

Antimicrobial Treatments to Preserve Packaged Ready-to-Eat Table Grapes

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Summary

Ready-to-eat table grapes are a product with severe shelf life problems. Mass loss, colour changes, accelerated softening and mould proliferation can greatly influence quality decay of berries. Hence, the effects of different dipping treatments on the quality of packaged ready-to-eat table grapes have been successfully assessed. Various antimicrobial compounds (*trans*-2-hexenal, potassium sorbate, eugenol, cinnamon bark oil and ethanol) were used at different concentrations for dipping grape berries prior to packaging. All the samples were packaged in biaxially oriented polypropylene and stored at (4±1) °C. During the storage period, headspace gas composition, spoilage microorganisms, appearance of visible moulds and sensory quality were monitored. The composition of the headspace gas was typical of a non-climacteric fruit, thus demonstrating that the antimicrobial compounds did not affect product respiration rate. For the entire storage period, the bacterial counts did not grow significantly. On the contrary, mould proliferation on the product surface affected sensory properties until provoking product unacceptability. Relevant differences were found between the dipped and undipped samples. In particular, 50 % ethanol or 20 % ethanol combined with potassium sorbate (3 %) seemed to be very effective in preventing mould proliferation, thus promoting an increase in shelf life by about 100 %.

Key words: antimicrobial compounds, ready-to-eat table grapes, packaging, shelf life

Introduction

Fruit and vegetables are important components of healthy diet and, if consumed daily in sufficient amount, could help to prevent major diseases, such as cardiovascular problems and certain cancers (1). The beneficial effects of fruit and vegetables have been attributed to the presence of vitamins and antioxidants that act as receptors for free radicals. A potential strategy to increase their use is the replacement of traditional snacks with new ready-to-eat fresh products.

Table grapes are a fruit with important constituents, such as soluble sugar in high percentage (around 18 %),

vitamin C and potassium. Moreover, table grapes contain copious amounts of polyphenols, flavonol glycosides and phenolic acids that generally exert antioxidant activity and health benefits (2,3). However, ready-to-eat grapes have a limited shelf life mainly due to mass loss, browning of the rachis and softening of berries (4). Additionally, berry decay is attributed to high incidence of mould development (5).

In recent years, the use of natural compounds, such as volatile plant products and essential oils, is an alternative to the traditional synthetic additives. Natural compounds can prevent bacterial and fungal growth and improve the shelf life of fresh-cut products (6). In par-

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ticular, volatile plant products, such as *trans*-2-hexenal, exhibited a significant inhibitory effect against pathogenic microorganisms frequently isolated from raw materials (7,8). It is also well known that the essential oils have a pronounced antimicrobial effect (9). In particular, literature data report the use of essential oils to improve the shelf life and safety of minimally processed fruits (10,11). Examples of application have also been reported in cheese (12), bakery products (13), meat (14) and fish (15). Among essential oils, eugenol and cinnamon bark oils exhibited good antimicrobial activity against bacteria, fungi and yeasts (16,17). The combination of natural compounds and traditional preservatives is a way to amplify the antimicrobial effects with a reduced concentration of synthetic compounds (18,19). Ethanol and potassium sorbate are common food additives with potent antimicrobial activity (19). Recently, it has been shown that dipping berries in ethanol prior to packaging inhibits their decay better than other washing treatments (20, 21). Moreover, a synergic activity of low concentrations of ethanol integrated with other compounds such as potassium sorbate to control mould development on table grapes was reported (19).

Headspace package conditions are another important factor to maintain freshness and extend the shelf life of ready-to-eat fruit. In particular, it has been widely demonstrated that an appropriate headspace gas composition, accounting for low oxygen and high carbon dioxide concentrations, achieved *via* the interaction between fruit respiration rate and gas transmission through the packaging film, can play an important role in preserving the quality of table grapes (22–24).

Therefore, the aim of this study is to evaluate the influence of different dipping treatments (*trans*-2-hexenal, potassium sorbate, eugenol, cinnamon bark oil and ethanol) on the extension of the shelf life of ready-to-eat seedless table grapes, packaged in a proper polymeric film. To assess the influence of both the washing treatments and packaging conditions on the investigated fresh produce, headspace gas composition, spoilage microorganisms and sensory quality were monitored during the storage period.

