressively decreased with longer exposure (Khadre et al. 2001).

Ozone andf Bacterial spore coats. Foegeding (1985) found that *Bacillus cereus* spores with coat proteins removed were rapidly inactivated by ozone, compared to intact spores. The researcher concluded that the spore coat is a primary protective barrier against ozone. Recently, Khadre and Yousef (2001b) found that spores of *Bacillus subtilis* treated with aqueous ozone showed heavily disrupted outer spore coats (Khadre et al. 2001).

Ozone and Enzymes. Several authors referred to enzyme inactivation as an important mechanism by which ozone kills cells. Sykes (1965) reported that chlorine selectively destroyed certain enzymes, whereas ozone acted as a general protoplasmic oxidant.

Ingram and Haines (1949), in view of their finding general destruction of the dehydrogenating enzyme systems in the cell, proposed that ozone kills *E. coli* by interfering with the respiratory system. Takamoto and others (1992) observed that ozone decreased enzyme activity in *E. coli* at a greater degree in case of cytoplasmic â-galactosidase than in case of the periplasmic alkaline phosphatase (Khadre et al. 2001). Inactivation of enzymes by ozone is probably due to oxidation of sulfhydryl groups in Cysteine residues (Chang 1971).

Ozone and Nucleic material. Reaction of aqueous ozone with nucleic acids *in vitro* supports the notion that it may damage nucleic material inside the cell (Khadre et al. 2001). Ozone modified nucleic acids *in vitro* , with thymine being more sensitive than cytosine and uracil (Scott 1975; Ishizaki and others 1981). In another study, ozone opened the circular plasmid DNA and reduced its transforming ability, produced single- and double-strand breaks in plasmid DNA (Hamelin 1985), and decreased transcription activity (Mura and Chung 1990). Studying *E. coli,* l'Herault and Chung (1984) found that ozone may induce mutations. However, other investigators did not detect any mutagenic effect of ozone on *Salmonella* spp. (Victorin and Stahlberg 1988). Compared to other known mutagens, ozone was found to be a weak mutagen on *Saccharomyces cerevisiae* (Dubeau and Chung 1982).

*Ozone and Viruses***.** Sproul and Kim (1980) and CK Kim and others (1980) found that aqueous ozone inactivated both f2 and T4 bacteriophages by attacking capsid protein, with liberation and inactivation of the nucleic acid. The RNA from f2 bacteriophage was partially inactivated prior to release from the capsid. They suggested that ozone breaks the protein capsid into subunits liberating RNA and disrupting virus adsorption to the host pili, and that the RNA may be secondarily inactivated (Khadre et al. 2001).

The DNA released from T4 bacteriophage was rapidly inactivated by ozone at about the same rate as that in the intact phage. CK Kim and others (1984) confirmed the results of Sproul and Kim (1980) on bacteriophage T4; they found that ozone randomly destroyed the head, collar, contractile sheath, end plate, and tail fibers and liberated the DNA from the head.

Yoshizaki and others (1988) found that aqueous ozone caused the coat proteins subunits of tobacco mosaic virus (TMV) to aggregate with each other and cross-link with the viral RNA. Despite their observation of a good correlation between loss of infectivity and decrease of recovery of viral RNA, Yoshizaki and others (1988) and Shriniki and others (1988) concluded that the major cause of TMV inactivation by ozone was the inability of the treated virus to

uncoat. Roy and others (1981) found that ozone altered two of the four polypeptide chains in the poliovirus protein coat. They, however, attributed the inactivation of the virus to the damage in its RNA by ozone. The observation by Herbold and others (1989) that 0.38 mg/mL aqueous ozone was needed for complete inactivation of hepatitis A virus (HAV) and only 0.13 mg/mL for complete inactivation of poliovirus may support the hypothesis that damage to viral envelopes is the main cause of inactivation of viruses by ozone. Enveloped viruses such as HAV are expected to be much more resistant to ozone compared to nonenveloped viruses such as poliomyelitis.

Efficacy of ozone. Efficacy of ozone is demonstrated more readily when targeted microorganisms are suspended and treated in pure water or simple buffers (with low ozone demand) than in complex systems such as food. The simplicity of low-ozone demand aqueous environment makes it possible to compare ozone efficacy against microorganisms within the same study, and occasionally among different studies. Ozone also may be compared with other sanitizers when experiments are done in the simple treatment environments just indicated, but differences in experimental designs, treatment conditions, and microbial strains tested should be considered (Khadre et al. 2001).

Inactivation spectrum

Bacteria. Studies shown that from 0.12 to 3.8 mg/mL aqueous ozone inactivated gram-positive bacteria by 1 to 7 log10 CFU/mL. When gram-negative bacteria were treated with 0.004 to 6.5 mg/mL aqueous ozone, their populations decreased 0.5 to 6.5 log10 CFU/mL (Khadre et al. 2001).

Sobsey (1989) reviewed studies to inactivate health-related microorganisms in water by several disinfectants and concluded that gram-positive bacteria, including *S. aureus* and *Bacillus* spp., and the *Mycobacteria* were more resistant than were gram-negatives.

Lee and Deniniger (2000) observed the dominance of grampositive bacteria among the surviving microorganisms in ozonated drinking water. When gram-positive and gram-negative bacteria were compared in side-by-side experiments, however, variable results were obtained. Restaino and others (1995) studying a group of food-related microorganisms, observed that gram-negative bacteria were substantially more sensitive to ozone in pure water than were the gram-positive ones including *L. monocytogenes*. Kim and Yousef (2000) and J-

G Kim and others (1999b) treated foodborne spoilage and pathogenic bacteria with ozone under identical conditions and found results inconsistent with the previous conclusion.

Resistance of bacteria tested in this study followed this descending order: *Escherichia coli* O157:H7, *Pseudomonas fluorescens*, *Leuconostoc mesenteroides*, and *Listeria monocytogenes*. Ozone is generally more effective against vegetative bacterial cells than bacterial and fungal spores.

J-G Kim and others (2001) studied inactivation kinetics of different microorganisms that commonly spoil fruit juices. Results of this study show that *Alicyclobacillus acidocaldarius* vegetative cells and *Zygosaccharomyces bailii* ascospores were inactivated rapidly with aqueous ozone.

Spores of *A. acidocaldarius* were the most resistant to ozone, and survivor's plot exhibited both a shoulder and a tail. Mold spores (*Neosartorya fischeri*) were intermediate in resistance to ozone, and tailing of survivor plots was apparent.

Khadre and Yousef (2001b) measured ozone efficacy against spores of 8 *Bacillus* spp. *B. stearothermophilus,* which is known for high resistance to heat, also possessed the highest resistance to ozone among the species tested.

Viruses. A limited number of studies on inactivation of viruses with ozone have been published. Researchers tested ozone concentrations in the range of 0.1 to 15.9 mg/mL against 8 different viruses; the treatment caused destruction of 0 to 7 log10-units. This may indicate that viruses are comparable to bacteria in sensitivity to ozone. Sobsey (1989), however, concluded that viruses are generally more resistant than vegetative bacteria and that bacteriophages are the most sensitive to ozone among the viruses tested. Other researchers (CK Kim and others 1980; Hall and Sobsey 1993) also reported the sensitivity of the bacteriophages MS2, and f2 to ozone. Based on the limited studies in Table3, it may be concluded that bacteriophages are the least resistant to ozone, followed by polioviruses, whereas human rotavirus was the most resistant to the sanitizer. This conclusion is in agreement with those reports by Herbold and others (1989) and Hall and Sobsey (1993).

Interaction between ozone and organic matrix

A number of environmental stresses, including pathogen attack, drought, and exposure to ultraviolet-irradiation, heavy metals and air pollutants such as sulfur dioxide and ozone (O3) , are thought to affect plants by causing the excess accumulation of active oxygen species (ROS).

Once O3, a very strong oxidant, enters the intercellular leaf space (apoplast) through stomates, it is converted into ROS such as $O_2^{\cdot -}$, HO· and H_2O_2 (Apel and Hirt, 2004). These ROS can react with membrane lipids to generate lipid peroxides that can initiate a series of reactions producing damaging reactive oxygen intermediates. These damaging free radicals and their products react with proteins, DNA and membrane lipids to cause the reduced photosynthesis, electrolyte leakage and accelerated senescence usually associated with O3 exposure. Plants respond to O_3-induced oxidative stress by activating a number of antioxidative stress-related defense mechanisms (Sharma and Davis,1997) .

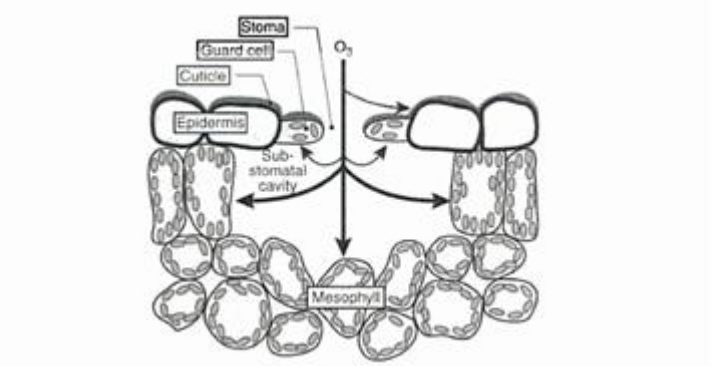


Fig. 1.7 Interaction between O3 and cuticle. Fonte Air pollution and plant life (J. N. B. Bell,Michael Treshow, 2002)

Some molecules, like phenolic compounds in the cell wall, could accelerate the formation of radical since considerable enhancement of $OH^{\cdot}$ production has been observed when ferulic or caffeic acid was added to ozonated water solution with pH 4.5-7.8 (Grimes et al. 1983).

Plants produce $O_2^{\cdot -}$ and H_2O_2 as a result of their normal aerobic metabolism in chloroplasts and mitochondria during photosynthesis and respiration. During evolution, plants have acquired a number of distinct biochemical mechanisms to efficiently and rapidly remove damaging ROS from different cellular compartments.

Non-enzymatic scavengers of ROS include a number of compounds with high reducing potentials such as vitamins C (ascorbic acid) and E, b-carotenes, polyamines and the tripeptide, glutathione.

Glutathione and ascorbic acid work in concert with enzymes such as ascorbate peroxidase (APX) , dehydroascorbate reductase (DHAR) and glutathione reductase (GR) to modulate the oxidation state of the cell. In the ascorbate-glutathione cycle (Fig.1.8) , H_2O_2 generated from ROS metabolism is converted to H_2O at the expense of NADPH. In addition to the ascorbate-glutathione cycle, there are other well studied antioxidant enzymes that play crucial roles in removing ROS.

These include multiple isoforms of superoxide dismutase (SOD) that convert O_2 i0 into H_2O_2 and catalases and peroxidases that further metabolize H_2O_2 to H_2O.While cat is primarily localized in peroxisomes, isoforms of peroxidase and SOD are distributed throughout the cell and can be found in cytosolic, mitochondrial and chloroplastic compartments.

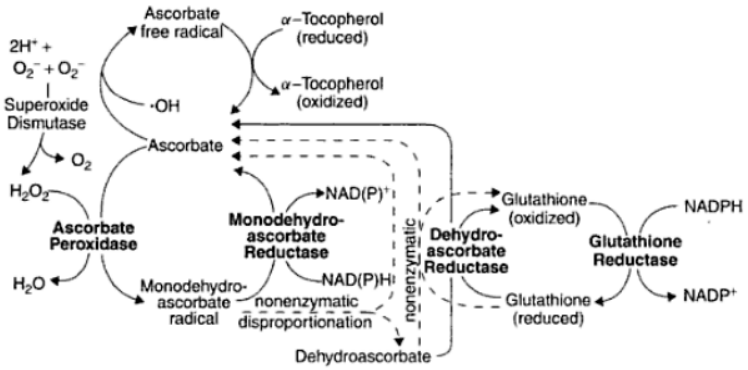


Fig.1.8 O3 Ascorbate-glutathione cycle (J. N. B. Bell,M. Treshow, 2002)

The direct reaction between ozone and ascorbate in cell wall 0.3-0.5 µm thick and at an ascorbate concentration of 0.5mM is able to detoxify 50-70% of the O3 that impinges on the wall surface (Moldau,1998). The inability of ascorbate to totally stop O3 has been confirmed by Jakob and Heber in 1998 by foliar fluorescence technique.

When in contact with ozone, lipids are subjected to “lyses”, the rupture of carbon double bond and the resulting reorganization of the molecule, forming the “Ozonide”, with three oxygen between the two carbons.

The light and dark reactions of photosynthesis might be impaired by ROS, lipid peroxidation and changes in membrane permeability (Andersen, 2003). As a consequence, plants have evolved physiological and biochemicalmechanisms, including increases in the activities of enzymes associated with general stress tolerance and increasing antioxidant concentration, which can avoid the effects of this pollutant (Conklin and Barth, 2004)

Stimulation of apoplatic SOD activity has been reported in few instances (Padu et al., 2006), while activation of apoplastic POD under ozone load is a well-documented phenomenon (Ranieri et al., 1999).

Cell wall polysaccharides were increased after 7 days and 2 month of ozone stress, while water-soluble pectins were elevated after 7 days but similar or lower after 2 month. Sod activity was lower after both treatment while POD activity was significantly higher in ozone-exposed leaves of Korona strawberries (Keutgen and Pawelzik, 2008).

Catechin, an allelochemical and antioxidant, is strongly induced by ozone in spruce and pine. The elevated levels of catechins and stilbenes persist over several months and may be related to the memory effect of ozone in conifers(Langebartels, C. et al., 1998). In addition to the furanocoumarin phytoalexins of parsley plants, ozone can also induce the genes, enzymes and metabolites of the UV-B-induced flavone malonyl-glycoside pathway13, which is known not to respond to fungal elicitor. The ability of ozone to mimic other stresses has been termed cross-induction (Eckey-Kaltenbach, H. *et al*.1994). This process can lead to cross-tolerance against pathogens as well as numerous abiotic stresses that are proposed to act via activated oxygen species (Schraudner et al.,1997)

Ozone induces the lignin biosynthesis enzyme, cinnamyl alcohol dehydrogenase, at the protein and transcript levels (Sandermann 1996), but the derived lignan- or lignin-like product has so far not been identified.

The oxidative stress caused by ozone appears to have two opposing effects: it is generally held responsible for the detrimental effects of ozone (Heath and Taylor 1997) and may serve as the initial signal for programmed cell death and HR.

This signalling may involve ethylene (Greenberg 1997), which was specifically induced in the ozone-hypersensitive biomonitoring plant tobacco Bel W3. The detrimental effect of activated oxygen species is well illustrated by an ascorbic acid-deficient *Arabidopsis* mutant that is hypersensitive to ozone, as well as to sulphur dioxide (SO2) and UV-B (Conklin, 1996).

Hydrogen peroxide and the superoxide anion radical are potential primary signalling species, only hydrogen peroxide being able to permeate over longer distances (Lamb 1997; Schraudner 1997; Heath and Taylor 1997).

Ozone, and to a lesser extent SO_2 and UV-B, cause an increase in transcript levels of the antioxidative enzymes catalase and glutathione peroxidase. There are small and delayed effects on transcript levels of several superoxide dismutase isoforms and ascorbate peroxidase (Willekens *et al.* 1994). In another study with tobacco, the cytosolic ascorbate peroxidase was

shown to be up-regulated by high-level ozone exposure as well as by methyljasmonate (Örvar 1997). Somewhat similar results were obtained for ozone in *Arabidopsis (*Kubo *et al*. 1995), where transcripts for the antioxidative enzymes Cu/Zn-superoxide dismutase, ascorbate peroxidase and GST-1, but not catalase, were induced by ozone (Sharma and Davis 1994; Sharma *et al.* 1996).

Only certain members of the antioxidative gene families seem to respond to ozone. Furthermore, compartmentation of antioxidative systems (e.g. apoplast, cytosol and chloroplast) has been shown to be of ey importance for ozone sensitivity and for generating ozone tolerance by gene transfer (Sandermann 1996; Schraudner 1997; Pell 1997).

Biosynthesis of phenilpropanoids, flavonoids and phytoalexins is elicited by ozone treatment, causing accumulation of different types of secondary metabolites as lignin and phenols (Saleem et al., 2001; Caban´e et al., 2004). In the last decades ozone (O_3) has been studied for its effects on fruit rheological properties. In tomato, Aguayo et al. analyzed the effect of cyclic exposures to ozone (4 μL/L for 30 min every 3 h) in minimally processed fruit, in which softening delay and high levels of sugars and organic acids was promoted

In 1996 Sarig et al found the phytoalexins resveratrol and pterostilbene were elicited at levels similar to those produced by uv-c irradiation. Resveratrol accumulated in greater quantities than pterostilbene.

In plants, phenylpropanoid metabolism is induced as a general response to stress.

Therefore, enhancement of key enzyme activities and accumulation of secondary metabolites are events that occur in order to improve the resistance against pathogen attack and/or tolerance to adverse environmental conditions and pollutants. PAL is an extremely sensitive indicator of stress conditions, and commonly considered as a biochemical marker indicating the activation of plant defenses which include the synthesis of both structural and protective compounds.

