

New Approaches for Postharvest Quality Retention of Table Grapes

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ABSTRACT

Major factors limiting table grape storage and shelf life and causing important economical losses to the industry are cluster dehydration (berry water loss and rachis browning), skin colour changes, accelerated softening and microbial spoilage, especially gray mould decay caused by the pathogen *Botrytis cinerea*. Appropriate environmental conditions during storage and/or transportation and the use of sulphur dioxide (SO₂) technologies have successfully alleviated these problems. However, postharvest treatments alternative to SO₂ are needed because of issues related to excessive sulphite residues and its phytotoxic effects such as berry bleaching and hairline cracks. In this article, active packaging based on the addition of antimicrobial essential oils, such as thymol, carvacrol, eugenol, or menthol, as in-package fumigants to modified atmosphere packaging (MAP) systems and development of natural antifungal edible coatings, like those based on *Aloe vera* gel, are reviewed as two of the most promising alternative approaches for cost-effective control of postharvest diseases and preservation of table grape physico-chemical and sensory quality and functional properties. Further research should focus on either the evaluation of these technologies for particular industry needs with specific table grape cultivars and postharvest handling conditions or their application in combination with other non-polluting alternatives as part of integrated disease management (IDM) programs.

Keywords: *Aloe vera* gel, essential oils, modified atmosphere packaging, natural edible coatings, sulphur dioxide

Abbreviations: CA, controlled atmosphere; CFU, colony forming units; CT, concentration x time product; GRAS, generally regarded as safe; IDM, integrated disease management; MAP, modified atmosphere packaging; MI, maturity index; N-OPP, non-perforated oriented polypropylene; RH, relative humidity; SSC, soluble solids concentration; SO₂, sulphur dioxide; TA, titratable acidity; US EPA, United States Environmental Protection Agency; US FDA, United States Food and Drug Administration; UV-C, far ultraviolet

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INTRODUCTION

For decades, the appropriate postharvest management of fruits and vegetables in relation to overall quality maintenance has been a challenge. It is generally believed that quality can only be maximised when the commodity is harvested mature or ripe and, on the contrary, shelf life is extended for less mature or unripe products (Toivonen 2007). However, produce are usually harvested at commercially mature stage (not over-ripe or senescent), but show a large number of problems that lead to a net reduction of shelf life or the so called "postharvest losses", basically due to quality deterioration and incidence of postharvest diseases. This is particularly important in the case of table grapes (*Vitis vinifera* L.), which are non-climacteric fruit, but are typically affected by severe problems during postharvest handling, storage and marketing. As other fresh fruits, postharvest quality deterioration of table grapes is mainly due to

fruit weight loss, colour changes and accelerated softening. Additionally, important product losses may also be attributed to rachis browning and high incidence of berry decay, especially when storage is prolonged (Nelson 1985; Martínez-Romero *et al.* 2003). This review covers recent literature on table grape ripening and problems related to product loss during postharvest handling and storage, as well as some innovative postharvest tools to control decay, maintain quality and extend shelf life.

TABLE GRAPE RIPENING PROCESS

Fruit ripening is a highly coordinated, genetically programmed process occurring at the later stages of maturation and involving a series of physiological, biochemical and sensory changes leading to the development of an edible ripe fruit with desirable quality parameters (Giovannoni 2001). The spectrum of biochemical changes is wide in-

cluding chlorophyll degradation, increased activity of cell wall-degrading enzymes, biosynthesis of carotenoids, anthocyanins, flavour and aroma components, accumulation of sugars and diminution of acidity. Two major classifications of ripening fruit (climacteric and non-climacteric) based on respiration and ethylene production rates have been distinguished. Climacteric fruit, such as apple, apricot, avocado, banana, peach, plum or tomato are characterised by their increased respiration and ethylene biosynthesis rates during ripening. In this sense, ethylene is considered the plant hormone responsible for the ripening process in climacteric fruit (Martinez-Romero *et al.* 2007a). Contrarily, in non-climacteric fruit such as citrus, eggplant, sweet cherry, strawberry, pepper or grape, ethylene is not required for the coordination and completion of ripening (Prassana *et al.* 2007). In grapes, ripening occurs at the second phase of berry development and results from the expansion of existing pericarp cells (Mailhac and Chervin 2006). Table grape quality refers to a range of attributes related to appearance, colour, texture, flavour and aroma. The evolution of these attributes starts at *veraison* and they are considered to reach their maximum level at the optimum ripening stage and immediately after harvest. The goal of an adequate postharvest handling is to maintain them unchangeable as long as possible after harvest, thus delaying fruit senescence. In the case of prolonged storage at low temperatures, it has been reported that the most important external and internal physico-chemical quality attributes of table grapes are not significantly affected by exposure to exogenous ethylene (Palou *et al.* 2003).