Materials and Methods

Sample preparation

A seedless cultivar of table grapes (*Vitis vinifera* L. cv. 'Supernova Seedless') was used. Fruit, immediately after harvesting, was directly transported to the laboratory and selected to obtain homogeneous clusters based on colour, size, lack of damage, healthy and greenish rachises. After the selection, the samples were washed for 1 min in chlorinated water (20 mL/L) to remove residues. Then, the berries were hand removed from the rachis and dipped for 5 min in different antimicrobial solutions containing *trans*-2-hexenal, potassium sorbate, eugenol and cinnamon bark oil, respectively. In particular, *trans*-2-hexenal, eugenol and cinnamon bark oil (all from SAFC, Sigma-Aldrich, Hamburg, Germany) were tested in concentrations of 1000, 2000 and 3000 mg/L. Dipping solutions with 1, 2 and 3 % of potassium sorbate (Fluka, Sigma-Aldrich, Seelze, Germany) with 20 %

ethanol (C. Erba, Milan, Italy) were also used. In order to enhance the water solubility, *trans*-2-hexenal, eugenol and cinnamon bark oil were dissolved in ethyl alcohol (96 %) and then diluted with distilled water. In particular, *trans*-2-hexenal was diluted with 20 % (by volume) of ethyl alcohol, cinnamon bark oil and eugenol with 50 % (by volume) of ethanol. As the controls, samples of berries dipped in 20 and 50 % ethyl alcohol were also used. As further control, berries without any dipping treatment were prepared, too. After dipping, the berries were air dried and packaged (100 g) in a biaxially oriented polypropylene film with a thickness of 60 µm (OPP 60), purchased from Icimendue (Napoli, Italy). All the samples were stored at (4±1) °C. For the sake of clarity, the investigated samples were grouped in two batches (batch 1 and batch 2). In particular, batch 1 denotes the set of samples dipped in *trans*-2-hexenal (Tra-1000, Tra-2000, Tra-3000), potassium sorbate (KSor-1, KSor-2, KSor-3) and in 20 % ethanol (Cnt-20). On the other hand, batch 2 includes the set of samples dipped in eugenol (Eug-1000, Eug-2000, Eug-3000), cinnamon bark oil (Cin-1000, Cin-2000 and Cin-3000) and 50 % ethanol (Cnt-50). As the controls, samples of berries without any dipping treatment (No-dip-1 and No-dip-2) were also labelled.

Analysis of headspace gas composition

Oxygen and carbon dioxide headspace volume fractions of packaged grapes were measured using a gas meter (Checkmate 9900; PBI Dansensor, Ringsted, Denmark). The volume taken from the package headspace for gas analysis was about 10 cm³. To avoid modifications in the headspace gas composition due to gas sampling, each package was used only for a single measurement. Three packages of each sample were used at each sampling time. Results were expressed as percentage of oxygen and carbon dioxide measured in each bag.

Permeation tests

It is still not possible to make permeation tests at a temperature lower than 10 °C by using the existing equipment; therefore, gas permeation tests were conducted at three different temperatures (10, 16 and 23 °C) and the permeability values at 4 °C were obtained by extrapolation using the Arrhenius equation (25). In particular, the oxygen transmission rate (OTR) was determined by means of an OX-TRAN[®] (Model 2/20; Mocon, Minneapolis, MN, USA). Two samples of each film with a surface area of 5 cm² were tested at 10, 16 and 23 °C and relative humidity (RH) was 0 % at the upstream and the downstream side of the sample. The carbon dioxide transmission rate (CDTR) was determined by means of a Permatran (Model C 4/41; Mocon). Two samples of each film with a surface area of 5 cm² were tested at 10, 16 and 23 °C and 0 % RH at the upstream and the downstream side of the sample. OTR and CDTR values were expressed as fm/(Pa·s).