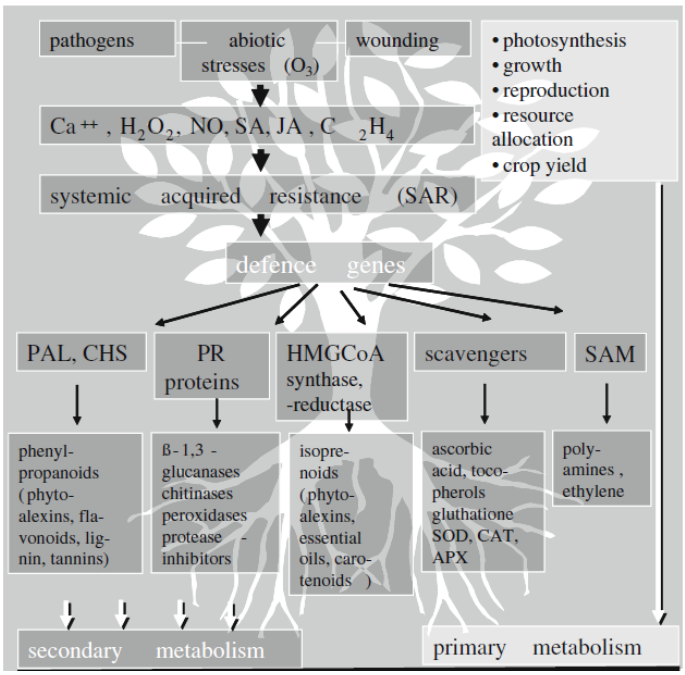


Fig.1.9 Influence of different stresses on plant metabolism. (Iriti and Faoro 2003)

In particular, ozone exposure elevates the level of flux through the phenylpropanoid pathway, thereby supplying carbon skeletons for secondary metabolites (Toumainen et al. 1996)

In Arabidopsis PAL mRNA is rapidly and transiently induced within 3h of ozone treatment (300 nL L−1 daily for 6 h), reaching a 3-fold higher levels than control plants (Sharma and Davis 1994). In parsley plants, a similar trend has been reported in which ozone treatment (200 nL L−1 for 10 h) induced an early 3-fold and 1.2-fold increase of PAL and CHS activity, respectively, followed by a 2-fold increase of total leaf furanocoumarins and flavone glycosides (Eckey-Kaltenbach et al. 1994).

Grape Cell wall and Postharvest dehydration

Cell wall composition

The shape of a plant cell is defined largely by its cell wall.

The plant cell wall is a dynamic compartment that changes throughout the life of the cell. The primary cell wall is born in the cell plate during cell division and rapidly increases in surface area during cell expansion, in some cases by more than a hundred-fold. The middle lamella forms the interface between the primary walls of neighboring cells. At the end many cells elaborate within the primary wall a secondary cell wall building complex structures uniquely suited to the cell's function.

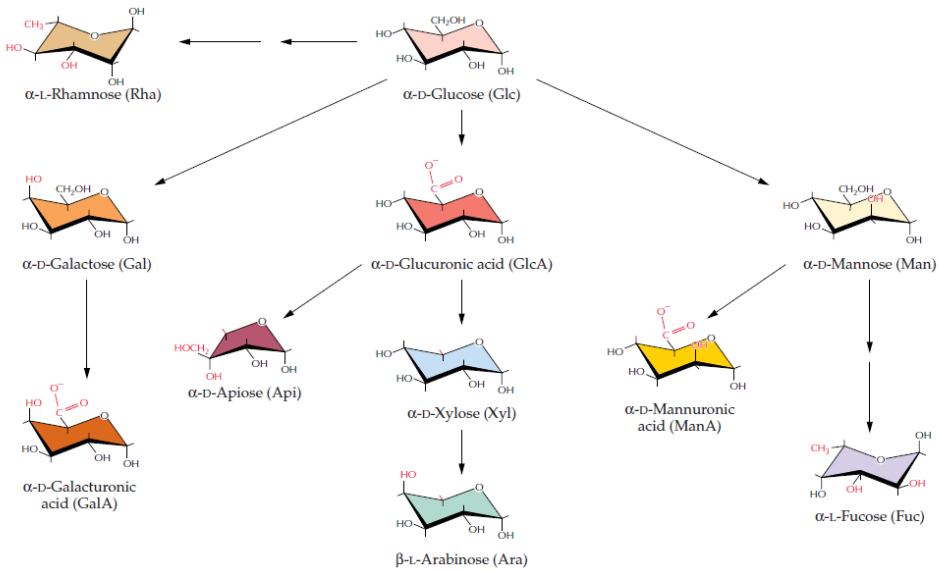
The plant cell wall is a highly organized composite of many different polysaccharides, proteins, and aromatic substances. Some structural molecules act as fibers, others as a cross-linked matrix, analogous to the glassfibers- and-plastic matrix in fiberglass (Carpita and McCann, 2000).

The wall provides support and shape for the plant, allowing it to stand upright. The plant cell wall also provides a barrier against the environment and potentially pathogenic organisms. However, in spite of the strong, rigid, and seemingly impenetrable properties of cell walls, they are metabolically active, allowing exchange of material and signals between cells, and are capable of expanding (Scheller and Ulvskov, 2010).

Cell wall Sugars

Polymers of sugar, polysaccharides are the principal components of the cell wall and form its main structural framework. Polysaccharides are consider like long chains of sugar molecules covalently linked at various positions, some being decorated with side chains of various lengths. Almost all cell wall sugars are aldoses.

Fig.3.1.1 Principal sugars contained in the cell wall(Carpita and McCann, 2000)



Sugars in polymers are always locked in pyranose or furanose rings. During sugar polymerization, the anomeric carbon of one sugar molecule is joined to the hydroxyl group of another sugar, a sugar alcohol, a hydroxylamino acid, or a phenylpropanoid compound in a glycosidic linkage.

Sugars are such important molecules for their ability to form linkages at multiple positions.

It has been found 11 different sugars in plant cell wall with 4 different linkage positions, 2 configurations with respect to the oxygen atom, the transformation of pentasaccharide structure exceed 5 billion, just think about that glucose can form about 15.000 different pantametric structures.

Cellulose

Cellulose is the most abundant organic chemical on the face of the earth.

Cellulose is the most abundant plant polysaccharide, accounting for 15% to 30% of the dry mass of all primary cell walls and an even larger percentage of secondary walls (Carpita and McCann, 2000).It is a glucan polymer of D-glucopyranose units, which are linked together by β-(1 → 4)-glucosidic bonds. In plants, on average, each microfibril is 36 individual chains thick in crosssection, but microfibrils of algae can form either large, round cables or flattened ribbons of several hundred chains. Microfibrils of angiosperms have been measured to be between 5 and 12 nm wide in the electron microscope.

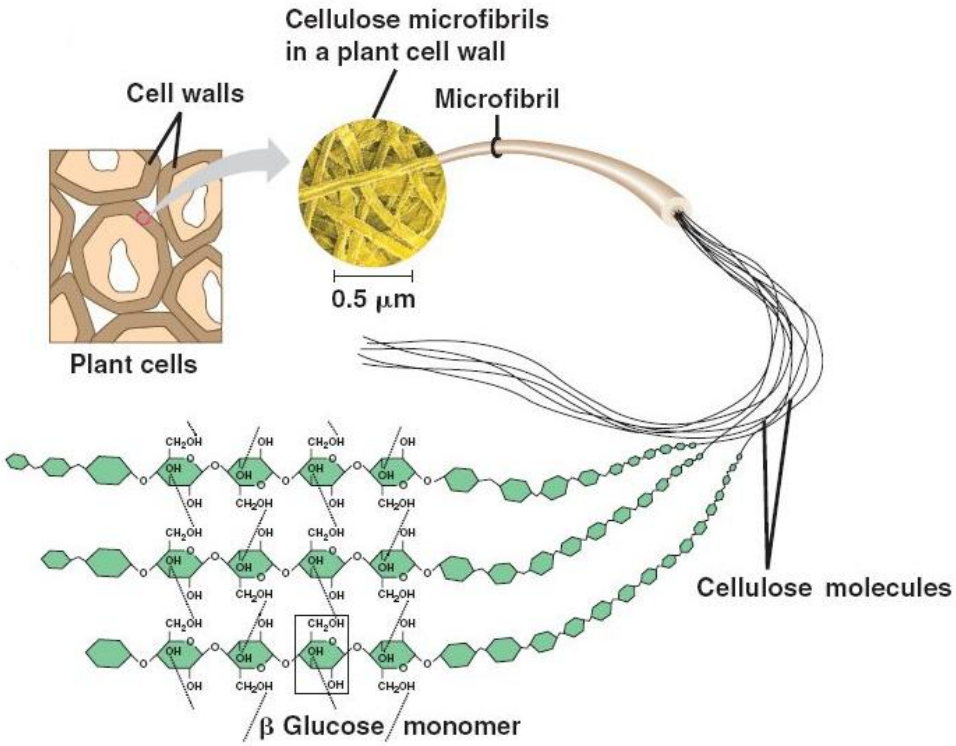


Fig.3.1.2 Cellulose in plants cell wall
(http://bio1151.nicerweb.com/Locked/media/ch05/cellulose.html)

Each (1→4)β-D-glucan chain may be just several thousand units (about 2 to 3 μm long), but individual chains begin and end at different places within the microfibril to allow a microfibril to reach lengths of hundreds of micrometers and to contain thousands of individual glucan chains. This structure is analogous to a spool of thread that consists of thousands of individual cotton fibers, each about 2 to 3 cm long.

Most cross-linking glycans are often called "hemicelluloses" (Carpita and McCann, 2000).

Hemicelluloses are polysaccharides in plant cell walls that have β-(1→4)-linked backbones with an equatorial configuration. Hemicelluloses include xyloglucans, xylans, mannans and glucomannans, and β-(1→3,1→4)-glucans. These types of hemicelluloses are present in the cell walls of all terrestrial plants, except for β-(1→3,1→4)-glucans, which are restricted to Poales and a few other groups (Scheller and Ulvskov, 2010).

The xyloglucans consist of linear chains of (1→4)β-D-glucan and is the most abundant hemicellulose in primary walls of spermatophytes except for grasses

Pectins

The pectic polysaccharides comprise a class of D-galacturonic acid-containing polysaccharides that are abundant in the plant cell wall; comprising as much as 30% of dicot, gymnosperm, and non-Poales monocot walls (Caffall and Mohnen, 2009).

Two fundamental constituents of pectins are homogalacturonan (HGA) and rhamnogalacturonan I (RG I).

HGAs are polymers of a-1,4-linked-D-galacturonic acid that can account for greater than 60% of pectins in the plant cell wall (Ridley et al., 2001). There are two kinds of structurally modified HGAs, xylogalacturonan and rhamnogalacturonan II (RG II).

The unmethylated C-6 of HGA GalA residues is negatively charged and may ionically interact with Ca2+ to form a stable gel with other pectic molecules if >10 consecutive unmethyl-esterified GalA residues are coordinated (Liners et al., 1989). The hypothesized in vivo structure of the HG–calcium complex is sometimes referred to as the egg-box model. The egg-box model describes the close packing of HG that occurs upon Ca2+-induced gelling, which accounts for ∼70% of the pectic gel in the cell walls of plants (Jarvis and Apperley, 1995).

Other polysaccharides, composed mostly of neutral sugars—such as arabinans, galactans, and highly branched type I arabinogalactans (AGs) of various configurations and sizes—are attached to the O-4 of many of the Rha residues of RG I. In general, about half of the Rha units of RG I have side chains, but this ratio can vary with cell type and physiological state (Carpita and McCann, 2000).

HG, RG-I, and RG-II are structurally diverse polysaccharides that contribute to primary wall function with regard to cell strength, cell adhesion, stomatal function, and defense response (Caffall and Mohnen, 2009).

Calcium crosslinking of HG contributes to wall strength by bringing blocks of unmethylesterified HG chains into a tightly packed conformation that is dependent on three characteristics: the intramolecular conformation of HG, the charge separation between two GalA molecules in a HG chain, and the efficiency with which HG chains pack together (Stolle-Smits et al., 1999).

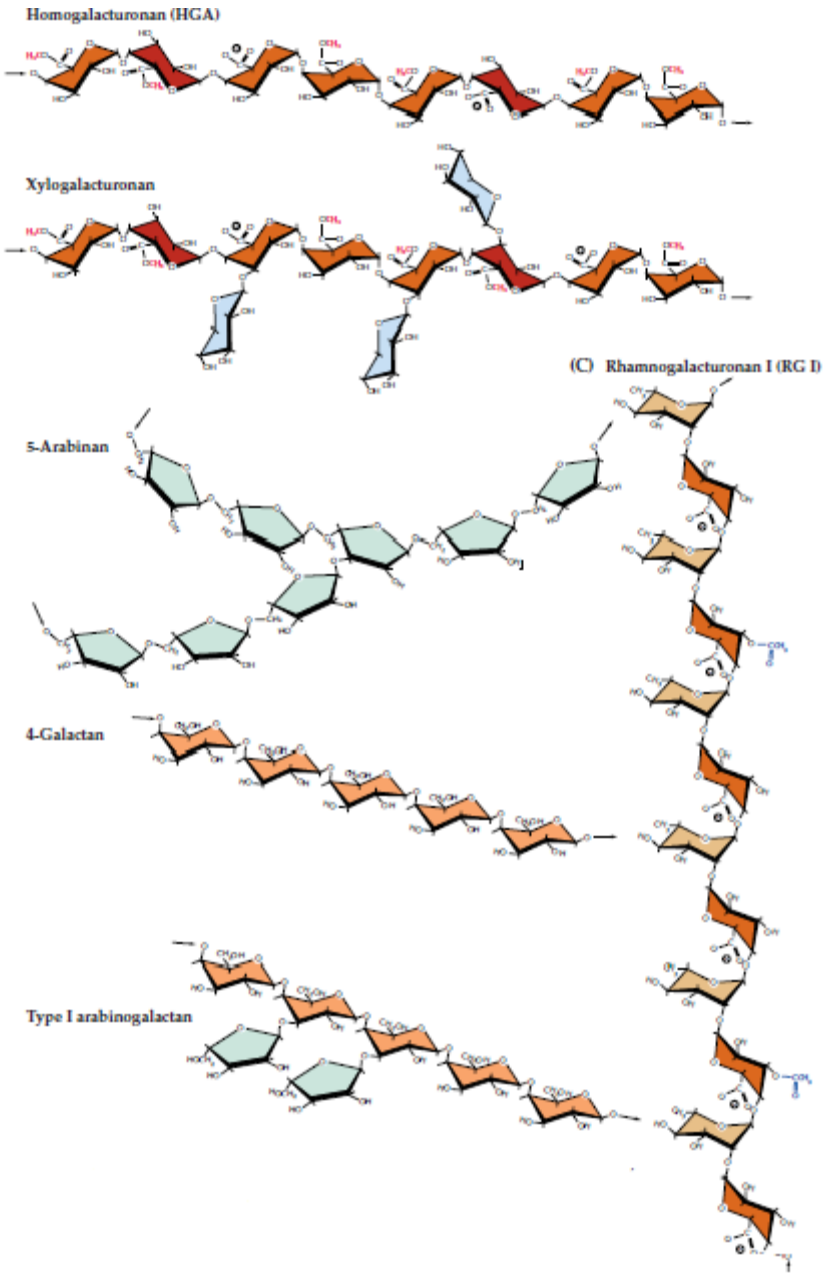


Fig3.13. Pectic polysaccharides of plants(*Carpita and McCann, 2000).*

Structural proteins

The structural proteins of the wall make up 2–10% of wall dry weight and comprise a variety of wall-associated proteins (Caffall and Mohnen, 2009).

There are four major classes of structural proteins, three of them named for their uniquely enriched amino acid: the hydroxyproline- rich glycoproteins (HRGPs), the proline-rich proteins (PRPs), and the glycine-rich proteins (GRPs) (Carpita and McCann, 2000).

The arabinogalactan proteins (AGPs), proline-rich proteins (PRPs), glycine-rich proteins (GRPs), and wall-associated kinases (WAKs) are wall-associated proteins and are hypothesized to aid in the wall structural reinforcement and regulatory pathways (Carpita, 1989; Hengel and Roberts 2003).

Extensin, encoded by a multigene family, is one of the best-studied HRGPs of plants and are important for secondary and tertiary structure.

Cell wall architecture

The primary cell wall is made up of two, sometimes three, structurally independent but interacting networks. The fundamental framework of cellulose and crosslinking glycans lies embedded in a second network of matrix pectic polysaccharides.

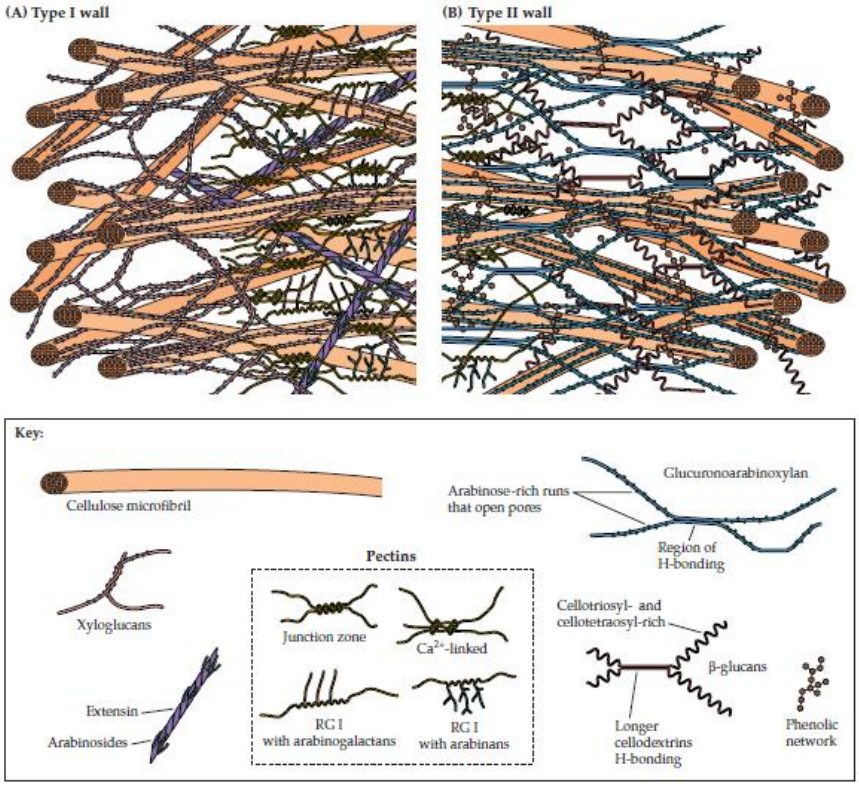


Fig.3.2.1 (A) A three-dimensional molecular model of the Type I wall shows the molecular interactions between cellulose, XyG, pectins, and wall proteins. A three-dimensional molecular model of the Type II wall shows the molecular interactions between cellulose, GAX, pectins, and aromatic substances (Carpita and McCann, 2000).

The third independent network consists of the structural proteins or a phenylpropanoid network. Evidence for these networks comes partially from direct imaging of walls.