Skin colour is one of the most important fruit quality attributes for consumer acceptance of table grapes. During ripening there is a degradation of chlorophyll and an accumulation of either anthocyanin in the case of red, purple and black grapes (Wrolstad *et al.* 2005) or carotenoids in the case of yellow-fleshed cultivars (Sajilata and Singhal 2006). Apart from their role in colouring fruits, both pigment groups have been claimed to induce, as biomolecules with functional properties, health benefits to consumers (Stintzing and Carle 2004). Berry softening occurs during ripening due to the activity of several enzymes that alter the structural components of the cell wall and diminish cell adhesion. According to Nunan *et al.* (1998), the main cell wall degrading enzymes responsible for table grape softening are β -galactosidase, polygalacturonase and pectin-methylesterase. As they greatly facilitate fungal infection and disease development and alter fruit quality and consumer palatability, cell wall modifications determine the fruit actual shelf life (Lashbrook 2005). An accumulation of sugars and a decrease of organic acid levels occur during ripening that confer to the mature berry a good balance in the relation sweetness/sourness in terms of flavour, aroma and overall taste acceptability. The most frequently used parameters to assess grape internal quality are soluble solids concentration (SSC) and titratable acidity (TA, as percent tartaric acid). The ratio between these two values, commonly known as maturity index (MI), is often used for commercial purposes as a quality criterion to properly harvest and market the fruit.

TABLE GRAPE DETERIORATION DURING POSTHARVEST HANDLING

Even with a non-climacteric physiological response, table grapes may deteriorate rapidly after harvest and reach the consumers not in proper condition if fruit handling, storage and transportation are not appropriately conducted to delay fruit senescence and preserve quality. The main causes of grape deterioration are dehydration, with the subsequent weight loss, colour changes, softening, surface pitting, browning, acidity loss, microbial spoilage and decay, among others. However, the deterioration rate is affected by different factors such as intrinsic characteristics of the product and environmental storage conditions in terms of temperature, relative humidity (RH), atmosphere gas composition,

ventilation, etc.

Gray mould, caused by the pathogen *Botrytis cinerea* Pers.:Fr., is the most economically important postharvest disease of table grapes. This fungus has a vigorous growth rate and ability to spread among berries even at temperatures as low as -0.5°C . Infections that cause postharvest losses can originate from spores on the surface of the berries, latent infections that occurred before harvest during the growing season, or visibly infected berries that escaped removal during packaging. In addition, any of these types of infection may easily cause gray mould nesting in packed produce as fungal mycelium spread from infected berries to adjacent healthy berries (Droby and Lichter 2004; Lichter *et al.* 2006). Depending mainly on growing conditions and postharvest handling, other postharvest diseases that can occasionally cause considerable economical losses in cold-stored table grapes are blue mould, *Alternaria* rot, *Cladosporium* rot and postharvest sour rot, caused by *Penicillium* spp. (mainly *P. expansum* Link), *Alternaria* spp. (mainly *A. alternata* [Fr.] Keissler), *Cladosporium* spp. (mainly *C. herbarum* [Pers.:Fr.] Link) and a variety of yeasts and/or bacteria (*Bacillus* spp., *Hanseniaspora* spp., etc), respectively. When the cold chain is broken or during retail display of the fruit, losses due to Rhizopus rot or postharvest black rot, caused by *Rhizopus stolonifer* (Ehrenb.:Fr.) Lind and *Aspergillus niger* van Tiegh., respectively, may also become important because these fungi grow very fast at ambient temperatures (Hewitt 1998; Franck *et al.* 2005; Palou *et al.* 2005; Lichter *et al.* 2006).