Microbiological analyses

For microbiological analyses, about 20 g of sample were aseptically removed from each package, placed in a stomacher bag, diluted with 0.9 % NaCl solution and homogenized with a stomacher LAB Blender 400 (Pbi

International, Milan, Italy). Serial dilutions in sterile saline solution were plated onto appropriate media. The media, all from Oxoid (Milan, Italy), and test conditions were the following: plate count agar (PCA), modified by adding 0.17 g/L of cycloheximide (Sigma-Aldrich, Milan, Italy) after autoclaving, incubated at 32 °C for 48 h, for total bacterial count; Sabouraud dextrose agar, supplemented with chloramphenicol (0.1 g/L) (C. Erba), incubated at 25 °C for 48 h, for yeasts; de Man Rogosa Sharpe (MRS) agar, modified by adding 0.17 g/L of cycloheximide after autoclaving, anaerobically incubated at 30 °C for 4 days, for lactic acid bacteria; and potato dextrose agar (PDA), supplemented with chloramphenicol (0.1 g/L), incubated at 20 °C for 7–10 days, for moulds. For replicates, three packages of each sample were used. Results were expressed as log CFU per g of table grapes. The value of $5 \cdot 10^7$ CFU/g was selected as the threshold for microbial acceptability, as suggested by the French Regulation (26,27).

Sensory analysis

A trained panel of 7 judges carried out a sensory analysis of the ready-to-eat table grapes. Panelists had at least several years of experience in the evaluation of fresh-cut fruit prior to this study. The panelists were retrained for ready-to-eat table grapes in two sessions held over two days (2-hour session per day). Appropriate descriptive terms for sensory evaluation were decided during the retraining sessions. Odour, colour, firmness and overall quality were selected as the main quality attributes. The intensity of each attribute was quantified on a scale from 1 to 5, where 1 corresponded to 'completely refused' and 5 to 'completely accepted', according to a similar procedure reported in the literature for fresh-cut products (28). Scores below 3 for any assessed attributes were considered as an indicator of the end of the acceptable quality. During the evaluation, the assessors were also asked to search for the presence of visual moulds, thus allowing determining the day between the latest storage time at which moulds were not visible and the earliest storage time at which moulds were visible, hereinafter referred to as VMT (visual mould time). Sensory evaluation was carried out using individual booths (located away from the sample preparation area) under normal light. Each sample was assigned with three-digit random numbers and served to each panellist. A glass of water was provided to cleanse the palate between samples.

In order to determine the sensory acceptability limit in terms of overall quality, the following modified version of Gompertz equation (26,27) was fitted to the sensory data:

$$OQ(t) = OQ_{\min} - A^Q \cdot \exp \left[-\exp \left\{ \left[\mu_{\max}^Q \cdot 2.71 \frac{\lambda^Q - SAL^{OQ}}{A^Q} + 1 \right] \right\} \right] + A^Q \cdot \exp \left[-\exp \left\{ \left[\mu_{\max}^Q \cdot 2.71 \frac{\lambda^Q - t}{A^Q} + 1 \right] \right\} \right] \quad /1/$$

where $OQ(t)$ is the table grape berry overall quality at time t , A^Q is related to the difference between the overall quality obtained at the stationary phase and the initial value of the berry sensory quality, μ_{\max}^Q is the maximal

rate at which $OQ(t)$ decreases, λ^Q is the lag time, OQ_{\min} is the threshold value, SAL^{OQ} is the sensory acceptability limit in terms of overall quality (*i.e.* the storage time at which the $OQ(t)$ equals OQ_{\min}), and t is the storage time. The value of OQ_{\min} was set equal to 3.

Statistical analysis

The SAL^{OQ} values as well as the shelf life values of all the investigated samples were compared by one-way ANOVA analysis. A Duncan's multiple range test, with the option of homogeneous groups ($p < 0.05$), was used to determine significance among differences. To this aim, STATISTICA v. 7.1 for Windows (StatSoft Inc., Tulsa, OK, USA) was used.

Result and Discussion

Headspace gas composition

Fig. 1 shows the headspace oxygen and carbon dioxide volume fractions plotted as a function of storage time for some samples of batch 1 and batch 2 (Tra-3000 and Eug-3000 as two treated samples and Cnt-20 and Cnt-50 as the two controls). As expected, for a nonclimacteric fruit such as table grapes, a slow decrease in oxygen volume fraction along with a slight increase in carbon dioxide were found. As also reported in the literature (22,29), the headspace gas composition is strictly related to both gas transmission rate of the film (Table 1) and respiration rate of the packaged grape. According to the results also reported in another work dealing with table grapes, the equilibrium gas volume fraction obtained in OPP 60 was better than that recorded in the films based on the same polymeric material but having different thickness (40 and 80 μm) (24), thus suggesting that OPP 60 is a proper packaging for table grapes. Comparable values of headspace gas volume fractions were also recorded for all the other dipped and undipped samples, thus confirming that the active compounds did not affect product respiration rate (4,21).