The walls of most dicotyledons and the noncommelinoid monocotyledons contain about equal amounts of XyGs and cellulose. These kinds of wall we denote as Type I walls.
In Type I walls, the cellulose-XyG framework is embedded in a pectin matrix that controls, among other physiological properties, wall porosity. Type II walls of commelinoid monocots contain cellulose microfibrils similar to those of the Type I wall; instead of XyG, however, the principal polymers that interlock the microfibrils are GAXs (Carpita and McCann, 2000).

Cell wall enzymes

Cell walls contain numerous enzymes capable of hydrolyzing the major components of the wall matrix, and it is attractive to think that some of these enzymes might function to loosen the wall by breaking load-bearing links between cellulose microfibrils, thereby allowing the wall to extend
Alternatively, wall hydrolytic enzymes might stimulate wall extension indirectly: by reducing the size and viscosity of matrix polymers, such enzymes could act synergistically to enhance the action of primary wall-loosening agents, such as expansin. A third alternative is that such hydrolytic enzymes have functions unrelated to wall loosening, e.g. in defense, in signaling, or in polysaccharide processing or breakdown to serve the cell's other metabolic or energy needs (Cosgrove, 1999).
The most copious RNAm transcript, after cell wall modification, are resulted to be the one that encoded for polygalacturonases and pectin methylesterases, and others for α and β galactosidase , pectinliase and cellulase (Nunan et al. 2001).

Pectin metilesterasi (PME, E.C. 3.1.1.11)
Pectin methylesterases (PME) catalyse the demethylesterification of cell wall polygalacturonans. In dicot plants, these ubiquitous cell wall enzymes are involved in important developmental processes including cellular adhesion and stem elongation(Micheli 2001).
PME is also produced by several types of fungi, most of all pathogen as B. cinerea (Lee et al., 1979).
PME acts on structural polysaccharides of cell wall catalyzing the hydrolysis of methyl groups in position six of galacturonic acid chains, releasing free methanol.

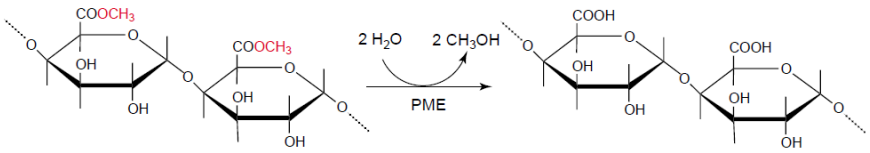


Fig.3.3.1 PME hydrolysis of methyl groups.

Once polymerized by PME, pectin could be attacked by polygalacturonase (PG) causing depolymerization and consequently the loss of consistence during fruits ripening.

it has been shown that the several PME isoforms detected in cell walls are encoded by a multigene family (Micheli, 2001). The study of this several PME isoforms has shown that they differed each other by molecular mass, optimal pH, optimal temperature and isoelectric point.It has also demonstrated that most of PME isoforms acts on neutral or alkaline pH (Bordenave, 1996) even though some of them seems to prefer acid pH (Ren & Kermode, 2000).

The systematic sequencing of the Arabidopsis genome has revealed the presence of the 67 PME-related genes in this species(Micheli,2001). The PMEgenes encode pre-pro-proteins that have peptide motifs considered to be signatures of PMEs. The pre-region or signal peptide is required for protein targeting to the endoplasmic reticulum. The pro-PME is secreted to the apoplasm via the cis, medial and trans Golgi cisternae, and the trans Golgi network, and only the mature part of the PME (without the pro region) is found in the cell wall.

PMEgenes can be divided into two classes, according to the systematic sequencing of the Arabidopsis genom. Genes in the first class contain only two or three introns and a long pro region, and genes in the other class contain five or six introns and a short or nonexistent pro region; these two classes have been called type I and type II, respectively (L. Richard, pers. commun.). The type II sequences have a structure close to that of the PMEs identified in phytopathogenic organisms (bacteria, fungi) and are involved in cell wall soaking during plant infection. Such data force us to consider the role and development of the PME pro region.

According to Markovic & Kohn (1984) this enzymes seems to have different types of action mechanisms, and it depends on the target, if they are acting on plant or on microorganisms.

In the first scenario, the alkaline isoforms of the enzyme operated linearly on the homogalacturonans causing the increase of free carboxyl groups that can interact with cations, especially with calcium.

Because acidic PMEs were thought to be essentially confined to fungi, the simplest hypothesis was that random demethylesterification depended on acidic PMEs, whereas linear

demethylesterification depended on alkaline PMEs. However, more recent studies have shown that PME activity also depends on pH and the initial degree of methylesterification of the pectins. Some isoforms can act randomly at acidic pH but linearly at alkaline pH. And, at a given pH, some isoforms are more effective than others on highly methylesterified pectins (Catoire, et al. 1998; Denès, et al. 2000).

The activity of this enzyme has shown to be influenced by cations concentration, which can influence the affinity for the substrate. Also, trivalent cations has revealed to have more efficacy than bivalents and monovalents cation (Micheli,2001). Therefore organic and inorganic cations content can affect PME activity, stability and its natural tendency to induce pectin aggregations.

Some studies (Lee et al., 1979; Barnavon et al., 2001) demonstrated that PME pH optimum is included between 7 and 7.5, it has also been shown how the enzyme activity rapidly raise up on Concorde grape variety(Lee et al., 1979) when pH increased from 5 to 7.5 and it decreases when pH goes further up.

Same trend was also confirmed on different species of tomato and citrus (Kertesz, 1938; Guyer et al., 1956).

In grape, Lecas and Brillouet in 1994 found that homogalacturonans methylesterification degree can change from 40 to 80% and it depends on cultivarripe stage and specific tissue; Vitis Vinifera species present lower activity when it is compared with Vitis Lambruscana and usually red grapes have higher activity than white grapes. As a consequence, methanol content in white wines is lower than red ones since PME and pectins are mainly located in grape skins (Lee et al., 1975).

Polygalacturonase (PG, EC 3.2.1.15)

Polygalacturonase (PG) are a class of enzymes involved on degradation of cell wall.

They usually catalyzed the hydrolysis of the α-(1,4) glycosidic bond of not-esterified galacturonic acids chains. Indeed, PG acts after methyl esterification of PME on pectins; releasing single unit of galacturonic acid molecules.

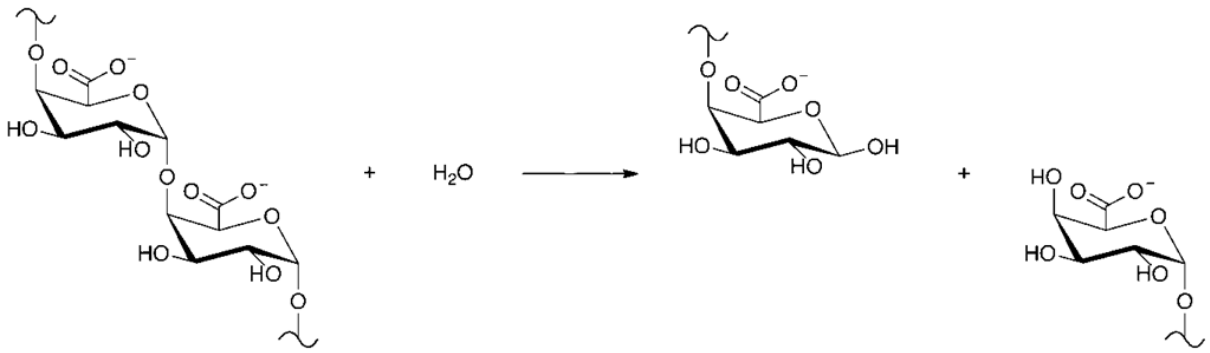


Fig.3.3.2 Releasing single unit of galacturonic acid molecules after PG action.

PG is also present on different types of plants pathogen bacteria, as *Erwinia carotovora* and *Ralstonia solanacearum* (Herlache et al. 1997), and in phatogen fingi, as *Aspergillus niger* (Bussink et al. 1992), when it is implicated on maceration and softening of plant tessues.

In grapes, the polygalacturonase are a multigenes family: the various isoforms are expressed in different tissues and respond to specific stimuli at different times. The grape is classified as a non-climacteric fruit because respiration is not fully and heavily influenced by ethylene, other substances such as acetic acid and abscissic acid seem to interfere much more on cellular respiration.

Deng et al. (2005) on grapes treated with different oxygen concentration (40% and 80%) and carbon dioxide (30%) demonstrated how those gases reduced the activity of cell wall enzymes (PME and PG).

The unbranched, non-esterified pectins can aggregate through calcium binding to form the junction zones that hold a gel together; pectin structure became more firm, similar to dense gel (Jarvis et al.,1984) causing limitation on enzymes mobility and activity.

Postharvest dehydration of wine grape

Grape postharvest dehydration or directly on the field, is a very ancient mediterranean technique.

Physical dehydration stars when saturation vapor pressure (SVP) inside the berry is different from the outside vapor pressure (VP); this difference is usually called vapor pressure deficit (VPD).

The water diffusion inside the tissue is a slow movement whose speed decreases as the relative humidity (RH) of the tissue decreases.

The formula for RH is:

(vapour pressure of air × 100)/vapour pressure at saturation point

For all practical purposes, it gives an indication of the capacity of air to pick up water.

The lower the RH of the environment, the faster the weight loss from the grapes, whatever temperature is used.

The main physical features (Mencarelli and Bellincontro, 2013) which affect water loss are:

- ratio of surface to mass or volume (berry size)
- berry size and shape
- berry skin surface
- micropores and cracks
- presence and physical condition of the rachid
- bunch density.

The main technical factors for grape dehydration are:

- initial moisture content
- sugar content
- berry volume
- berry surface area and surface-to-volume ratio (S/V) values (Barbanti *et al.*, 2008).

The environmental factors affecting water loss are mainly temperature, RH, and air flow.

Sunlight can be another factor for dehydration in open air but its role is a combination of infrared and UV radiation, which are not studied at all.

Bellincontro *et al.* (2009) have shown that the lower the temperature (with fixed RH and air flow), the better the grape structure during dehydration, and it is known that fruit pigment is more likely to remain intact with a lower dehydration temperature (Del Caro *et al.*, 2004).

When cellular structure of the berry is maintained, it's possible to obtain the following advantages: first, water stress can be postponed and be less traumatic; second, the secondary metabolism for the production of polyphenols (Mencarelli *et al.*, 2010) and volatile compounds can proceed slowly (Santonico *et al.*, 2010), releasing important compounds into the cell sap; third, less oxidation occurs; and fourth, slower aerobic fermentation takes place with lesser formation of metabolites dangerous for berry quality such as ethanol, acetaldehyde and acetic acid (Cirilli *et al.*, 2012).

General dehydration techniques

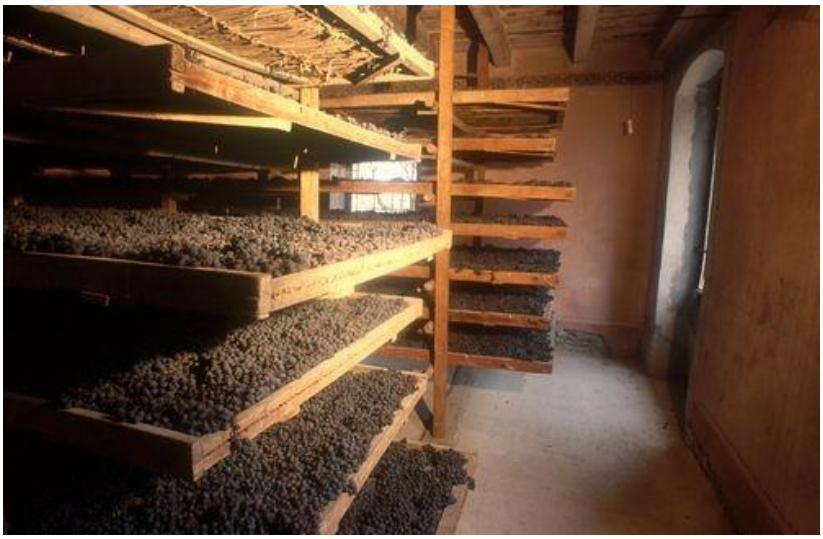
On-vine withering

In regard to on-vine withering, all the practices adopted have the purpose, directly or indirectly, of delaying the harvest of wine grapes.

In Italy, which is the country with the most varied typology of grape drying processes, on-vine withering is used for 17% of the total grapes treated (Fregoni, 2005). This technique is widespread and consists of leaving the grapes on the vine beyond the regular stage of technological ripening.

Natural withering

The general term of natural withering describes all the procedures of wine grape dehydration that are based on the favourable actions of the environmental conditions like direct grape exposure to the sun or to wind, together with the use of house garrets and house floors arranged for grape drying.



In Italy, natural withering covers 78% of the total drying procedures for grapes destined for wine production (Fregoni, 2006), considering that the specific techniques employed differ from country to country and from vineyard to vineyard.

Forced withering

The definition of forced withering is usually used to describe the grape drying procedures that take place in closed environments which frequently have technology installed for the partial or total control of air ventilation, RH and temperature (Fregoni, 2006).

Water loss: Important changes on wine grape

Water loss is responsible for large and significant changes in the metabolism of fruits and vegetables (Kays, 1997). With 0.5% water loss, cell wall enzyme activity is already increased, and a further increase in the rate of water loss accelerates respiration and ethylene production together with the loss of volatiles.

The over-ripening stage in on-vine berries leads to a magnification of the changes occurring during ripening, in particular concerning sugar concentration, organic acid evolution, aroma and polyphenol compound profiling.

That postharvest dehydration is effective in modulating berry metabolism through changes in gene expression was initially demonstrated by Versari *et al.* (2001) and Tonutti *et al.* (2004), who reported increases in the expression of stilbene synthase (*STS*) and phenylalanine ammonia lyase (*PAL*) genes, respectively.

Dehydration technology applied to wine grapes used to make dry and sweet wine has been seen to significantly increase the amount of the majority of phenols (Mencarelli et al., 2010) as suggest by the increased PAL activity.

Quality characteristics and volatile compounds of Malvasia, Trebbiano and Sangiovese (Bellincontro *et al.*, 2004) and Aleatico (Cirilli *et al.*, 2012) grapes changed markedly following different postharvest dehydration rates. These rates are also effective in modulating the activity of alcohol dehydrogenase (ADH) and lipoxygenase (LOX) enzymes and the expression of ADH and carotenoid cleavage dioxygenase 1 (CCD1) genes (Chkaiban *et al.*, 2007; Cirilli *et al.*, 2012).

Carotenoids content, express as lutein and β-carotene, were found to decreased during dehydration of Grechetto grape, while was stimulated the production of the two most important grape isoflavones, genistein and daidzein (De Sanctis et al., 2012)

Also, anaerobic metabolites such as ethanol, acetaldehyde, and ethyl acetate increase significantly when berry water loss reaches ~20% under closed, controlled, environmental conditions (Costantini et al. 2006),

PG increase its activity during grapes ripening (Cabanne e Donèche, 2001) and the same result has been shown for PME, with VvPME1 induction (Deitieux-Belleau et al., 2008). At the beginning of ripening was also observed an increasing of the VvPG1 gene expression, related to fruit softening and polysaccharide chains depolymerization, while the same behavior was shown onto the mesocarp during veraison.

Aims of the study

This PhD thesis research project is aimed to study metabolic, biochemical and structural changes on white wine grape, red wine grape and raisins using ozone post-harvest treatments without addiction of sulfites.

In wine production, sulfur dioxide is currently used for its positive effects on wine stabilization but it's even known its negative effects on grape and human health (Bush, 1986).

Recognized from US EPA (Environmental Protection Agency) as a safe agent for food contact, ozone was approved in 2001 by FDA (Food and Drug Administration) as GRAS (generally recognized as safe) for food sanitation and it started to be used in many food industry as natural alternative to chemicals disinfection process. The use of ozone in the food industry represent an important step forward towards technological innovations.

It has been found a significant rule in food manufactory and packaging industry for its efficacy on B. cinerea, S. enteritis, E. coli (Smilanick et al., 2010; Rodriguez-Romo and Yousef, 2005; Achen and Yousef, 2001).

Several authors (González- Barrio et al., 2006; Artés-Hernández et al., 2007; Cayuela et al., 2009) demonstrated the numerous benefits of ozone treatments on table grapes, increasing resveratrol and other bioactive phenolics. Defense pathways was also found to be stimulated by the gas (Srivastava 1999, Sharma and Davis, 1997).

Biosynthesis of phenilpropanoids, flavonoids and phytoalexins is elicited by ozone treatment, causing accumulation of different types of secondary metabolites as lignin and phenols (Saleem et al., 2001; Caban´e et al., 2004).

Several studies have been done and are going on to understand the multiple effects of O_3 treatment on table grapes but apparently no study has been done actually on wine grapes. A recent EU pending patent 12704901.3-1357 has been release for the production of wine without addition of sulfites, technology called Purovino®

Based on this technology, in this PhD research I present the results of different experimental studies conducted using ozone as postharvest treatment on different types of wine grapes even in combination with grape dehydration, in order to evaluate its effect on metabolites formation and texture changes.

Materials and Methods

Effects of ozone on grape cell wall, phenolic content and volatile organic compounds (VOCs)

Grape clusters variety Sauvignon Blanc (Castello de "La Sala" winery, Orvieto, Italy) and Cabernet Sauvingon (Fresno State University, California) were carefully hand harvested, selected and placed in a single layer into perforated crates (60 x 40 x 15 cm) until 6 kilograms Kg (±500 g) each, and divided into thermohygrometric controlled rooms (12 m^3) at 10 (±1)°C and UR 70%.

The O_3 exposure effects on Cabernet S. was studied on three different stages of grape ripening, 21,3°BRIX (unripe stage), one week later for 23,6°BRIX (ripe stage) and two more week later for 26°BRIX (overripe stage).

Fig.4.1.1 Cabernet sauvignon grapes after ozone treatments.

Cabernet Sauvignon grapes were treated with 300 (A), 800 (B) and 1000 (C) ppb of ozone (Purfresh® generator, Purfresh Inc, Freemont, CA) for 18 hours at 10°C and 70% RH.