Because of the etiology of gray mould and other postharvest diseases, an effective control of table grape decay relies in integrated disease management (IDM) programs based not only on appropriate fungicide usage in the field, but also on the application of antifungal treatments just after harvest and/or during fruit storage. Currently and for many years, acceptable control levels have been achieved with technologies involving the use of sulphur dioxide (SO_2). Fumigations with this gas were developed and first used in California more than 75 years ago (Nelson 1985), whereas in-package SO_2 generating pads were also developed in California in the late 1960s (Gentry and Nelson 1968) and today are commercially used worldwide. In these pads, gaseous SO_2 is released after reaction of sodium metabisulphite with environmental moisture. Different types of pads, where the rate of gas release is controlled (one or two different phases with quick- and/or slow-release devices), have been developed according to industry needs (Palou *et al.* 2002a). SO_2 is highly effective to kill both mycelia and spores of *B. cinerea* (Šmilanick and Henson 1992), but chemical residues and phytotoxicity (bleaching of fruit colour and hairline cracks) are major problems associated with both SO_2 fumigation and generating pads. SO_2 was removed from the list of compounds generally regarded as safe (GRAS) by the United States Food and Drug Administration (US FDA) and classified as a pesticide by the United States Environmental Protection Agency with a tolerance of 10 $\mu\text{L/L}$ (ppm) for sulphite residues in table grapes (US EPA 1986). This change was due to excessive residues that can occur along the food chain causing hypersensitive reactions to certain consumers. Furthermore, the use of SO_2 is thus banned for organic produce. Bleaching is an early-described physiological disorder that occurs when the gas penetrates at too high concentrations into the berry stem end or through lenticels or skin wounds causing bleached or sunken areas (Ryall and Harvey 1959; Nelson and Ahmedullah 1972). Hairline cracks are microscopic, longitudinal splits often followed by an exudation of berry juice also caused by excessive SO_2 concentrations (Zoffoli *et al.* 2008). According to recent research, the incidence of this disorder after cold storage is related to the cuticle content and porosity of the berry and might be predicted at harvest by dipping the fruit in a citric acid solution and staining with methylene blue (Zoffoli *et al.* 2009).

The general incidence of these SO_2 injuries is greatly dependent on the cultivar, fruit condition, type of SO_2 tech-

nology and application mode, postharvest handling, and storage environmental conditions. In any case, research efforts have been devoted to reduce SO₂ doses while maintaining a satisfactory balance between decay control, sulphite residues and fruit injury. For instance, in California it was recommended to perform, when possible, precooling and initial SO₂ fumigation simultaneously in a forced-air precooling room under the total utilization system. This method uses about 75% less SO₂ than traditional initial fumigations (Luvisi *et al.* 1992). Regarding produce gassing with cylinders of compressed SO₂ (pressurized liquid gas), a practice that is typically used in California for export marine container fumigation at the loading point (shipping), Crisosto *et al.* (2002b) concluded that the usual commercial doses of 1.3-2.2 kg SO₂ were clearly excessive and it was enough a dose of 0.2 kg SO₂ for effective decay control. In their evaluation of SO₂ fumigations, Smilanick and Henson (1992) found that the CT product (gas concentration x exposure time) required in the air spaces surrounding the berries to effectively control gray mould at 0°C was as low as 100 µL/L-h. This CT dose can be obtained with an average concentration of 100 µL/L for 1 h, 200 µL/L for 0.5 h, 25 µL/L for 4 h, or an equivalent combination of concentration and time. Palou *et al.* (2002a) proposed the use of an emission rate (measured in µmol SO₂ per kg of fruit exposed per h of exposure) to assess the minimum constant amount of SO₂ that a generating pad should emit during table grape storage or shipment at 0°C. They reported that a rate of 3.6 µmol/kg h was sufficient to inhibit the aerial mycelial growth of *B. cinerea* and consequently prevent gray mould nesting. Other workers used this knowledge to enhance SO₂ existing technologies and, especially in the case of in-box gas generators, new improved pads or combinations with liners or other devices have been developed (Opperman *et al.* 1999; Crisosto *et al.* 2001; Petrel *et al.* 2006; Zutahy *et al.* 2008).

Despite these approaches to more suitable uses of SO₂ technology, the important problems derived from its utilization, the public perception that, as other chemical pesticides, this gas is harmful to human health and environment, and the lack of new available postharvest reduced risk fungicides for table grapes have motivated worldwide the search for alternative non-polluting strategies for decay and microbial control, fruit quality optimisation and reduction of losses along the postharvest chain. Among them, the use of natural antimicrobial compounds for active packaging and the development of new natural edible coatings currently appear as especially promising.