Table 1. Values of oxygen transmission rate (OTR) and carbon dioxide transmission rate (CDTR) of OPP 60 at different temperatures

Temperature/°C	OTR/($\text{fm}/(\text{Pa}\cdot\text{s})$)	CDTR/($\text{fm}/(\text{Pa}\cdot\text{s})$)
4	41.45	126.91
10	72.84	167.51
16	103.80	237.03
23	142.72	338.55

Shelf life evaluation

To determine a product shelf life, microbial quality, sensory properties and proliferation of visible moulds were taken into account. In accordance with scientific literature, shelf life was determined as the lowest value among the three above mentioned quality indices (26).

Table grapes generally do not suffer from microbial proliferation but the appearance of visible moulds on the surface (5,21). Different microbial cell load concen-

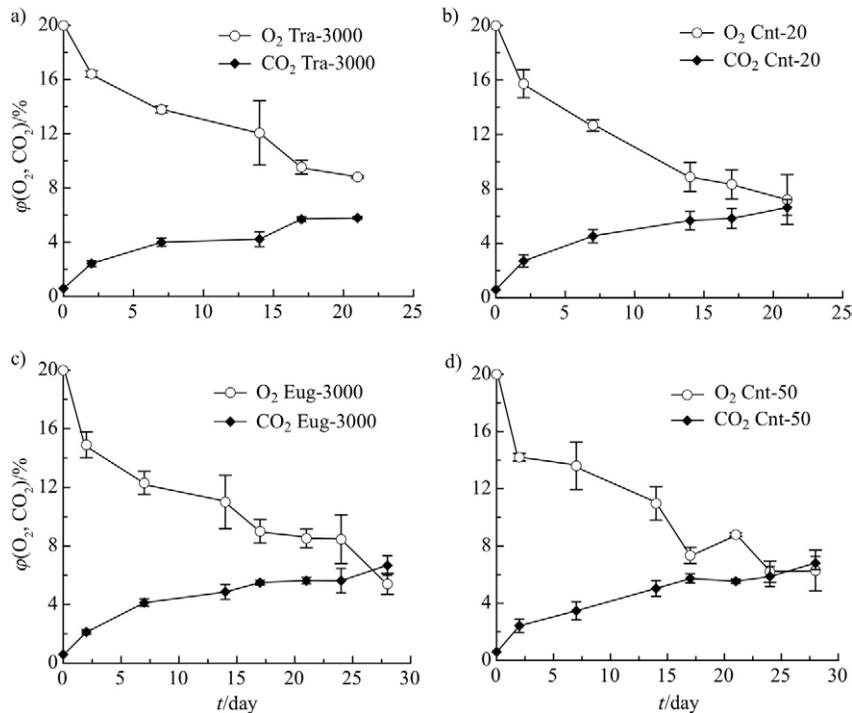


Fig. 1. Headspace O_2 and CO_2 volume fractions plotted as a function of storage time for grape berries dipped in: a) *trans*-2-hexenal 3000 mg/L and 20 % ethanol (Tra-3000), b) 20 % ethanol (Cnt-20), c) eugenol 3000 mg/L and 50 % ethanol (Eug-3000) and d) 50 % ethanol (Cnt-50)

trations have been reported in the literature for various table grape cultivars. This has been attributed to numerous factors, such as ambient conditions, postharvest handling procedure and natural variability of fruit (30). Moreover, grapes possess a very hard and smooth skin, which can protect the vulnerable inner tissue from yeasts and fungal invasion (31). According to most works in literature, also in the current study, cell loads of total mesophilic bacteria, lactic acid bacteria, yeasts and moulds, as revealed by plate counting, remained very low in all samples for the entire storage period. As an example, Fig. 2 shows the viable cell concentration of yeasts plotted as a function of storage time for batch 1 and batch 2 at the highest tested concentrations, along with their controls. As can be inferred from the data reported in the figure, for both batches the undipped samples show a high final cell load if compared to the treated samples. It is worth noting that some samples of batch 1 and batch 2 (*i.e.* No-dip-1, No-dip-2, Cnt-20 and Tra-3000 samples) were monitored for a short period of time, due to the detection of visible moulds on the product surface. Concerning batch 1, a better efficacy against yeast development was achieved by combining ethanol (20 %) with potassium sorbate (3 %). According to other works, no antimicrobial effects were detected when using ethanol 20 % (19). Results from batch 2 highlight that 50 % ethanol exerted a very pronounced effect on yeast growth, as also assessed by other authors (20,21,29,32). It is interesting to note that no differences were detected between Cnt-50 and the samples dipped in eugenol and cinnamon bark oil at 3000 mg/L. Moreover, no synergistic effect was found by combining these essential oils with 50 % ethanol. Similar trends of yeast proliferation were also obtained for all the other samples dipped in solutions at lower concentrations of active compounds.