The control sample consisted in grape bunch placed in another cold room without O_3 treatment.

Fig. 4.1.2 Thermohygrometric cells for multiples ozone concentration treatments.

For Sauvignon blanc the grape bunches were treated with Purovino treatment(European pending patent 12704901.3-1357 PC Engineering srl, Uggiate Trevano, Italy) immediately at harvest and after 4, 8 and 16 hours of treatment .

Fig.4.1.3 Sauvignon blanc grapes after ozone treatment (16 hours)

Ozone and Postharvest Dehydration

Red grape clusters variety Pignola, were carefully selected and placed in a single layer into 36 perforated crates (60 x 40 x 15 cm) until 6 kilograms Kg (±500 g) each, and divided into small tunnels, wich were placed in three thermohygrometric controlled rooms at 10 (±1)°C and 70%(±5%) of relative humidity and an air flow of 1.5 m/sec.

Fig. 4.2.1 Pignola grapes during dehydration.

The following treatments were performed:

- O_3 fumigation, 1.5 g/h in continuous flow (ozone generator A series, PC Engineering, Uggiate Trevano, Como, Italy) for 18 hours (A) followed by dehydration;
- O_3 fumigation, 1.5 g/h in continuous flow followed by dehydration with 0.5 g/h of O_3 fumigation for 4 hours (B), every day, during dehydration;
- control treatment (CK) without ozone fumigation for 18 h and then dehydration

Sampling was performed at the beginning and then at 20 and 35% wl. Mycological tests were also performed to ensure the efficacy of ozone treatment during long term proofs.

Fig. 4.2.2 Pignola cluster at 35% of w.l

Chemical analysis

Weight loss and Sugar content

Weight was carefully measured using a technical balance (Adam Equipment Co. Ltd., Milton Keynes, U.K.), and the SSC of the juice obtained from the berries was measured using a digital refractometer (Atago, Tokyo, Japan).

Titratable acidity

Titratable acidity (TA) was measured by titration of 5 g of juice obtained from grape berries, to pH 8.1 with 0.1 N NaOH, using phenolphthalein as a colorimetric indicator.

Color

Berry colour was assessed at the beginning of the experiment and at 20% and 35% weight loss with a CM-2600d colorimeter (Konica Minolta, Ramsey, NY) set at SCE (specular component excluded). Each berry was measured twice according to CIELAB coordinates L, a, and b. The hue angle (h°) was calculated as h° = arctan (b/a).

Respiration rate

500 g of fruits for each replicates were placed inside a 1L air tight glass containers equipped with a septum for sampling oxygen and carbon dioxide; analysis were carried out in triplicate, following the trend of the same fruits throughout the tests.

The rate of CO_2 production was monitored by gaschromatographic method using a GC Clarus 400 (shincarbon ST 80/100 column, TC detector, Perkin Elmer). The activity was expressed as mL of CO_2 per grape kilogram in one hour.

Total carotenoids

Total carotenoids expresses as μg/ml extract solution of xanthophylls and carotenes per mg of dry weight were extracted using a solution of acetone with 20% (v/v) water (Lichtenthaler H.K., 1987). After 30 min in the dark , the samples were centrifuged using a BECKMAN JA-21 at 6000 rpm for 15 min at 10°C and subsequently spectrophotometrically analyzed by a Perkin Elmer Instr. (Lambda 25 UV/VIS Spectrometer).

Total phenolc compounds

The Total phenols content was determined according to the Folin- Ciocalteu spectrophotometric method (Di Stefano & Cravero, 1991). 200µL of extract, 1mL of the Folin- Ciocalteu reagent and after 5 minutes, 4 mL of 10% sodium carbonate solution were added. This method is based on oxidation of phenols by the Folin-Ciocalteu reagent and a color change of the reagent (from yellow to blue), which is monitored by measuring absorbance at 750 nm. The final results were expressed as milligrams of catechins per kilogram of dry weight. Total anthocyanins were evaluated according to Di Stefano & Cravero, 1991 method.

Specific Phenolic content

The pedicels were removed and the berries were manually skinned. Skins and flesh were separated using a scalpel, berry by berry.

Skin and pulp samples were extracted with methanol slightly agitated, for 4 hours in the dark and analyzed by Hplc system (Agilent Technologies, equipped with LiChrospher C18 column, 4x250mm, 5µm particle size) with three solvent pumps and a diode array UV-visible detector was used, as described by Ritchey and Waterhouse in 1999. For detection and quantification of compounds, the chromatograms were recorded at 280, 316, 365 and 520 nm. All extractions for each grape sample were performed in triplicate

Volatiles compounds

Volatile and glycoconjugated organic compounds (VOCs) were extracted from fresh berries in triplicates (100 berries each) by solid phase extraction (SPE) using packed cartridges and aglycones of the glycoconjugated forms were liberated using glycosidase enzymes and then identified and quantified using a Thermo GC-MS according to Matarese et al.,2013. Volatile compounds were tentatively identified by comparing the mass spectra with those available in the data system library (NIST 08, National Institute of Standards and Technology, Gaithersburg, MD, 2008) and using published retention indices (Mateo et al. 1997). Positive characterization was achieved when a volatile compound was identified with a probability of >70% in at least three independent samples.

Mycological study

After each treatment fungi on grapes were determined by enrichment culture in liquid media followed by plating on agar media. Individual grape berries were randomly and aseptically

removed from each cluster o bunch to get a sample of about 50 g . Grape Berries were suspended in 90 mL of sterile 0.1% Bacteriological peptone (Oxoid, Milan, Italy) solution containing 0.01% Tween 80 (Sigma, Milan, Italy), for 30 mi at 25°C. Rinses were serially diluted in 0.1% Bacterial Peptone solution and spread onto mal extract agar (MEA (Pitt and Hocking, 1997) and YEPD medium (Valero et al, 2007). The latter was used to count yeasts, while MEA was used to enumerate general fungal community. All media were supplemented with 100 mg/L chloramphenicol (Sigma, Italy) to inhibit bacterial growth. Plates were incubated at 28 °C for 5 days for colony development.

Cell wall analysis

Grape cell wall isolation was performed as described by Vicente et al. in 2007.

Skin and pulp were immediately placed in 300mL 95% (v/v) Ethanol, homogenized in a Ultraturrax and boiled to ensure enzymes inactivation.

The filtered material was sequentially washed with 100 mL of ethanol, followed by 100 mL of methanol: chloroform (1:1 v/v) and 100 mL of acetone, and finally air dried at 35°C overnight.

NS, UA and Cellulose measurement

2 mg of dry cell wall were hydrolyzed, (based on Albersheim et al., 1967 and Blakeney et al., 1983) with 0.5 mL 2N trifluoroacetic acid (TFA) containing 100 µg/mL myoinositol (as internal standard) at 120°C for 1 hour. The dried TFA samples were washed with methanol and the supernatant was collected and blow dried for sugar content measurement while TFA-insoluble pellet were saved for cellulose contents assay.

After reduction with 100µL 1N NH_4OH containing 20 mg/mL of $NaBH_4$, samples were acetylated with 200 µL of acetic anhydride and 20µL of 1-methyl-imidazone, chloroform washed and blow dried.

Fig. 4.4.1 Reduction phase of sugar grape extracts with 100µL 1N NH_4OH containing 20 mg/mL of $NaBH_4$.

The dried chloroform phase was then re-suspended in 200µL of acetone just before GC analysis.

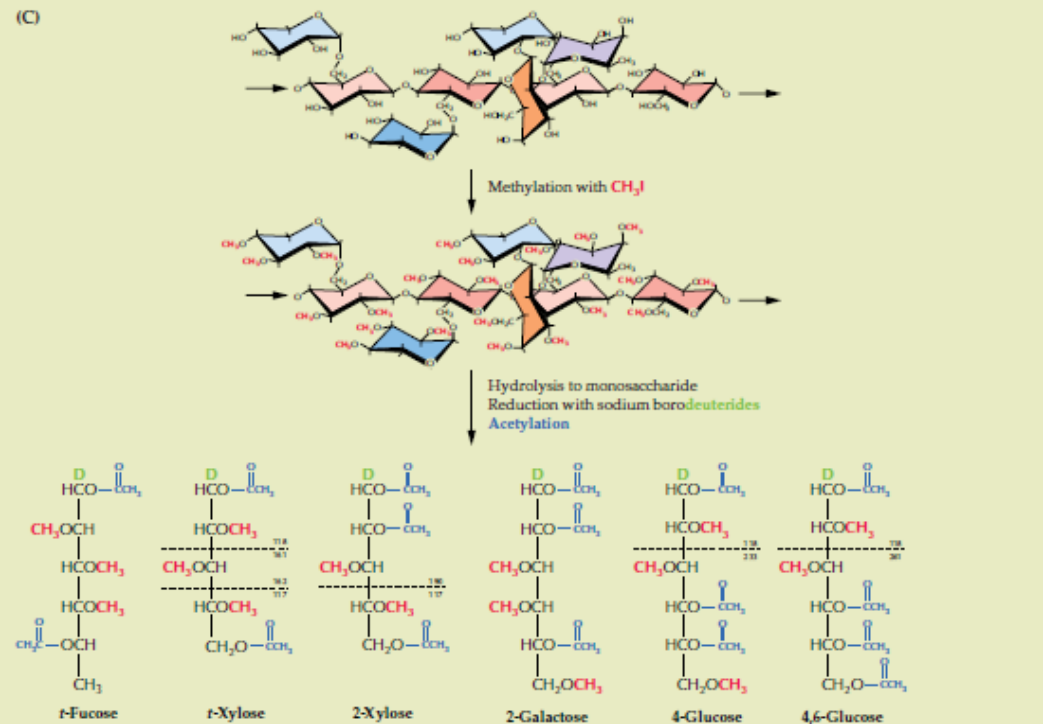


Fig. 4.4.2 Different phases of neutral sugars method of analysis at GC (Carpita and McCann, 2000)

Cellulose measurement (based onDische,1962) were performed by spectrophotometric method.

The 2N TFA-insoluble material were dissolved in 2 mL 67% H2SO4 and boiled for 8 min with 1mL of 2mg/mL anthrone powder dissolved in conc. H2SO4. The absorbance was determined at 620nm.

Uronic acid were measured according to Ahmed & Labavitch, 1977. The A520 of 200µL cell wall sample, after reaction with 1.2mL of sodium tetraborate and 20µL of metaphenylphenol were recorded. Three independent replicates for each samples were analysed and measurements were done in triplicates. UA content were evaluated against standard curve of polygalacturonic acid prepared simultaneously with the samples.

Fig. 4.4.3 Uronic acid standards.

Enzymatic assays

PME activity was evaluated by applying Hagerman (1986) method, with some modifications. The method is based on the color change of a pH indicator, bromothymol blue, during the PME reaction. The production of acid groups, as a consequence of the ester bonds hydrolyzation, causing a pH decrease and a color change of the indicator (from blue to yellow). The continuous color change was constantly monitored spectrophotometrically, and the initial rate of the reaction is set. All of the reagents without any specific references are from Sigma Aldrich Co. 4.5 g of powder for each sample, obtained from frozen and ground grape berries, was suspended in 5 mL of 3mM phosphate buffer (7.5 pH), contained NaCl 8.8% w/v, PVPP 1% w/v and 1mM EDTA. The homogenate was centrifuged at 20000 rpm for 10 min at 4 °C. The reaction mixture contained 2 mL of pectin citrus 0,5% w/v, 150µL of bromothymol blue 0.01% w/v (prepared in 0.003 M, pH 7.5, potassium phosphate buffer) and 60 µL of extract. All solutions was carefully adjusted to pH 7.5 with NaOH and checked just before the assay. Enzyme activity was measured at 620 nm, with a Lambda 3B UV-vis spectrophotometer (Perkin-Elmer Instruments Ltd., Seer Green, Beaconsfield, U.K.) against water. The enzymatic activity was express as µmol/min*g of fw

PG activity was measured by applying Lohani et al.'s method (YEAR), with some modifications.

Five grams of powder, obtained from frozen and ground grape berries, was suspended in 5 mL of 50mM sodium acetate buffer (4.5 pH) contained EDTA 1mM, 5% w/v PVPP, 0.5 M NaCl and 2% w/v PeG 8000 . The homogenate was centrifuged at 17000 rpm for 1 hour at

4°C. The reaction mixture contained 1.2mL sodium acetate (200mM, pH 4.5), 1.2mL of polygalacturonic acid (PGA, 0.5% aqueous solution adjusted to pH 4.5) and 0.4mL of enzyme extract in a total volume of 2.8mL. The mixture was incubated at 37 ◦C for 1 h and then followed by addition of 20 μL of DNS (dinitrosalicylic acid). The reaction was terminated by heating the reaction mix in a boiling water bath for 15min. The DNS reagent, composed by dinitrosalicylic acid, Rochelle salt, sodium bisulfite, phenol and sodium hydroxide, reacted with reducing sugar to form 3-amino-5-nitrosalicylic acid, which absorbs light at 540 nm. The difference between the A_{540} of this mixture and the A_{540} of control tubes, in which the substrate was added after the heat treatment, was recorded as ΔA_{540}. The enzymatic activity was express as μmol/min*g of fw.

Results and discussion

Effects of ozone on wine grape: var Sauvignon blanc

Chemical analysis

The application of O3 (European pending patent 12704901.3-1357 PC Engineering srl, Uggiate Trevano, Italy) on white wine grape was studied on Sauvignon blanc grape accurately harvested at 20°BRIX.

Sampling occurred after 4, 8 and 16 hours but no difference in sugar content was found after each time of sampling. Similar results were reported in Napoleon table grapes continuously exposed to 0.1 ppm of O3 for 38 days (Arte´s-Herna´ndez et al. in 2003), in Autumnseedless table grapes in macro-perforated polypropylene container and treated with 0.1L L−1 of O3 (Art´es-Hern´andez et al.2004) and in grape juice ozonated with processing variables of ozone concentration (1.6–7.8% w/w) from 0 to10 minutes (Tiwari et al., 2009) where color parameters, firmness, total SSC, pH and TA remained quite constant.

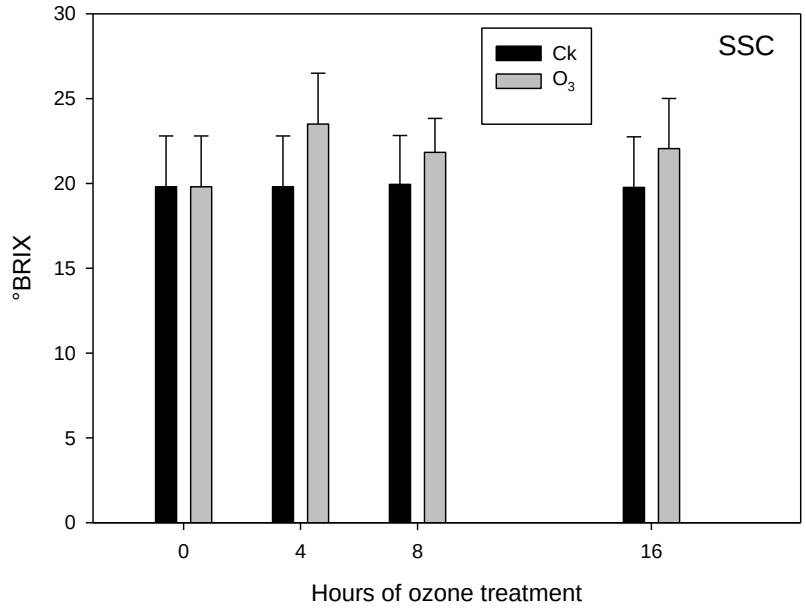


Fig.5.1.1 Sugar content after O3 treatment in Sauvignon blanc grape.

A similar result was found for titratable acidity (TA).

In accordance, no significant effect of ozone on TA was reported by Perez, Sanz, Rios, Olias, and Olias (1999) for the storage of strawberry. Low-level ozone enrichment has been reported to have no effect on organic acid composition of treated tomatoes and citrus (Smilanick,

2003). In general, levels of acids decline during ripening, presumably due to their consumption in respiration.

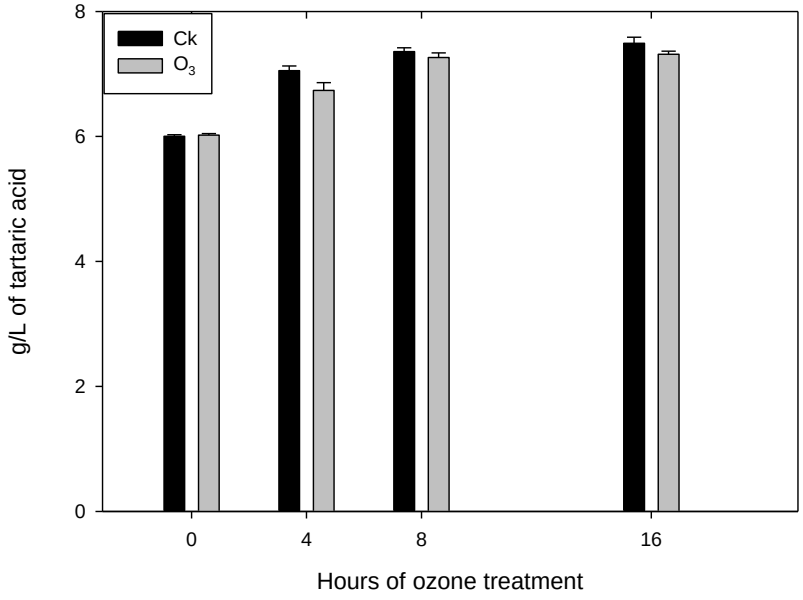


Fig5.1.2 Titratable acidity (TA), expressed as g per liter of tartaric acid in Sauvignon blanc grape

CO_2 production rate decreased in the first 4 h in ozone-treated and control samples due to the lower temperature; then after 12 h, when the tissue are acclimatized, a significant increase in both samples occurred; anyway, after 16 h the ozone-treated sample showed higher CO_2 production rate.