RECENT FINDINGS TO RETAIN POSTHARVEST GRAPE QUALITY AND SAFETY

In recent years, extensive research work on the possibilities to delay the ripening process, prevent decay and microbial spoilage and in turn increase table grape postharvest life has been conducted. Postharvest technologies evaluated for this purpose include the application of physical methods such as low doses of far ultraviolet (UV-C) irradiation (Nigro *et al.* 1998; Shama and Alderson 2005; González-Barrío *et al.* 2006), treatments with gaseous ozone, ozone in water or storage in ozonated atmospheres (Sarig *et al.* 1996; Palou *et al.* 2002b; Smilanick *et al.* 2002; Artés-Hernández *et al.* 2004; González-Barrío *et al.* 2006), hot water or vapour heat treatments (Karabulut *et al.* 2004; Lydakis and Aked 2003a, 2003b), short hypobaric or hyperbaric treatments (Romanazzi *et al.* 2001, 2008), carbon dioxide (CO₂) or oxygen (O₂) shocks or storage in controlled atmospheres (CA) (Crisosto *et al.* 2002a; Retamales *et al.* 2003; Artés-Hernández *et al.* 2004; Deng *et al.* 2007; Romero *et al.* 2008) or modified atmosphere packaging (MAP). MAP has been used since the late 1980s to preserve the quality of fruit and vegetable products (Kader *et al.* 1989). Traditionally, effective MAP, known today as passive MAP, relies on the natural evolution of the concentration of CO₂ and O₂ in the atmosphere surrounding the produce packed in different types of liners. Although some benefits from the

use of passive MAP on table grapes have been reported (Artés-Hernández *et al.* 2004, 2006; Lichter *et al.* 2005), traditional MAP is not usually enough to ensure quality and safety preservation to fulfil consumer demand (Lichter *et al.* 2005, 2006). In this sense, active MAP packaging is an innovative concept for food packaging as a response to new market trends and continuous challenges for consumer satisfaction (Vermeiren *et al.* 1999; Suppakul *et al.* 2003). Besides the addition of antifungal essential oils, which will be later discussed in this section, other compounds that have been added to MAP to develop active packaging for table grapes include (*E*)-2-hexenal (Archbold *et al.* 1999), chlorine dioxide (Zoffoli *et al.* 1999) and ethanol (Chervin *et al.* 2005; Lurie *et al.* 2006).

The development of edible coatings for fruits and vegetables is an innovative and interesting technology suitable for commercial application and, at the same time, a natural alternative to the use of postharvest chemical treatments, particularly in the case of high perishable fruits. As other alternatives for shelf life extension, such as the use of exogenous polyamines that compete with ethylene and preserve quality (Valero *et al.* 2002), edible coatings have been successfully used in a wide range of fleshy fruits, giving promising results. These coatings act as barriers during processing, handling and storage, retarding food deterioration and enhancing its physical, biochemical and sensory quality. Furthermore, they may be environmentally-friendly alternatives to disinfect food, control spoilage and assure food safety because of their inherent biocidal activity or the incorporation of antimicrobial compounds as ingredients of the formulations (Valencia-Chamorro *et al.* 2008).

Integrated treatment with essential oils and MAP

Essential oils are aromatic oily liquids obtained from many plant organs: flowers, buds, seeds, leaves, twigs, bark, herb wood, fruit and roots. The term "essential oil" is thought to derive from the word *Quinta essentia* with medical use attributed to Paracelsus. Until recently, essential oils have been used as flavouring ingredients due to their flavour and fragrance, but nowadays essential oils and their pure components are gaining increasing interest from the point of view of their safe status, wide acceptance by consumers and their exploitation for multi-purpose uses (Cowan 1999). In addition, the antimicrobial properties of essential oils obtained from plant organs have been empirically recognized for centuries, but only came to scientific attention recently (Appendini and Hotchkiss 2002; Burt 2004). Many essential oils having antifungal activity belong to the genus *Thymus*, *Origanum*, *Syzygium*, *Mentha* and *Eucalyptus*, for which the main compounds are thymol, carvacrol, eugenol, menthol and eucalyptol, respectively. These compounds have granted GRAS status approval by the US FDA (US FDA 2009) and, in general, essential oils are exempted from the US EPA registration process (US EPA 2008). Some of these compounds have been tested, particularly in *in vitro* conditions, against different fungi causing postharvest diseases, including *B. cinerea* (Tripathi and Dubey 2004; Tripathi *et al.* 2008). One of the main advantages of essential oils is their bioactivity in the vapour phase, a characteristic that makes them attractive as possible fumigants for protection of stored commodities as well as for active MAP.

Control of gray mould, at least at similar levels than those obtained with the use of SO₂, is essential for the development of any feasible active packaging system for table grapes. For evaluation of the antifungal activity of essential oils, potato dextrose agar (PDA) plates, previously inoculated with 75 spores of *B. cinerea* (10 µL of a spore suspension containing 7,500 spores/mL), were deposited inside 2-L plastic packages (20-µm thickness non-perforated oriented polypropylene [N-OPP] bags) and then the five volatiles mentioned above were impregnated in gauzes at 0 (control), 0.1, 0.4, 1.0 or 2.0 mL (vapour phase) and added before sealing hermetically the packages. Concentrations over 0.4 mL killed all the spores and no mycelium growth

was observed after 4 days of incubation at 25°C. At 0.1 mL, one colony of *B. cinerea* was obtained for menthol, eugenol and carvacrol, 50 colonies for eucalyptol and none for thymol. In addition, at the dose of 0.1 mL, the diameter of the colonies was significantly smaller in treated packages than in control non-treated ones (Fig. 1A). Very similar results were obtained in *in vivo* experiments with 'Autumn Royal' berries artificially injured, inoculated with 75 spores of *B. cinerea*, treated in the same type of N-OPP packages and incubated at 25°C for 4 days. At the lowest dose of 0.1 mL, thymol was able to kill all the spores and thus no decayed berries were observed, while menthol, eugenol and carvacrol significantly reduced the percentage of decayed berries compared with control and eucalyptol, which was found to be ineffective (Fig. 1B). Therefore, the effectiveness of the oils was higher *in vitro* in PDA dishes than in grapes. This result is in agreement with previous reports, in which considerably higher concentrations of essential oils were needed in food systems than *in vitro* to gain the same efficacy level. In other cases, oils that showed good inhibitory activity *in vitro* failed to effectively control postharvest diseases *in vivo*, basically due to different environmental conditions and complex interactions between the host and the pathogen (Burt 2004; Tripathi and Dubey 2004).

Although the exact mechanism of action of the essential oils was not completely clarified, some authors have attributed it to their hydrophobicity, which enables them to partition in the lipids of the cell membrane disturbing its integrity and the inorganic ions equilibrium (Lambert *et al.* 2001; Bagamboula *et al.* 2004). Additionally, it has been postulated that the presence of the phenolic ring may be necessary for the antimicrobial activity of eugenol and thymol (Ultee *et al.* 2002). Moreover, the site(s) and number of hydroxyl groups on the phenol ring are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity (Cowan 1999). For those essential oils with absence of phenolic groups like menthol, it has been speculated that the mechanism of action involves membrane disruption by the lipophilic compounds (Mendoza *et al.* 1997). Disease symptoms in control berries inoculated with *B. cinerea* were softening and watering of the infected zone as well as mycelium growth. As shown by lower external diameter, internal height, external area and internal volume of infection, the intensity of these symptoms was reduced after the addition of the essential oils inside the packages (Martínez-Romero *et al.* 2007b). The effectiveness of the oils in reducing gray mould decay in the fruit was higher at external than internal level, since the essential oils were applied as vapours and then the primary contact zone was the berry epidermis. As it happens with SO₂ applications (Palou *et al.* 2002a) and other gaseous treatments for table grapes, one of the factors restricting the use of fumigations with essential oils is the limited proportion of the vapour that is sorbed by the fruit and thus the limited control of gray mould from latent field infections that develop internally after harvest.

The addition of essential oils to the MAP also induced significant physiological effects in treated berries since both respiration rate (Fig. 1C) and ethylene production (Fig. 1D) were lower in treated fruit than in control fruit. In these experiments, CO₂ and ethylene production were measured by placing 15 'Autumn Royal' berries from each package (passive MAP (control) or MAP with thymol, menthol, eugenol, eucalyptol or carvacrol) in 0.25-L glass jars hermetically sealed with a rubber stopper for 1 h. One mL of the holder atmosphere was withdrawn with a gas syringe, and the ethylene was quantified using a gas chromatograph equipped with a flame ionization detector. Results were the mean of 4 determinations for each package and expressed as nL g⁻¹ h⁻¹. For respiration rate determination, another sample of 1 mL of the same atmosphere was withdrawn and CO₂ quantified using a gas chromatograph with a thermal conductivity detector. Results were the mean of 4 determinations for each package and expressed as mg CO₂ kg⁻¹ h⁻¹. Table grapes are categorised as non-climacteric fruit (Martí-