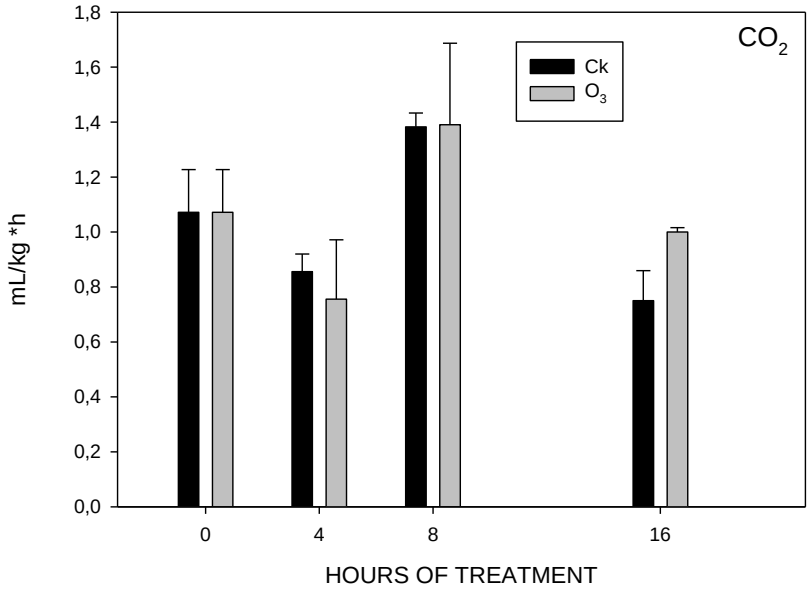


Fig.5.1.3 Respiration rate of Sauvignon blanc grape after O3 exposure

Grape color was unaffected by ozone treatment. Changes of color due to ozone was not observed in Strawberries cv. Korona by Keutgen and Pawelzik in 2008, but ozone may cause physiological injury of produce due to its strong oxidising activity.

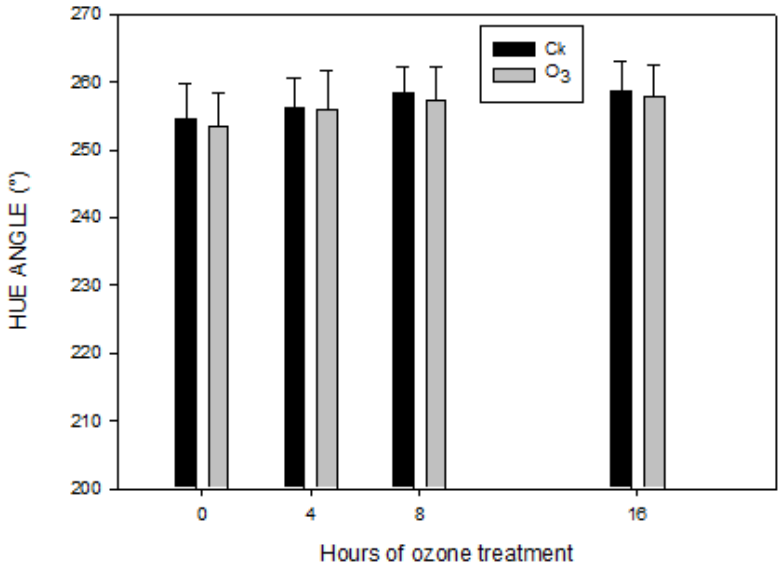


Fig.5.1.4 Color change express as hue angle. Data represent the mean of 30 berries (two readings for berry) for each thesis. Vertical bars indicate SE

For instance, bananas treated with ozone developed black spots after 8 days of exposure to 25–30 ppm gaseous ozone (Parish et al., 2003). This was not our case, indeed no harm to the rachis or browning to the berries were found.

Despite what previously studies suggested (Iglesias et al.,2006; Calatayud and Barreno, 2004; Francini et al., 2007;Ali et al., 2014), no differences were found for carotenoids content after ozone exposure.

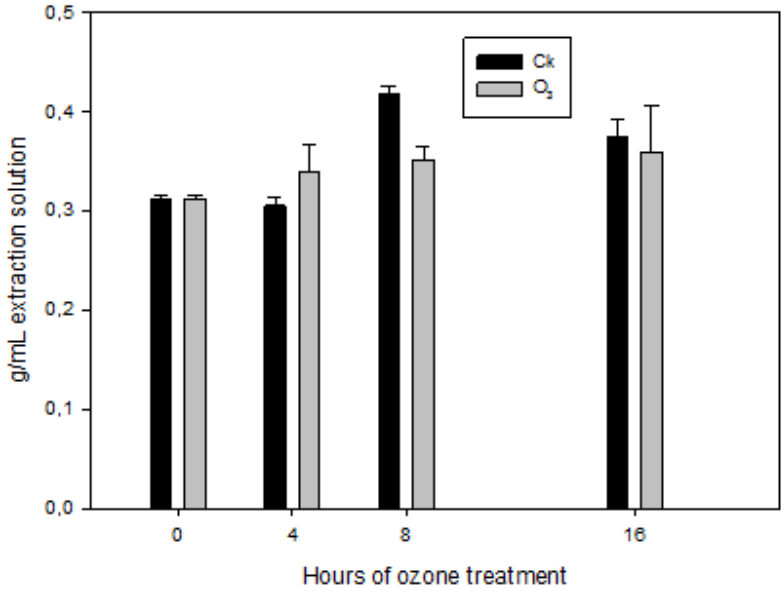


Fig.5.1.5 ***Carotenoids content*** *expressed as µg of Xanthophylls and carotenes/ml of extract solution *mg dw.*

Sauvignon blanc phenolic content shown an increase of 12% after 8 hours and a 5% more after 16 hours, indeed from the initial value of 359 mg/kg, a final concentration of 416 mg/kg was reached (fig).

Quantitative analysis of phenolic fraction shown as caftaric and coutaric acid are positively affected by ozone whereas catechin and epicatechin are resulted to be more susceptible.

Sgarbi et al., in 2003 demonstrated how phenol metabolism is differentially affected by ozone in two cell lines from grape leaf. Indeed, they found out that caffeic acid, ferulic acid and gallic acid increased when ozone is applied for different hours while catechins drops.

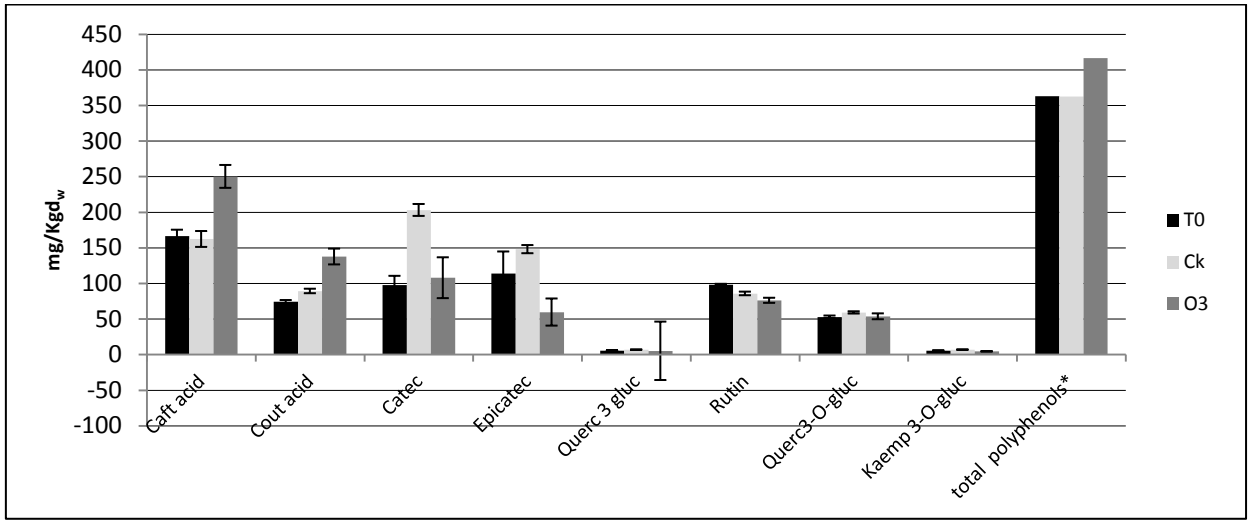


Fig.5.1.6 Quantitative analysis of polyphenols of Sauvignon blanc after 16h of O_3 treatment

Analysis of aromatic potential of S. blanc grape showed significant variation among the thesis. In fig.5.1.7 (A and B) are reported the most evident variation of aromatic compound we found.

Ozone treatment, from a general overview of the total VOCs, increased the glycosilated form. The presence of glycosilated form is important for vinification because the yeast breaks the bound and make free the odorant compound.

Glycosilated volatiles organic compounds (VOCs) increased significantly in Sauvignon blanc treated samples expecially benzyl alcol, methoxyeugenol, Coniferol 2, 7-OH a-terpineol and Isogeraniol; while decreased in the untreated one.

The increase of benzyl alcohol, coniferol, isogeraniol, terpineol in ozone-treated berries provides floreal and balsamic notes which in glycosilated form are unexpressed but, during fermentation, will be released in the must and wine.

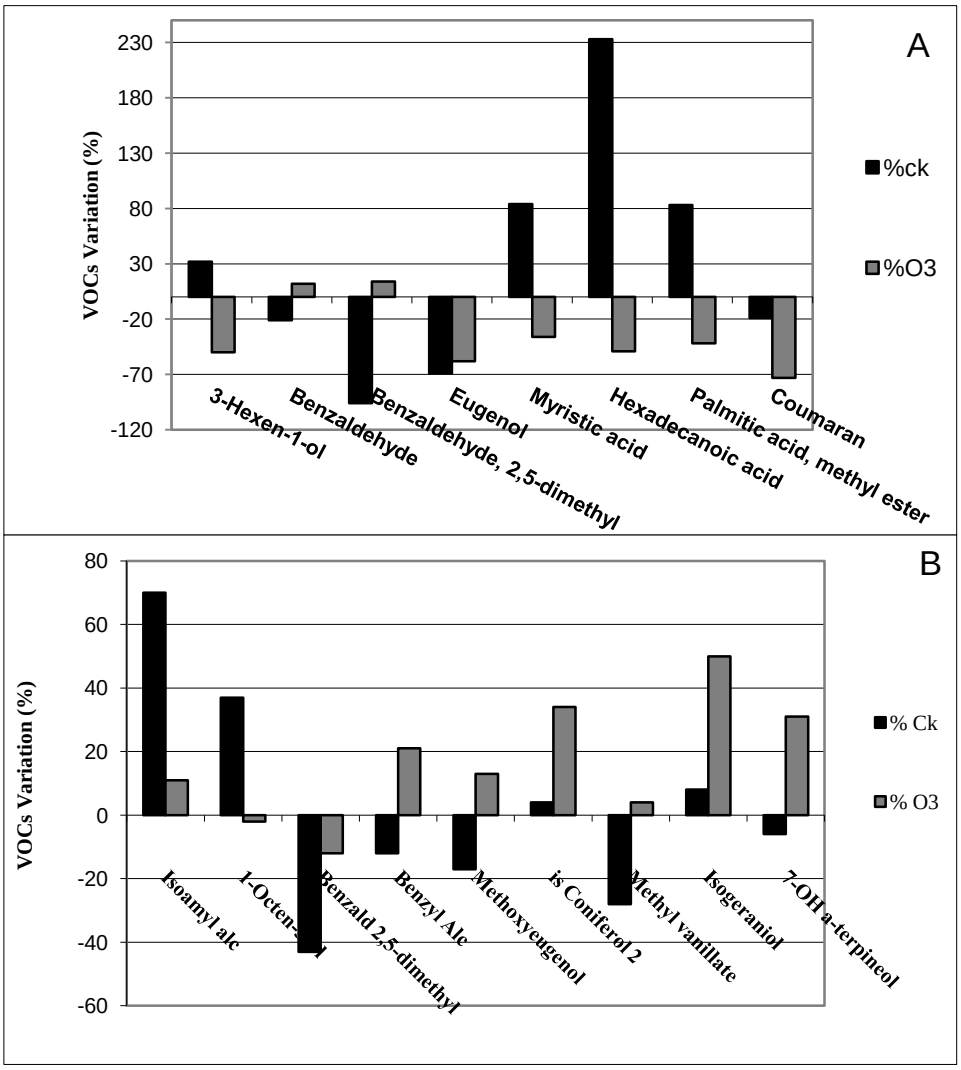


Fig.5.1.7 (A) % of Sauvignon blanc free (VOCs) after 16h of O3 treatment. (B) % of Sauvignon blanc glycosilated(VOCs) after 16h of O_3 treatment

Free VOCs, whom are perceptible directly from the berry, as expected after ozone treatment fallen, while the untreated sample shown a marked increase of volatile fatty acid derived from cellular membrane (Hexadecanoic acid, Myristic and Palmitic acid) whom bestow to the berry the unpleasant flavor of fat. 3-hexen-1-ol comes from the oxidation of fatty acid, thus

the increase of all these membrane related compounds in control berry, indicates an oxidation process.

Thiols, typical compounds in Sauvignon blanc grape, as 4-mercapto-4-methylpentan-2-one, 3-mercaptohexyl acetate, 4-mercapto-4-methylpentan-2-ol, 3-mercaptohexan-1-ol and its acetate, and 3-mercapto-3-methylbutan-1-ol, which are usually present in low concentration did not have shown significant changes after treatment.

Effects of ozone on grape cell wall and phenolic compounds

Phenolics content

HPLC analysis of Cabernet Sauvignon polyphenols showed an increase of flavonols when grapes were treated at 21.3 ºBrix with A and B ozone if compared to untreated one; moreover, when B conc was used an increase of anthocyanins was recorded. At 23 ºBrix, as shown in table 5.2.1 , the use of C conc of O_3 had a negative effect. At the final harvest, all ozone concentrations reduced flavan-3-ols content of the skin while an opposite trend was shown by pulp. Total cinnamates seems to be less sensitive when grapes were treated at an early stage whereas, at both the other stages, they were lost. Flavonols have shown a favorable effect, almost doubling their content when grape was treated with A conc at the an early stage. Total anthocyanins resulted to be the less sensitive to the treatments, indeed, an increase was shown for the early grape exposed to B conc of ozone, while the others samples remained quite stable after the exposure.

Table 5.2.1 Cabernet Sauvignon ***polyphenols HPLC analysis.*** *The results, expressed as mg/L, are the mean of three replicates.*

	tot flavan-3-ols		**tot cinn**		**tot flavonols**		**tot antho**	
21.3BRIX	skin	pulp	skin	pulp	skin	pulp	skin	pulp
ck	14.21 b	8.11 b	50.89 a	24.45cd	73.08 c	1.93 a	127.79 b	0.28 b
O_3A	12.9 c	7.55 c	40.92 b	27.68 c	134.57 a	1.6 b	125.37 b	0.35 ab
O_3B	12.45 cd	7.34 c	49.57 a	28.44 c	90.14 b	1.6 b	142.21 a	0.35 ab
O_3C	13.14 b	6.78 c	51.18 a	18.18 d	72.72 c	1.67 b	129.56 b	0.34 ab
23.6BRIX								
Ck	11.68 d	8.25 ab	27.18 c	37.93 a	85.65 b	2.2 a	106.23 c	0.33 ab
O_3A	13.98 bc	9.07 a	28.07 c	36.91 a	78.01 bc	2.01 a	104.79 c	0.33 ab
O_3B	12.71 c	8.74 ab	28. 45 c	33.11 b	72.82 c	2.31 a	103.36 c	0.32 ab
O_3C	11.95 d	8.7 ab	24.95 cd	31.8 b	63.18 d	1.98 a	79.68 d	0.29 b
26BRIX								
Ck	16.74 a	7.6 c	29.82 c	22.28 cd	66.55 cd	1.37 c	103.71 c	0.27 b
O_3A	14.99 b	8.85 ab	30.06 c	31.03 b	65.09 cd	1.98 a	105.8 c	0.28 b
O_3B	14.46 b	9.37 a	23.01 d	31.63 b	75.26 bc	1.96 a	101.71 c	0.53 a
O_3C	14.06 b	9.07 a	19.59 d	31.28 b	71.23 c	1.35 c	85.65 d	0.37 ab
LSD 5%								

Studies have shown that phenylpropanoid metabolism can be stimulated by O_3. For example, the activity of phenylalanine ammonia-lyase (PAL), the first enzyme in the general phenylpropanoid pathway, increased in soybean (Tingey et al., 1975), Scots pine (Pinus sylvestris L.) (Rosemann et al., 1991), and parsley (Petroselinum crispum L.) (Eckey-Kaltenbach et al., 1994) soon after treatment with 150–200 nmol O_3 mol^{-1}.

Phenolic compounds is reported to accumulate in leaf tissue in response to O_3 included hydroxycinnamic acids, salicylic acid, stilbenes, flavonoids, furanocoumarins, acetophenones, and proanthocyanidins (Howell and Kremer, 1973; Keen and Taylor, 1975; Hurwitz et al., 1979; Rubin et al., 1983; Kicinski et al., 1988; Sandermann et al., 1989; Jordan et al., 1991; Eckey-Kaltenbach et al., 1994; Yalpani et al., 1994; Booker et al., 1996).
Soybean plants grown for 6 weeks in charcoal-filtered (CF) air and then treated with CF air plus 100 nmol O_3 mol^{-1} 7 h daily for up to 13 d in chambers in the greenhouse, showed an increased levels of cell-wall-bound total phenolics, acid-insoluble lignin and lignothioglycolic acid (LTGA) extracted from leaf on average by 65%. The activities of general phenylpropanoid pathway enzymes (phenylalanineammonia-lyase and 4-coumarate5CoA ligase) were stimulated by O_3 after 6 h (Booker and Miller, 1998). Also on wine grape the postharvest ozone treatment (Purovino technology) has been seen increasing significantly the polyphenols (Mencarelli F. et al., 2011).