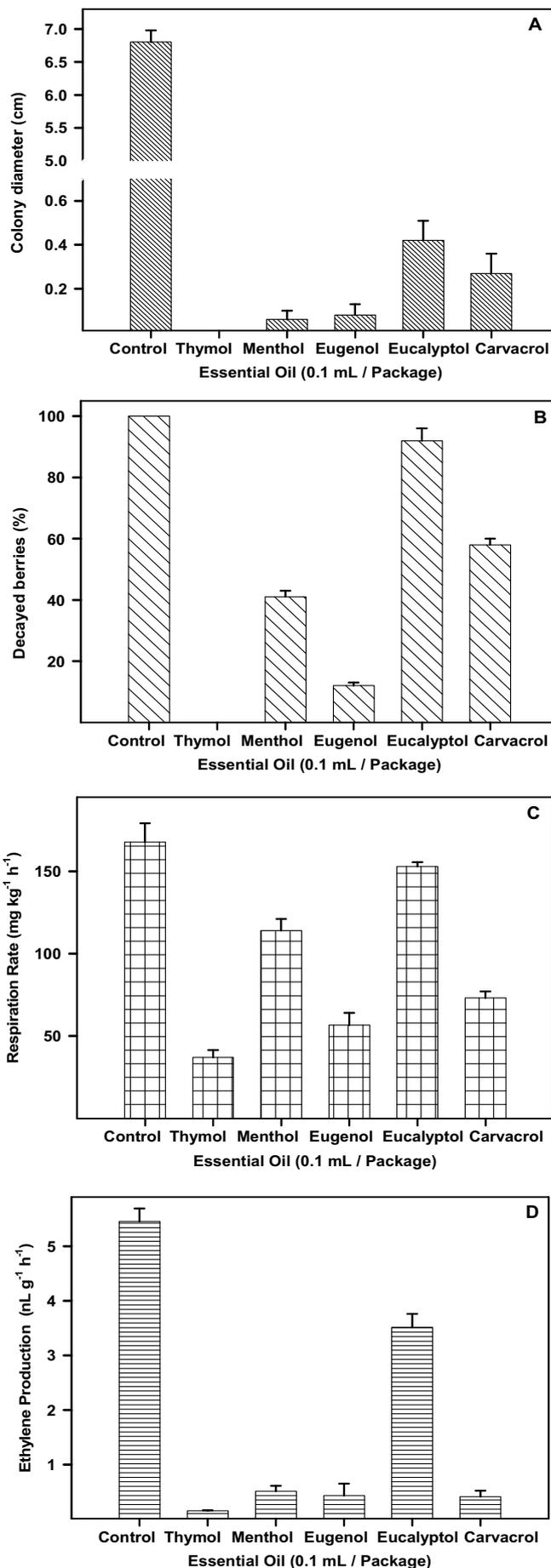


Fig. 1 Reduction of *in vitro* colony diameter of *Botrytis cinerea* (PDA plates, A), gray mould decayed berries (B), respiration rate (C) and ethylene production (D) on 'Autumn Royal' table grapes packed in MAP with the addition of 0 (control) or 0.1 mL of the corresponding essential oil and incubated at 25°C for 4 days.

nez-Romero *et al.* 2003), and then the observed increase in ethylene production might probably be a consequence of fungal growth. Accordingly, it has been reported that *B. cinerea* produced greater amounts of ethylene as the concentration of conidia inoculated *in vitro* or in the climacteric tomato fruit increased (Cristescu *et al.* 2002). The respiration rate of 'Autumn Royal' grapes was clearly reduced by the application of essential oils, particularly thymol, eugenol and carvacrol, which could be attributed to lower disease development. In fact, there are evidences supporting the relationship between respiration rate and fungal decay in fruits. For instance, a substantial increase in respiration rate prior to the appearance of rots during storage of grapes has been reported (Bower 2001).

Based on these and similar results, an active packaging has been developed for several grape cultivars based on the addition of essential oils to MAP (Valverde *et al.* 2005; Valero *et al.* 2006; Guillén *et al.* 2007; Martínez-Romero *et al.* 2007b). At the oil concentrations selected, the characteristic aroma of the oils dissipated shortly after opening the sealed packages and the sensory attributes of the grapes were not adversely affected. With MAP technology, variable increases in CO₂ concentration and decreases in O₂ concentration occur inside the packages depending primarily on film permeability and product respiration. In previous work (Martínez-Romero *et al.* 2003), the MAP system, used alone, was found to be effective to maintain the quality of table grapes, but the action of CO₂, at the actual concentration inside the packages, could not be competent enough to satisfactorily inhibit microbial growth. In contrast, the active MAP packaging resulting from the combination with selected essential oils may not solely improve the overall fruit quality, especially in terms of reducing softening, delaying colour evolution and maintaining organoleptic properties, but also effectively control decay and reduce microbial spoilage.

Natural edible coatings

Food packaging, an important discipline in the area of food technology, concerns preservation and protection of all types of foods and their raw materials, as well from oxidative and microbial spoilage. Wax was the first coating used on fruits. The Chinese applied wax coatings to oranges and lemons in the 12th and 13th centuries. Although the Chinese did not realize that the full function of fruit coatings was to slow down respiratory gas exchange, they found that wax-coated fruits could be stored longer than non-waxed fruits (Park 1999). Edible coatings preserve the quality of fruits and vegetables and enhance their appearance by forming a film over the product that serves as a partial barrier to gases (CO₂ and O₂), water vapour and aroma compounds, creating a modified atmosphere around the commodity, decreasing the respiration rate of the fruit and the water loss, and preserving texture and flavour. Edible coatings are also useful as carriers of different food additives, such as antioxidants, antimicrobials, nutrients, etc, that may help to preserve or even improve the quality of intact or minimally processed fruits and vegetables (Olivas *et al.* 2008).

Components of edible films and coatings can be divided into three categories: hydrocolloids, lipids and composites. Hydrocolloids include proteins, cellulose derivatives, alginates, pectins, starches or other polysaccharides. Suitable lipids include waxes, acylglycerols and fatty acids. Composites contain both hydrocolloid and lipid components. The most important characteristic of any coating is its permeability properties and they are determined by the type and proportion of these components. Hydrophilic coatings are excellent O₂, aroma and oil barriers and provide strength and structural integrity, but are not effective moisture barriers due to their hydrophilic nature. Good O₂-barrier properties in hydrophilic coatings are due to their tightly packed, ordered hydrogen bonded network structure and low solubility. These coatings may retard ripening and increase the shelf life of coated produce, but can cause, under

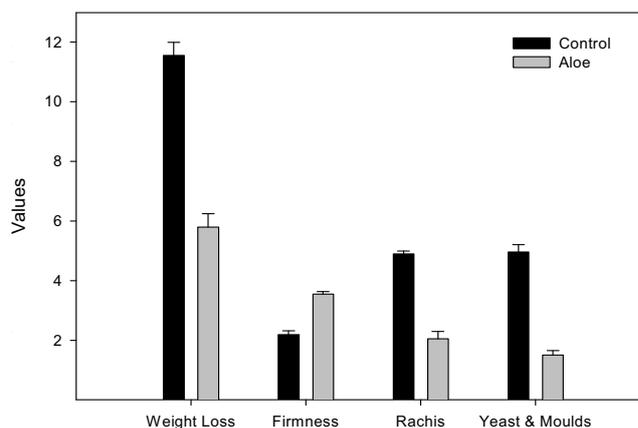


Fig. 2 Effect of *Aloe vera* on weight loss (%), berry firmness (N), rachis score dehydration (0 = none, 5 = severe browning) and population of yeasts and moulds (log CFU/g) on uncoated (control) and coated 'Crimson Seedless' table grapes after 28 days of cold storage at 1°C.

UV-C illumination (Romanazzi *et al.* 2002, 2006, 2007; Xu *et al.* 2007; Meng *et al.* 2008). A novel natural edible coating based on *Aloe vera* gel developed in Spain has been successfully used to maintain the quality and safety of table grapes during cold storage and subsequent shelf life (Valverde *et al.* 2005). This coating was very effective in reducing weight loss, which was related to the lower respiration rate of coated fruit, and delaying the natural evolution of other ripening-related attributes such as softening, colour changes and acidity loss. However, the most important benefits in treated grapes were the important reduction of rachis dehydration and the control of microbial spoilage, with a net reduction of the colony forming units (CFU) of yeasts and moulds present in the fruit. The results obtained after 28 days of cold storage at 1°C and 95% RH on uncoated controls and Aloe-treated 'Crimson Seedless' table grapes are presented (Fig. 2). In these tests, weight loss was obtained as the mean of 5 individual clusters (replicates) that had been weighed on the day of harvesting. Flesh firmness was determined in N using a texture analyser interfaced to a personal computer. For each berry, 1 cm² of the skin was removed and penetration force measurement was individually recorded using a 2 mm diameter probe. Penetration rate was 20 mm min⁻¹ for 10 mm after contacting the flesh, and the results were the mean of 50 determinations (10 berries for each cluster). For rachis, symptoms of dehydration and browning for primary and secondary branches were evaluated on a ranked scale of 1 to 5, where scores 1, 2, 3, 4, and 5 referred to absence of these symptoms, slight occurrence, moderate, severe, and extremely severe browning and dehydration, respectively. For the microbiological analysis, samples of 10 g from each cluster were obtained under sterile conditions and homogenized in sterile peptone water using a stomacher. Serial dilutions were carried out, and 1 mL was added to plate count agar for yeast and mould counts. Samples were prepared in triplicate and the plates were incubated for 5 days at 25°C. The same procedure was performed with recently harvested berries and after 28 days of cold storage at 1°C. The sensory analysis of the berries rendered higher scores on treated bunches for visual aspect, firmness, crunchiness, juiciness and sourness, although sweetness was higher on control berries due to increased sugar concentration caused by increased weight loss. Apart from these organoleptic quality parameters, additional research (Serrano *et al.* 2006) showed that the application of *Aloe vera* gel coating was also an effective postharvest technology to maintain the functional properties of table grapes during cold storage. While the contents of total phenolics and ascorbic acid in coated 'Crimson Seedless' grapes were effectively maintained after 35 days of storage at 1°C followed by 4 days of shelf life at 20°C, with a higher retention of total antioxidant activity, the loss of these compounds was clear in con-