Cell wall analysis result

The cell wall of grape berries have a common set of structural polysaccharides, with a cultivar-depending distribution. Previous studies have shown how cell wall composition consists primarily of cellulose, pectin and hemicelluloses. The presence of pectic polysaccharides could be inferred from the large amount of uronic acids, arabinose, galactose and rhamnose (Ortega-Regules et al., 2008) while the presence of glucose, xylose and fucose was indicative of the occurrence of cellulose and hemicellulosic polysaccharides.
Gas chromatographic analysis of neutral sugar content revealed glucose, galactose and arabinose as the most abundant sugars in both skin and pulp grape berries cell wall. Ozone treatment affected skin sugars composition and its action was not only dose dependent but above all it depends on the stage of grape berries development. For all the sugars, except glucose, when grapes were treated at an early stage an increment was found. At the ripe stage, berries are more sensitive to ozone concentration. Indeed, even though the content of rha, fuc

and xyl increased when grapes were treated with 800ppb of ozone, sugars tended to decrease and glu content decreased using C conc of O_3.

At the last stage of ripening most of the sugars, whether or not high ozone concentration were used, decreased their levels; glu showed a proportional response to ozone treatment: the higher was the concentration, the higher was glu content.

Table 5.2.2 Percentage of Cabernet sauvignon skins neutral sugars. Different letters within the same column represent significant differences according to LSD test. Rha Rhamnose, Fuc Fucose, Ara Arabonose, Xyl Xylose, Man Mannose, Gal Galactose, Glu Glucose.

	% of SKINS NEUTRAL SUGARS						
21,3 BIRX	Rha	Fuc	Ara	Xyl	Man	Gal	Glu
ck	7.60 c	2.09 b	23.48 c	7.13 d	6.60 c	17.70 d	35.41 a
A	10.41 a	2.89 a	24.80 b	9.75 b	9.25 a	24.04 a	18.86 d
B	8.60 b	2.45 a	23.46 c	8.22 c	8.24 b	24.19 a	24.83 c
C	7.52 c	2.89 a	24.81 b	9.53 b	8.66 ab	22.97 b	23.62 c
23,6BRIX							
ck	3.92 d	2.18 ab	27.09 a	8.88 c	6.95 c	22.43 b	28.54 b
A	7.07 c	2.41 a	25.90 b	9.31 b	5.28 d	21.43 c	28.60 b
B	6.93 c	1.57 c	23.69 c	9.32 b	6.67 c	20.82 c	31.01 b
C	7.76 c	2.86 a	28.02 a	11.06 a	7.18 c	21.59 b	21.53 d
26BRIX							
ck	10.33 a	1.92 b	20.69 d	9.48 b	9.03 a	18.98 cd	29.57 b
A	8.95 b	1.97 b	24.20 b	10.35 a	6.51 c	18.00 d	30.02 b
B	9.15 b	1.82 b	19.56 d	8.50 c	8.07 b	18.52 d	34.38 a
C	7.00 c	1.74 c	22.96 cd	8.36 c	6.46 c	17.54 d	35.95 a

Pulp sugars composition showed a different result from skin. As a matter of fact, treating grapes during an early stage decreaseed much all sugars content with some exceptions indeed glu, man and ara tended to increase when B or C conc of ozone were used.

The ripe and overripe stage showed less sensitivity to treatments.

% of PULP NEUTRAL SUGARS							
21,3 BIRX	Rha	Fuc	Ara	Xyl	Man	Gal	Glu
ck	10.37 a	1.49 a	26.70 c	10.10 a	5.33 a	27.79 c	18.21 c
A	8.30 b	nd	27.90 b	7.00 c	2.23 c	34.64 a	19.93 c
B	8.79 b	nd	28.06 b	6.94 c	5.57 a	30.65 b	19.99 c
C	6.76 d	0.66 b	28.04 b	6.28 c	4.86 a	28.77 c	24.62 b
23,6BRIX							
ck	8.37 b	0.95 b	26.54 c	8.01 b	3.98 b	30.91 b	21.23 bc
A	9.87 a	1.20 a	29.72 a	8.23 b	4.23 b	30.14 b	16.61 d
B	8.21 b	1.31 a	29.42 a	6.50 c	5.64 a	31.67 b	19.93 c
C	8.62 b	1.06 b	26.73 c	8.41 b	5.62 a	29.82 b	17.05 d
26BRIX							
ck	7.00 cd	0.54 b	24.27 d	5.02 d	5.45 a	26.24 d	31.48 a
A	7.66 c	1.36 a	23.11 d	6.41 c	4.11 b	28.85 c	28.49 a
B	7.03 cd	0.43 c	23.98 d	5.38 d	4.95 a	28.00 c	30.22 a
C	7.06 cd	nd	25.91 cd	6.09cd	4.77 ab	25.92 d	30.25 a

Table 5.2.3 Percentage of Cabernet sauvignon pulp neutral sugars. Different letters within the same column represent significant differences according to LSD test. Rha Rhamnose, Fuc Fucose, Ara Arabonose, Xyl Xylose, Man Mannose, Gal Galactose, Glu Glucose.

During fruit ripening process, a series of genetically-programmed biochemical events alters the characteristics of the unripe fruit from hard, green, acid-tasting tissue into one with an attractive appearance, sweet taste, fragrant aroma and soft texture (D.A.Brummell, 2006).

Cellulose and uronic acid in grapes showed a positive trend during ripening, increasing their levels of 6% and 24% respectively, while when grape undergoes the overripe stage, those cell wall constituent start to decrease of about 4% and 23%. Total neutral sugars instead, increased during all fruit stage of ripening of about 5.5%.

Cellulose and uronic acid content decreased at each ripening stage when ozone is used. Therefore, C conc of ozone seems to stop the loss of constituents, for both cellulose and uronic acid, as suggested by Aguayo et al. (2006) in tomato fruit treated with ozone where softening was delayed. An et al., 2007 reported that the levels in both cellulose and

hemicelluloses contents increased significantly during storage of fresh-cut green asparagus in 1 mg/l aqueous ozone.

Neutral sugars followed similar trend for early and ripe stage, while at overripe stage grapes are less sensible to the treatment. Pulp neutral sugars presented a different trend. When grapes were treated at an early stage with the lower ozone concentration neutral sugars tended to increase while at ripe and overripe stage the use of C conc of O3 caused a greater release of sugars.

	Cellulose[a]		UA[b]		Tot NS[*]	
21.3BRIX	Skin	pulp	skin	pulp	skin	pulp
ck	30.36 b	23.77 a	44.93 b	7.38 a	280.09 c	217.43 c
A	29.19cd	22.32 b	39.84 c	5.44 b	243.61 d	240.83 b
B	28.43 d	22.68 b	36.09 c	4.89 b	232.07 d	207.72 c
C	29.53 c	21.67 c	42.82 b	3.62c	237.74 d	221.62 c
23.6BRIX						
ck	32.33 a	21.56 cd	59.27 a	4.85 b	291.32 b	188.94 d
A	20.84 b	22.22 b	58.86 a	3.35 c	265.85 c	191.78 d
B	20.83 b	21.17 d	53.27 a	3.92 c	281.43 c	207.74 c
C	20.05 b	21.72 c	56.01 a	5.57 b	291.22 b	245.97 b
26BRIX						
ck	31.01 ab	21.53 cd	45.58 b	5.21 b	296.24 b	239.37 b
A	30.44 b	22.21 b	39.36 c	4.25 c	287.84 bc	232.07 b
B	29.016 cd	21.79 c	32.64 c	4.65 bc	303.88 a	205.07 c
C	29.10 cd	21.80 c	43.52 b	5.53 b	290.68 b	287.55 a

Table 5.2.4. ***Cabernet sauvignon cell wall compositions*** *after O_3 treatment calculated as % of a or b (uronic acid) per g^{-1} of cw. *NS total neutral sugars expressed as µg per g^{-1} of cw.*

The changes in cell wall structure and composition result from the composite action of hydrolytic enzymes produced by the fruit, namely, polygalacturonase(PG), pectinesterase (PE), b-galactosidase (b-GAL), pectate lyase (PL), and cellulase (Brummell & Harpster, 2001).

Changes in the cell wall composition and degrading enzyme activity have been reported in detail during the aging or ripening of other types of grapes (Ishimaru & Kobayashi, 2002; Nunan, Sims, Bacic, Robinson, & Fincher, 1998).

On Cabernet Sauvignon PME and PG activity are both affected by the ripening stage.

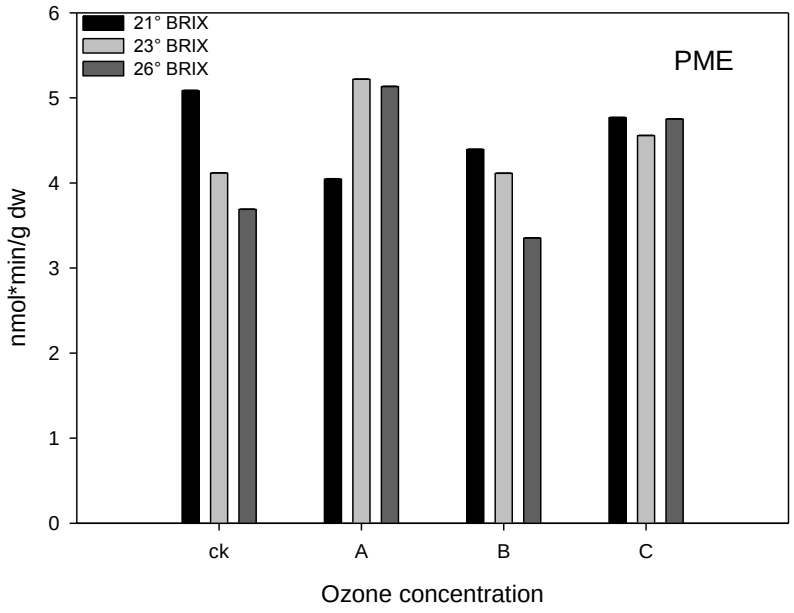


*Fig.5.2.1 Activity (expressed as µmol*min/g dw) of* ***pectinmethylesteras(PME)*** *during the test. Data represent the mean of tree 60 berries batch analysis of each sample. Vertical bars indicate SE.*

Pectin methyl esterase, after ozone exposure, responded increasing its activity when the grapes were treated at ripe and overripe stage while a decrease occurred when treated at an early stage. PME activity increased using A conc on ripe and overripe grapes while it reached its lower activity when the same concentration was applied to early grapes.

Similar trend for Polygalacturonase activity; the highest activity was reached using A conc O_3 on ripe grapes or using B conc on overripe grapes. Ozone exposure at an early stage was found to decrease the PG activity proportionally to the O_3 concentration.

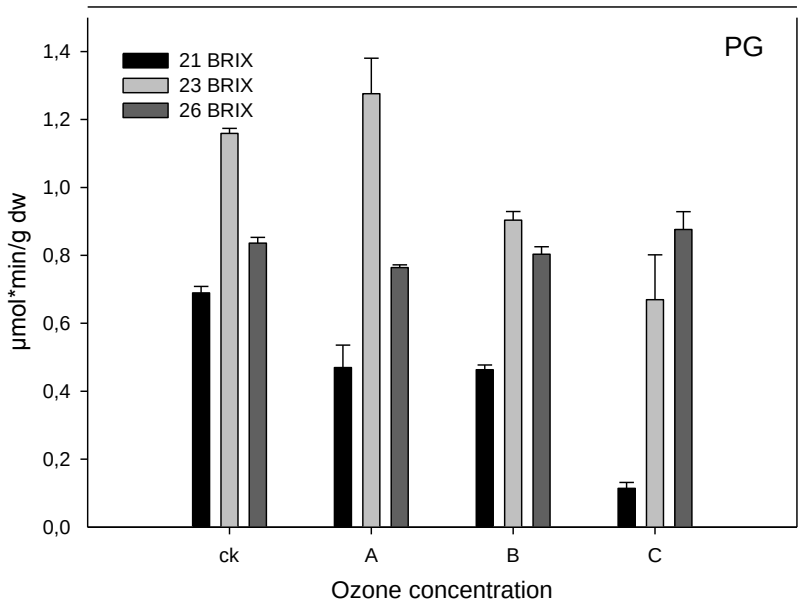


*Fig.5.2.2 Activity (expressed as µmol*min/g dw) of* ***polygalacturonase (PG)*** *during the test. Data represent the mean of tree 60 berries batch analysis of each sample.*
Vertical bars indicate SE.

In tomato, Aguayo et al. (2006) analyzed the effect of cyclic exposures to ozone founding that the treatments did not alter the activity of the pectin-degrading enzymes polygalacturonase (PG) and β-Galactosidase (β-Gal), while a clear decrease in pectin methyl esterase (PME) was found.

PG activity of kyoho grapes conserved in high oxygen atmosphere showed a significant rise even though the levels stopped increasing after 30 days (Deng et al., 2005).

Effects of ozone during postharvest dehydration of Pignola grapes

Chemical and metabolic changes due to the effect of drying procedures and ozone treatments have been studied on grapes treated with Purovino treatment (European pending patent 12704901.3-1357 PC Engineering srl, Uggiate Trevano, Italy) for 18 hours, then some of those were continuously treated with 0.5 g/h of O_3 for 4 hours/day and all the grapes were brought up to 35% of weight loss. Sampling was performed at the beginning and then at 20 and 35% of wl.

Chemicals analysis

Weight loss was almost linear as we verified in the plot of mass loss (kg of water/kg of dry matter) against dehydration time, which provided straight decreasing linearity with R^2 0,9615, 0,9685 and 0,9503 for control, 18 hours treated and nostop treated, respectively.

Palou at all. in 2002 confirmed that no differences in weight loss were found between 0.3 ppm ozone and control treatments on 'Flame Seedless' table grapes stored for 4 weeks at 5 °C and 90% RH.

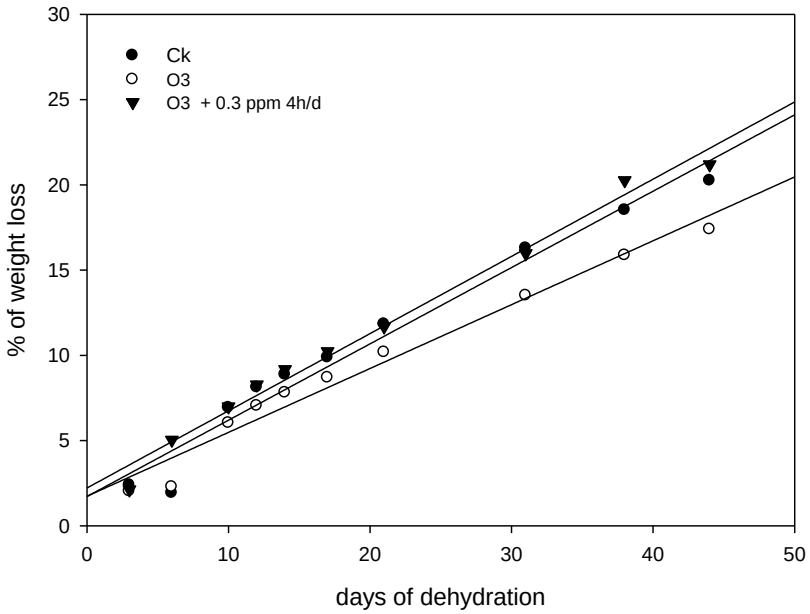


Fig 5.3.1. ***Dehydration kinetics*** *of Pignola grape clusters. Data represent the mean of 12 kg (± 1) of grape for each sample.*

The SSC increased linearly from 25,6°Brix to 35-38 °Brix without significant differences between the samples.

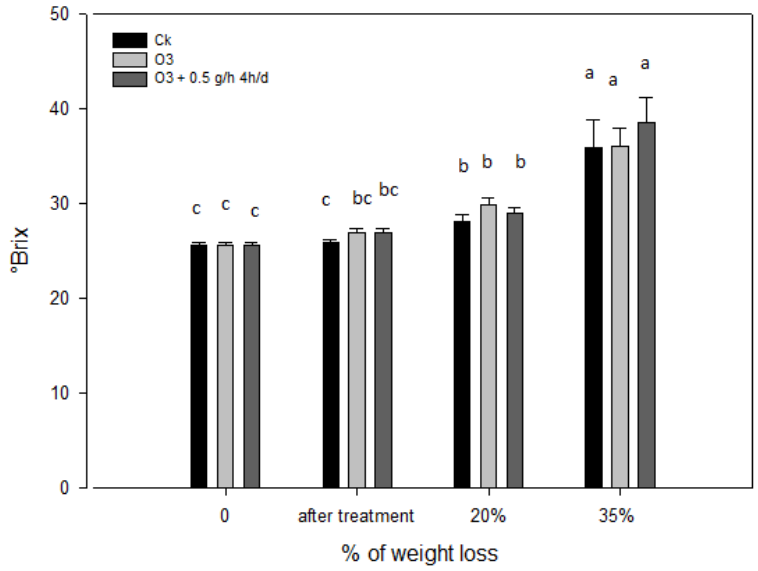


*Fig.5.3.2 Changes in **soluble solids content SSC**. Data represent the mean of 25 berries from different clusters. Vertical bars indicate SE*

Full-ripe tomato fruit exposed to ozone (0.05 or 1.0 mol mol−1) for between 1 and 6 days did not show any difference in soluble sugar content (fructose and glucose) and did not affect fruit weight loss, antioxidant status, CO_2/H_2O exchange, ethylene production or organic acid (Tzortzakis et al., 2007).

With respect to values at harvest, slight or no change in titratable acidity was found (fig. 3) until the 20% of weight loss.

During dehydration only fruits treated continuously with ozone shown a decrease in TA which became less evident at 35% of weight loss.

No change in organic acid composition was observed in ozone-treated tomato fruit, a finding at odds with the study of Aguayo et al. (2006), but consistent with the reported effects of ozone treatment on citrus (0.1 mol mol^{-1}; Smilanick, 2003), pear and apple (1.7mol mol^{-1}; Skog and Chu, 2001).

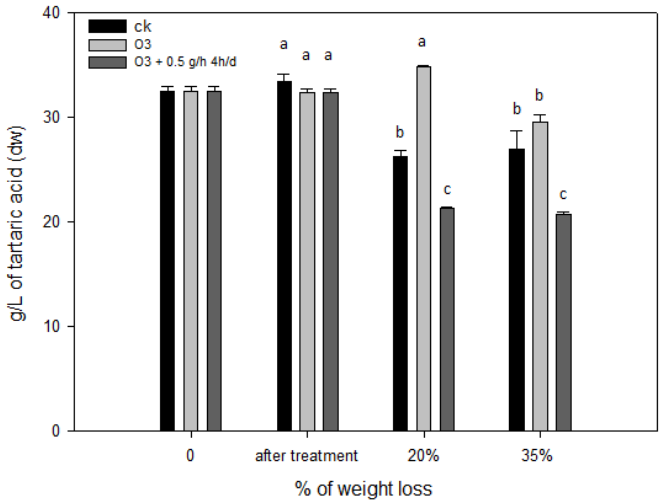


Fig.5.3.3 Changes in tritrable acidity. Data represent the mean of tree different readings. Vertical bars indicate SE. Different letters indicates significant difference between the treatments.

Kiwi fruits stored at 0 °C inside industrial ozone chambers for 29 weeks have shown a strong decrease in acidity content, especially for the two most abundant kiwi acids, citric and quinic acid but no effect was found for tartaric acid, the predominant acid in grape fruit (Barboni et al., 2010).

An increase in hue angle value was found in each thesis, however, no significant changes or browning for any treatment at any sampling time were found as suggested by several authors (Beltraän et al.2005, Arteäs-Hernaä ndez et al., 2003, Skong and Chu, 2001) .

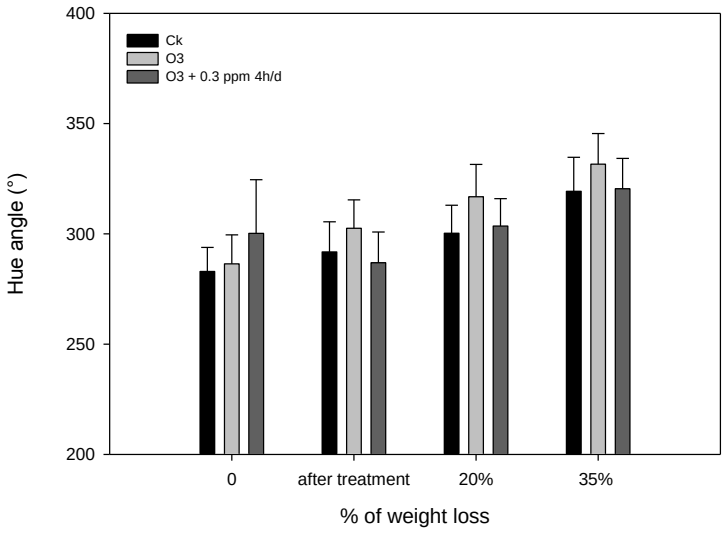


Fig.5.3.4 Color change expressed as hue angle. Data represent the mean of 30 berries (two readings for berry) for each thesis. Vertical bars indicate SE

This is in agreement with previous studies where washing fresh-cut lettuce using three different ozonated water dips [10, 20, and 10 activated by ultraviolet C (UV-C) light mg L-1 min total ozone dose] maintained the initial visual appearance and controlled browning during storage in air (Beltraä n et al., 2005) and with Ikeura et al.(2013) in which, bubbling OMCB treatment, had no effect on the colour and pulling strength of the persimmon leaves.

CO_2 production seems to slightly decrease after 18 hours of treatments but at the end of dehydration period no difference were found between treated and not treated grapes.
After dehydration, when compared to those at harvest, significant changes in CO_2 of all samples were found, related to the water loss stress as reported from Costantini et al. in 2006 , in which an increased of respiration rate was found in wine grapes(cv. Malvasia) starting from weight losses of about 10%, with maximum peaks reached in correspondence of 22%. No change was recorded among the theses. The high CO_2 value reached at 35% of wl are probably due to the change from aerobic to anaerobic metabolism in which glucose and malic acid are metabolized to ethanol and carbon dioxide (Romieu et al., 1992).

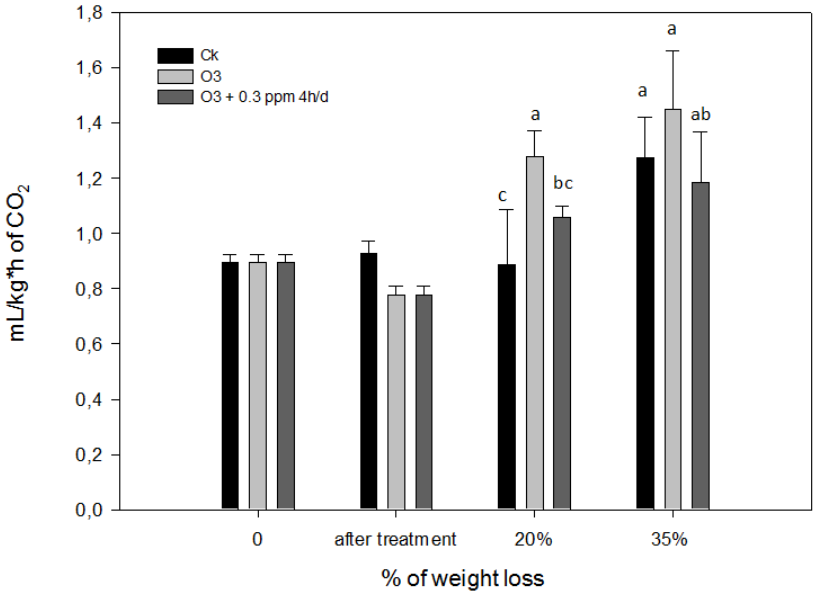


Fig.5.3.5 Respiration rate. The results represent the mean of tree different readings on six clusters for each thesis. Vertical bars indicate SE. Different letters indicates significant difference between the treatments

The initial value of carotenoids content determined in samples was 1,14 µg /ml of extract solution per mg $_{dw}$. After 18 hours of ozone treatment, a slightly increase in carotenoids content of treated samples was observed, without significant differences among the fruits.

Dehydration has been shown to have a negative effects on pigments, effects that became more evident at the end of dehydration time, especially for those berries treated all time with O_3.

Same results was found on Clementine mandarin leaves (Iglesias et al.,2006) where chlorophyll and carotenoids content was reduces as ozone concentration increased.

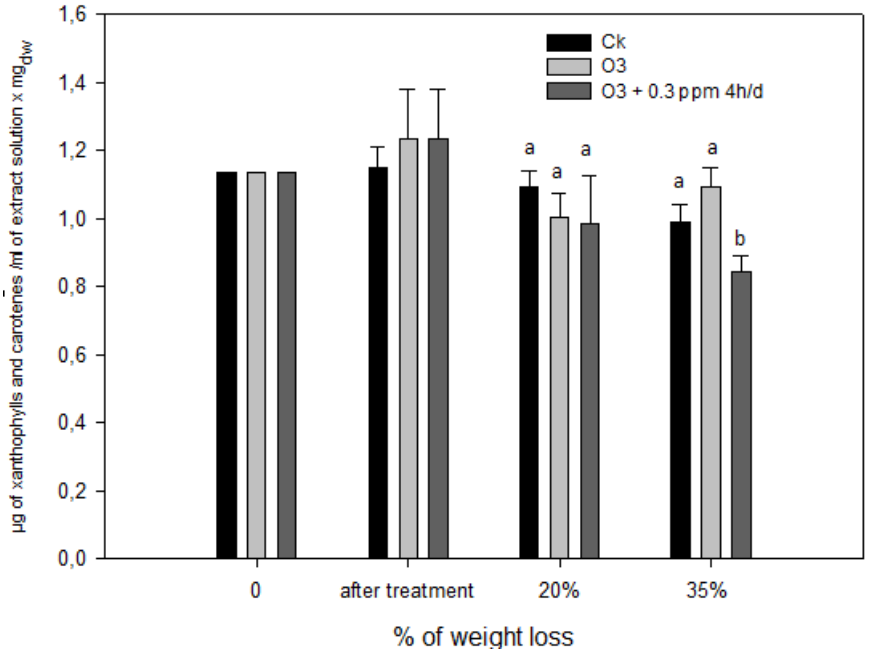


Fig. 5.3.6 ***Xanthophylls and carotenoids content*** *expressed as µg/ml of extract solution *mg dw. Vertical bars indicate SE. Different letters indicates significant difference between the treatments.*

In general, it has been shown that due to the high oxidative capacity of O_3 and its ability to generate toxic molecular species, it can acts as potent phytotoxic agent.

It elicits plant defense reactions such as the production of phytoalexins, including ascorbic acid (AA) in strawberries (Perez et al., 1999) and resveratrol in grapes (Gonzalez-Barrio et al., 2006). Vitamin C and phenolic compounds are able to scavenge oxygen radicals and avoid oxidative stress (Klopotek et al., 2005). Thus, changes in AA of O_3-treated strawberries can be attributed to the activation of an antioxidative system that promotes the biosynthesis of vitamin C from the carbohydrate pool (Perez et al., 1999).

Total phenolic content was observed to be unaffected by 18 hours of ozone exposure, however at 20 and 35% of w.l. it's interesting the combined effort of dehydration and ozone

gas. Indeed, a phenolic degradation occurs for all samples but while O_3 sample was able to kept an higher content of phenolics (respect to the others), grapes continuously expose to the gas shown a significantly ($p < 0.05$) 3 times lower value.

The behavior of phenolic molecules during dehydration is controversial. In some variety is well know that the use of low temperature (10°C and 20°C) until 20% of wl increase phenol content and some specific fractions of them (Mencarelli et al., 2010; De Sanctis et al., 2012). In other cases is reported how postharvest dehydration for more than 10-15% caused a phenolic degradation (Borsa and Di Stefano, 2000) or a decrease of specific fractions (Moreno et al., 2008). However, a general activation of phenylpropanoid pathway in dried samples has also been confirmed by the up-regulation of cinnamate 4-hydroxylase (C4H) and 4-coumarate-CoA ligase (4CL) genes (Zamboni et al. , 2008, 2010; Bonghi et al. , 2012).

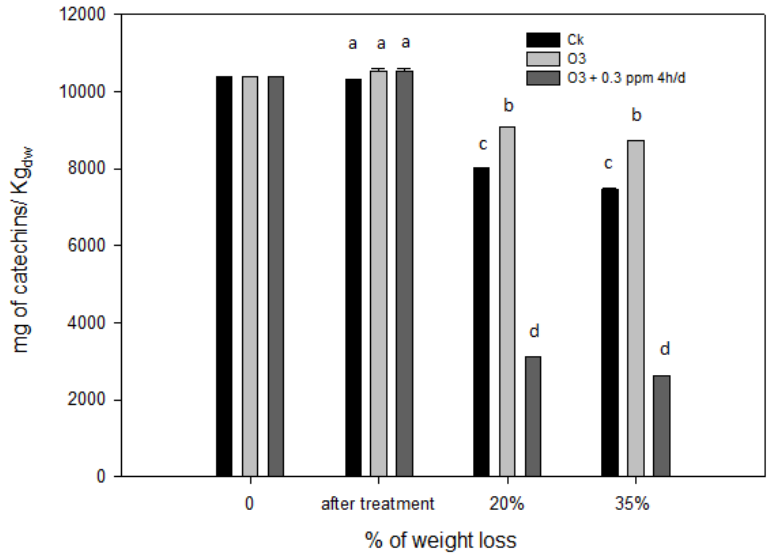


Fig.5.3.7 Variation in polyphenols content. The results represent the mean of tree 50 berries batch for each thesis. Vertical bars indicate SE. Different letters indicates significant difference between the treatments.

However, the observed decrement could be probably due to the different grape variety and to the higher UR respect to the one commonly used (45%). This could have prolong the dehydration time inducing a minor water stress and a major oxidative one.

This assumption seems to be supported by ozone data which can induce phenolic synthesis in grapes after short treatments (Artes-Hernandez et al., 2007; Mencarelli et al.,2011), while for long time exposure can cause a significant loss, especially when dehydration is applied.

On fresh-cut honey pineapple, banana 'pisang mas', and guava exposed to ozone at a flow rate of 8±0.2 ml/s for 0, 10, 20, and 30 min, Alothman et al., (2010) observed that total phenol

and flavonoid contents of pineapple and banana increased significantly when exposed to ozone for up to 20 min, with a concomitant increase in FRAP and DPPH values. The opposite was observed for guava. Ozone treatment significantly decreased the vitamin C content of all three fruits. The study shows promising results for enhancing antioxidant capacity of some fresh fruits by ozone treatment although the positive effect is compromised by a reduction in vitamin C content.

Treating with O_3 for short time did not affect anthocyanin content, as also reported by Barth et al. in 1995. During all dehydration time an anthocyanin loss was observed with differences among the thesis Significant differences were found at 20% of wl for all the samples. As shown in fig.5.3.8 from the initial content of 746 ± mg of malvidin/Kg dw a strong decrease was observed, with lower value in control fruits (512± mg of malvidin/Kg dw) than in continuous ozonated fruits (626± mg of malvidin/Kg dw). Afterwards lower values for control, O_3 18 hours and nonstop O_3 samples of 477± mg of malvidin/Kg dw, 411± mg of malvidin/Kg dw and 407± mg of malvidin/Kg dw respectively, were recorded.

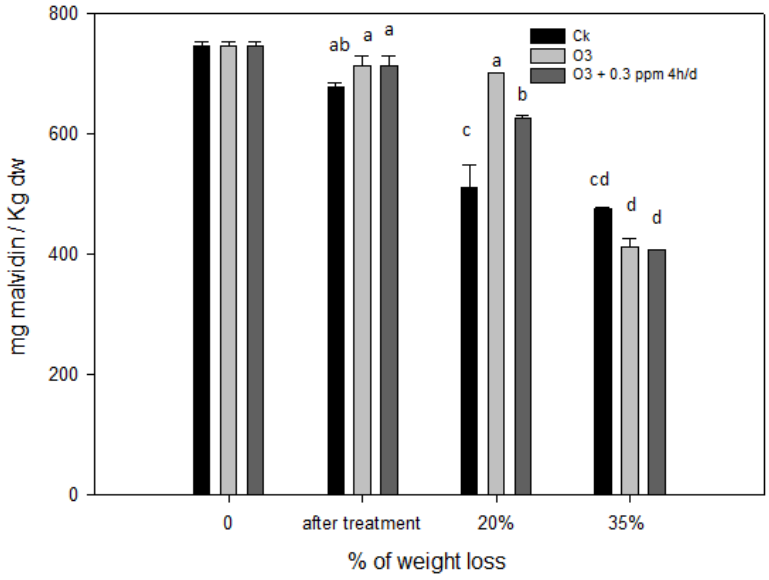


Fig.5.3.8 Variation in anthocyanins content. The results represent the mean of tree 50 berries batch for each thesis. Vertical bars indicate SE. Different letters indicates significant difference between the treatments

In the thesis treated continuously with ozone, stress is resulted to be stronger and there was a greater decrease in anthocyanin content, while, in the thesis treated for 18 hours with ozone, the decrease is less evident also due the higher extraction caused by the activity of the cell

wall enzymes. At 35% of weight loss, anthocyanins in the three thesis continued to drop, especially for the shock caused by the evident water loss and the consequent phenomena of cell lyses which are generated. In this context, in the samples treated with ozone, content of these pigments drops to values around 0.4 mg / g, underlining how, in addition to the dehydration stress there is also a stress due to the long time treatment.

Mycological study

Fungi content of grapes after harvesting was $6.25*10^1$ CFU/gr fw. The ozone treatment for 18 hours caused a 50% fungal reduction. During dehydration both samples treated with ozone retarded fungal growth until the end of dehydration period. No differences were found for control grapes throughout the tests, suggesting how fungal development is temperature dependent. Also, once reduced by the treatment, fungal content was pretty much stable during all period without significant differences between non-stop treated and one time treated samples.

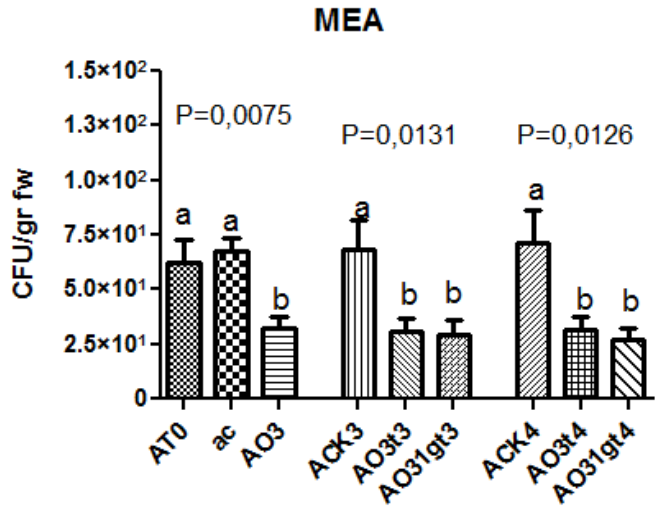


Fig.5.3.9 General fungal community on grapes during dehydration. AT0: time zero, ac: control grapes,AO3: Grapes treated with ozone for 18 hours, ACK3: control at 20%wl, AO3t3: ozone for 18 hours + 0.5g/h 4h/d at 20% wl, AO31gt3: ozone for 18 hours at 20%wl, ACK4: control at 35%wl, AO3t4: ozone for 18 hours + 0.5g/h 4h/d at 35% wl, AO31gt4: ozone for 18 hours at 35%wl.

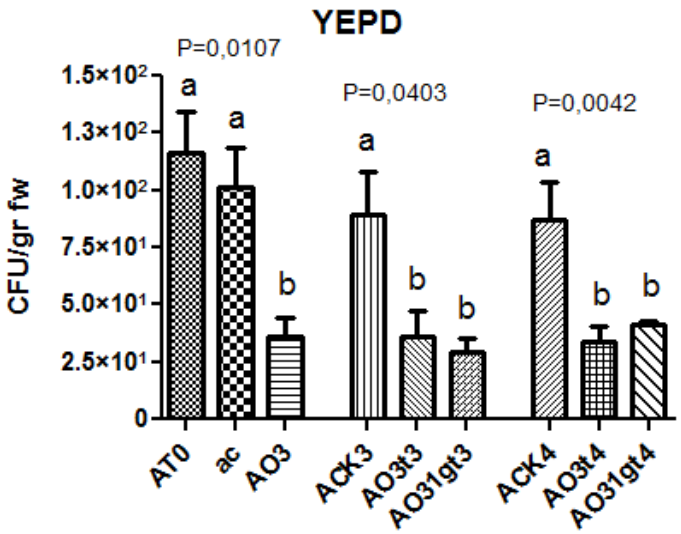


Fig.5.3.10 Yeasts community on grapes during dehydration. AT0: time zero, ac: control grapes,AO3: Grapes after ozone for 18 hours, ACK3: control at 20%wl, AO3t3: ozone for 18 hours + 0.5g/h 4h/d at 20% wl, AO31gt3: ozone for 18 hours 18h at 20%wl, ACK4: control at 35%wl, AO3t4: ozone for 18 hours + 0.5g/h 4h/d at 35% wl, AO31gt4: ozone for 18 hours at 35%wl.