trol uncoated berries, which showed an accelerated ripening process during storage revealed by a significant increase in anthocyanin content.

CONCLUSIONS AND FUTURE TRENDS

Table grapes are non-climacteric fruit that, depending on the cultivar, can be stored for several months at temperatures surrounding 0°C and high RH conditions. However, major factors limiting grape storage and shelf life are cluster dehydration and microbial spoilage. Moreover, important fruit quality attributes such as rachis condition, skin colour, flesh firmness, or MI are soon detrimentally affected by adverse environmental conditions during storage and/or transportation. Gray mould, caused by *B. cinerea*, the most economically important postharvest disease of table grapes worldwide, has been successfully controlled for many years by the use of different sulphur dioxide (SO₂) technologies. Nevertheless, issues related with excessive residues and high incidence of phytotoxicities caused by this pesticide (berry bleaching, hairline cracks) are increasingly promoting extensive research to, in the first place, reduce the commercial SO₂ dosages and, secondly, find and implement safe and cost-effective alternative treatments.

Among the physical, chemical and biological alternative methods for decay control and table grape quality preservation, active packaging based on the addition of antifungal essential oils to MAP systems and development of antifungal natural edible coatings are two of the most promising and interesting approaches. Recent research results showed that the release of pure essential oils such as thymol, carvacrol, eugenol, or menthol in N-OPP packages significantly controlled the development of *B. cinerea* in both *in vitro* and *in vivo* experiments. Furthermore, this innovative active packaging effectively reduced respiration rate and ethylene production of treated berries delaying fruit softening and colour evolution and maintaining organoleptic properties. Further studies are needed to better understand the mechanism(s) by which these essential oils affect the grape physiology modulating the ripening process as well as their ability to inhibit microbial growth. Likewise, other possible use of essential oils could be their combination with other tools that modified the atmosphere surrounding the fruit such as edible coatings or some physical postharvest treatments.

An innovative natural edible coating based on *Aloe vera* gel effectively preserved the most important quality attributes (fruit weight loss, flesh softening, peel colour changes, rachis browning, TA, MI, sensory quality) of table grapes during storage at low temperature while satisfactorily reduced microbial populations in the fruit and controlled postharvest decay. In addition, the application of the coating significantly retained functional properties of cold-stored table grapes like total antioxidant activity. Following this track, there is currently a clear trend for the development of simple or composite natural edible coatings with either inherent microbicidal activity or the addition of antifungal ingredients (essential oils, food preservatives, antagonistic microorganisms, etc.) in order to provide, on the one hand, significant fruit senescence retardation and, on the other hand, effective disease control and overall food safety. Despite the substantial research progress that has been accomplished, a strong impulse in the development of these novel technologies is required in the future for commercial application, since antifungal active packaging, natural edible coatings, combinations of both methods or their integration with other non-polluting physical, chemical or biological postharvest treatments in IDM programs are emerging concepts in horticultural technology that may confer many benefits which fulfils consumer demand for safe products avoiding the use of contaminating synthetic chemicals as a means of preservation. In contrast to these conventional pesticides, typically characterized for high curative and preventive fungicidal activity and persistence, the mode of action of natural antimicrobials is rather fungistatic and not

so persistent, which may restrain their use as stand-alone treatments and made their performance more variable and dependent on species, cultivar, and physical and physiological condition of treated fruit. New oriented research efforts should provide appropriate tools to suit the application of these natural treatments to commercially important table grape cultivars and particular postharvest handling conditions according to specific industry needs.

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