Yeast community followed the same trend showed for fungi. From an initial yeast content of $1.17*10^2$ CFU/gr fw a three times reduction was observed for samples after 18 hours of ozone treatment. During dehydration yeast content was stable as the initial one for control grapes while both ozonated grapes yeast count was always three times lower.
Those data seems to be supported by several authors (Palou et al., 2001; Spalding 1966 and 1968; Ridley and Sims,1967; Krause and Weidensaul, 1977; Mlikota Gabler et al., 2010; Smilanick et al., 2010; Cullen et al., 2009; Khadre et al. 2001) confirming the strong antimicrobial power of ozone.

Cell wall enzymes

During all dehydration time a low PME and PG activity were observed.

Ozone treated samples kept an higher activity throughout the dehydration period showing an opposite trend compared to studies on tomato where Rodoni et al. in 2010 have found a clear decrease in the de-esterification of polugalacturonic acid after ozone treatments, revealing an increase of methyl-esterification degree on polygalacturonic chains by the enzyme.

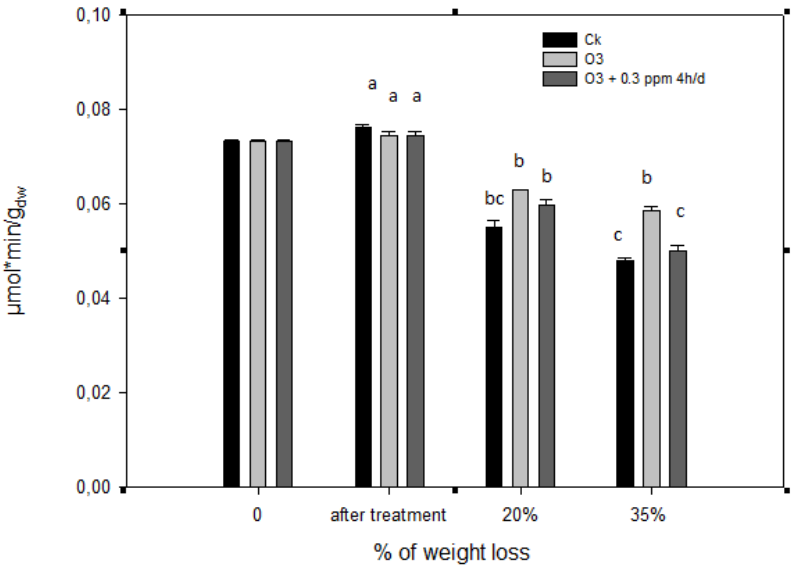


*Fig.5.3.11 Activity (expressed as µmol*min/g dw) of* ***pectinmethylesteras(PME)*** *during the test. Data represent the mean of tree 60 berries batch analysis of each sample.*
Vertical bars indicate SE. Different letters indicates significant difference between the treatments.

The PG activity decreased immediately after the 20% of wl in nontreated and continuosly treated samples and it remained low at the end of drying time, whereas in 18 hours treated sample the activity remained stable as the initial one and increased at 35% w.l. Those data partially confirmed what observed for PME.

The O_3 short treatment reduces the decrease of PME activity during dehydration and increases the activity of PG at 35% to depolymerize poligalacturonic acid chains. In previous work (Botondi et al. 2010) we observed an increase of PME activity during Aleatico grapes dehydration at 20°C meanwhile the PG shown a 30% higher activity until 30% of wl. It appears that the combination dehydration and short ozone treatment favour the cell wall degradation and this could be worthwhile for the extraction of cell compounds during vinification process.

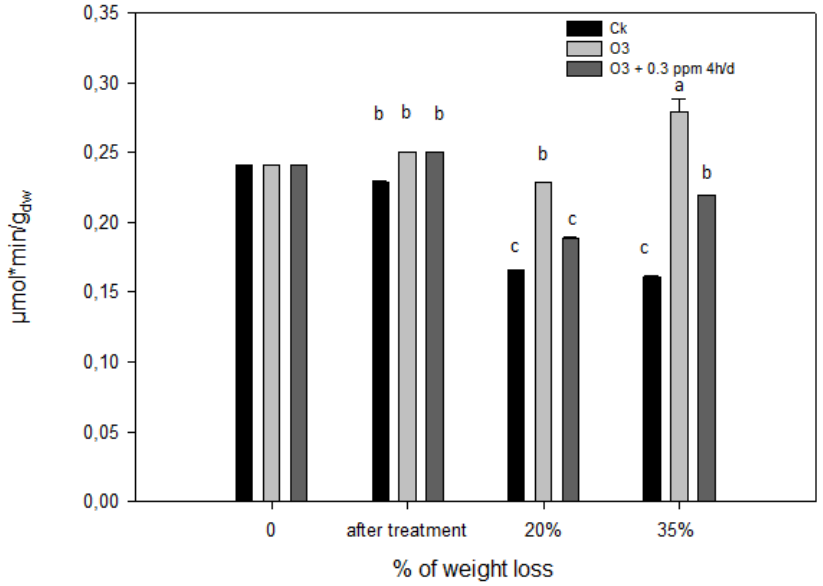


*Fig.5.3.12 Activity (expressed as µmol*min/g dw) of* ***polygalacturonase (PG)*** *during the test. Data represent the mean of tree 60 berries batch analysis of each sample.*
Vertical bars indicate SE. Different letters indicates significant difference between the treatments.

Conclusion

During this research aimed to understand the main outcomes of treating wine grapes with ozone as a postharvest treatment, grape color (red or white), stage of ripening (unripe, ripe or overripe), dehydration (with one ozone exposure or no-stop exposure) and of course ozone concentration have influenced, depending on the case, the ability of the gas to promote or to degrade some beneficial factors.

- First important thing was to understand if the gas exposure could cause imbalances on sugar and acidity content, the main aspects of a future grapes vinifications. As a matter of facts, regardless grape type and ozone concentration, no changes of those parameters were found. Also, analyzing respiration rate, weight loss and grape color no visible injuries or negative effects of treatments were observed. During dehydration, usually, acidity trend is to drop, but when ozone is continuously applied TA falls.
- Carotenoids content, despite what other studies suggest, was observed to be not directly connect to the exposure itself, indeed, just after ozone for 18 hours in both white and red grapes, any decrease was observed. On the other hands, when dehydration is applied and the exposure to the gas is uninterrupted, carotenoids were degraded by the strong oxidative power of O_3.
- Volatile compounds were analyzed only for white grape and their behavior after the treatment was very interesting even though not all the main classes were found to be affected by ozone.

  Glycosilated and free VOCs shown a complete different behavior. As expected free VOCs were oxidized by ozone, while glycosilated VOCs increased significantly especially phenols and monterpens.

- The total content of phenolics was notably higher in all the performed tests when grapes were subjected to ozone. Phenolic metabolism is differentially affected by O_3 as suggested by our results. Some fraction as cinnamates and flavonols resulted to be positively affected by the treatment whereas catechins and epicatechin are resulted to be more susceptible. Ozone can also induce phenolic synthesis after short treatments,

while for long time exposure can cause a significant loss, especially when dehydration is applied.

Our data suggest how important is chose the right ozone concentration especially according to the ripening stage. Unripe grapes seems to respond better to medium-high O_3 concentration for most of the phenolic classes with the exception of flavonols, which respond doubling their content with low concentration. Ripe and overripe grapes react better instead to medium-low concentration.

- Total anthocyanins content are resulted to be the less sensitive to the treatments remaining quite stable after the exposure. 800ppb of ozone on unripe stage was the only concentration able to increase anthocyanins content.

  A different behavior was found during dehydration in which an anthocyanin loss was surely observed for all the samples even though, ozonated grapes have delayed the loss until the end of drying.

- Glucose, galactose and arabinose have revealed to be the most abundant sugars in both skin and pulp grape berries cell wall. Ozone treatment affects skin sugars composition and its action is dose dependent but above all it depends on the stage of grape berries development. At unripe stage an increment was found for all the sugars, excepted glucose. During ripe stage, berries are more sensitive to ozone concentration. Indeed, even though rha, fuc and xyl increase their levels when grapes are treated with 800ppb of ozone sugars tend to decrease. At the last stage of ripening most of the sugars, whether or not high ozone concentration were used, decrease their levels.

  Pulp berries sugars decreased during unripe stage, with some exceptions; glu, man and ara tend to increase when 800 or 1300 ppb of ozone were used.

  The ripe and overripe stage showed less sensitivity to treatments despite this time the increment are related to the lower ozone concentration.

- Cellulose and uronic acid followed a positive trend during ripening, increasing their levels of 6% and 24% respectively, while when grape undergoes the overripe stage, those cell wall constituent start to decrease of about 4% and 23%.

Cellulose and uronic acid content decreased at every ripening stage when ozone is used. Therefore, 1300ppb of ozone seems to stop the loss of constituents, for both cellulose and uronic acid.

- During all tests a low PME and PG activity were observed.

  On cabernet sauvignon Pectin methyl esterase and Polygalacturonase activity are both affected by the ripening stage. PME, after ozone exposure, responds increasing its activity when the grapes are treated at ripe and overripe stage while a decrease occurs when treated at unripe stage. PME activity increase using 300ppb on ripe and overripe grapes while it reached his lower result when the same concentration is applied to unripe grapes.

  Similar trend for PG activity; the highest activity was reached using 300ppb of O3 on ripe grapes or using 800ppb on overripe grapes. Ozone exposure at unripe stage was found to proportionally decrease the PG activity.

  Throughout the drying period ozone treated samples kept an higher PME activity. The PG activity decreased immediately after the 20% of wl in nontreated and nostop treated samples, whereas in 18 hours treated sample the activity remained stable as the initial one.

Future prospective may look to increase knowledge on how cell wall is affected by ozone, focusing our attention on pectin, the most important component of grape cw, and how its composition changes, increasing the extractability of important specific components essential for grapes vinifications.

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Appendix: Lists of published papers, poster communications included

F. De Sanctis , M. G. Silvestrini, R. Luneia, R.Botondi, A. Bellincontro and F. Mencarelli. 2012. Postharvest dehydration of wine white grapes to increase genistein, daidzein and the main carotenoids. Food Chemistry 135, 1619–1625.

Abstract
Wine white grape bunches of the Grechetto variety were dehydrated at 10, 20 and 30°C, RH 45% and forced air ventilation of 1.5 m/s. Chemical and metabolic changes due to the effect of dehydration were studied at various stages of weight loss: 10%, 20%, 30% and 40%. Berry colour at 10 and 20°C tended to become greener with dehydration but at 30°C, at the final sampling, the colour darkened. Acidity decreased in all samples, while sugars increased. Total phenol content increased at 10°C until 30% weight loss was reached and then declined, while at 20 and 30°C the concentration decreased immediately. The contents of lutein and b-carotene (respectively 68 and 58 mg/kg d.w.), representing the 80% of total carotenoids, did not change significantly until the 30% of weight loss, when at 30°C the value increased above all for lutein while at 10 and 20°C, the contents decreased significantly. Daidzein, at 10 °C, rose significantly from about 150 lg/kg d.w. to 1434 lg/kg d.w. at 20% weight loss and then declined; at the same weight loss percentage, the genistein concentration began to increase. At 20°C both isoflavones rose until the end of the experiment, reaching values similar to the sample at 10°C. A temperature of 30°C was deleterious to grape isoflavones. A discussion on the changes in isoflavones related to temperature and time is reported.

I. Nicoletti, A. Bellincontro, A De Rossi, F. De Sanctis, D. Tiberi, P. Pietromarchi, R. Botondi, D. Corradini and F. Mencarelli.2013. Postharvest dehydration of Nebbiolo grapes grown at altitude is affected by time of defoliation. Australian Journal of Grape and Wine Research 19, 358–368.

Abstract
Background and Aims: Sfursat is an Italian wine produced with partially dehydrated Nebbiolo grapes in the Valtellina region which is located at high altitude. The research aims to understand the influence of fruit exposure on the rate of water loss by harvested fruit and the influence of exposure and dehydration on the content of phenolic substances and anthocyanins in the wine.
Methods and Results: Clusters of Nebbiolo grapes from control vines (ND) and from vines that were defoliated at fruitset (DFS) or post-veraison (DPV) were harvested at a sugar concentration of about 230 g/L (there was no significant difference among the treatments), and dehydrated at 10, 20 or 30°C and at 60% relative humidity (RH), and air flow. Fruit were sampled at 10 and 20% weight loss (WL). Leaf removal had little effect on the physical characteristics of bunches and on the anthocyanins content and profile of harvested Nebbiolo

grapes but affected dehydration. At 10 and 20°C, DFS fruit lost mass more slowly than DPV and ND fruit. In ND fruit, 20% WL reduced anthocyanins from 554 mg/kg fresh mass (FM) at harvest to 458, 432 and 396 mg/kg FM at 10, 20 and 30°C, respectively. In DFS and DPV berries, anthocyanins increased during dehydration at 10°C. At harvest, ND berries had a lower content of total stilbenes than those from defoliated vines. Dehydration (10°C, 20% WL) increased stilbene concentration in ND and DPV fruit. Dehydration at 10°C induced a rise in the flavonol concentration in fruit from defoliated vines. Catechin concentration was 106.5 mg/kg DM (dry mass), the highest value in ND berries at harvest.
Wine was produced only from grapes dehydrated at 10°C. Wine from DFS fruit had a higher content of phenolic substances (2704.8 mg/L) and anthocyanins (104 mg/L) than that from DPV (2454.9 and 96.2 mg/L, respectively) and ND (2301.9 and 100.5 mg/L, respectively) fruit.
Conclusions: Postharvest dehydration was slower where vines had been defoliated and resulted in changes in the ratios among groups of phenolic substances as well as among single phenolic components.
Significance of the Study: In Nebbiolo grapes for Sfurzat wine production, defoliation at fruit set enables fruit to reach phenolic maturity at a lower sugar concentration allowing dehydration to increase sugar concentration without producing excessively alcoholic wines.

R. Botondi, F. De Sanctis, S. Bartoloni and F. Mencarelli. 2014. Simultaneous application of ethylene and 1-MCP affects banana ripening features during storage. Journal of the Science of Food and Agriculture (Early view, online version)

Abstract

Background
In order to avoid the ripening blocking effect of 1-MCP (1-methylcyclopropene) on bananas when applied before ethylene commercial treatment, 1-MCP in combination with 'CD ethylene' (ethylene–cyclodextrin complex) was used in gas formulations: 300 nmol mol^{-1} 1-MCP + 1200, 2400 or 4800 nmol mol^{-1} ethylene (ETH). Control bananas received 1-MCP alone or 4800 nmol mol^{-1} ethylene alone or no treatment. Treatments were done on overseas shipped bananas, at 14 °C, 90% relative humidity (RH), for 16 h; the bananas were stored under the same atmospheric conditions. After 4 or 12 days the bananas were commercially treated with 500 µmol mol^{-1} ethylene.
Results
A 300 nmol mol^{-1} 1-MCP treatment significantly blocked banana ripening in terms of physiological and technological parameters, inhibiting ethylene production and respiration, despite the commercial ethylene treatment. The application of 300 nmol mol^{-1} 1-MCP + 1200 or 2400 nmol mol^{-1} ethylene delayed ripening but with a regular pattern. A 300 nmol mol^{-1} 1-MCP + 4800 nmol mol^{-1} ethylene application did not delay ripening as did 4800 nmol

mol^{-1} ethylene treatment. The development of black spots was closely associated with advanced ripening/senescence of fruits.
Conclusion
The combined 300 nmol mol^{-1} 1-MCP + 1200 or 2400 nmol mol^{-1} ethylene treatment appears to be a promising treatment to extend banana storage, following overseas shipping.

XVI Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology, Lodi, 2011. Metabolic and structural effects of ozone postharvest treatments on wine grape. Poster

XVIII Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology, Conegliano, 2013. Metabolic and Structural Effects of Dehydration and Ozone Postharvest Treatments on Wine Grape. Oral dissertation.

PUROVINO®: a Fumigation Postharvest Treatment for the Quality of Wine Grape. Poster

Abstract

High quality wine requires high quality grape. High quality grape means good aromatic properties and texture, excellent phenols panorama, and salubrity. Thus, addition of sulfites to the wine reduces the quality level because affects the salubrity of the product. Ozone is known as a great sanitizing agent but in Italy cannot be used directly on food. Purovino® is a special fumigation treatment based on oxygen and its activated form, which has been developed to avoid the use of sulfites on wine but, in addition, to increase the phenolic fraction and aroma of grapes. Here some research data on Sauvignon Blanc grape are reported regarding the use of Purovino® postharvest treatment. Grapes were hand harvested at the end of day in Castello de "La Sala" winery, placed in perforated boxes in one single layer and then stacked in a cold room at 10°C and 70% RH. The Purovino® started immediately and bunches were sampled before and after treatment which lasted 4,8, and 16 h. The control sample consisted in grape bunch treated in similar way, placed in another cold room without Purovino® treatment. Total phenols increased of 10-20% after treatment compared to the control sample. Total carotenoids raised slightly, non significantly. Glycosilated volatiles organic compounds (VOCs) increased significantly in Purovino® treated samples while decreased in untreated one; in contrast the free VOCs, which means the perceptible aroma compounds, were higher in untreated than in Purovino® treated grape. This is an important results because the grape for vinification must have mainy glycosilated VOCs which are released during the process by yeasts. Changes of specific phenols and VOCs are reported.