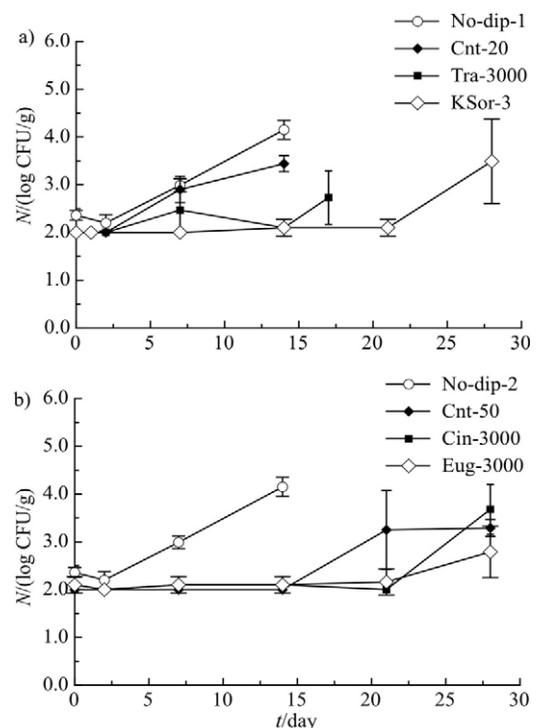


Fig. 2. Yeast growth plotted as a function of storage time for some samples of: a) batch 1 and b) batch 2

No-dip-1=undipped sample of batch 1; No-dip-2=undipped sample of batch 2; Cnt-20=sample dipped in 20 % ethanol; Cnt-50=sample dipped in 50 % ethanol; Tra-3000=sample dipped in *trans*-2-hexenal 3000 mg/L and 20 % ethanol; KSor-3=sample dipped in 3 % potassium sorbate and 20 % ethanol; Cin-3000=sample dipped in cinnamon bark oil 3000 mg/L and 50 % ethanol; Eug-3000=sample dipped in eugenol 3000 mg/L and 50 % ethanol

Tangible differences amongst samples were recorded in terms of the appearance of visible moulds (VMT in Table 2). As can be seen in Table 2, No-dip-1 and Cnt-20 became unacceptable because visible moulds appeared within two weeks of storage, whereas the use of *trans*-2-hexenal retarded the proliferation by some days, regardless of the compound concentration. Better results were recorded with potassium sorbate, which preserved the product for more or less one month. Other authors (19, 33) also proved that a combination of potassium sorbate and ethanol is effective against mould proliferation on table grapes. As regards samples of batch 2, it is interesting to note that ethanol alone at 50 % was enough to preserve grape berries for about one month. The addition of essential oils and in particular of eugenol at 3000 mg/L further delayed mould development by about 10 days. These results confirm the antimicrobial effects described for essential oils (4,10). The mechanism of action

Table 2. Overall quality (SAL^{OQ}) and values of visual mould time (VMT) of ready-to-eat grape samples

	Sample	SAL^{OQ} day	VMT day
Batch 1	No-dip-1	(12.3±1.4) ^a	12
	Cnt-20	(14.1±1.7) ^a	15
	Tra-1000	(16.8±1.2) ^a	19
	Tra-2000	(16.7±1.2) ^a	19
	Tra-3000	(16.6±1.3) ^a	19
	KSor-1	(18.9±2.3) ^{a,b}	26
	KSor-2	(21.7±2.0) ^b	26
	KSor-3	(24.4±4.7) ^b	31
Batch 2	No-dip-2	(12.3±1.4) ^a	12
	Cnt-50	(23.4±1.7) ^b	26
	Cin-1000	(26.5±2.9) ^b	31
	Cin-2000	(25.8±2.6) ^b	31
	Cin-3000	(23.7±3.8) ^b	31
	Eug-1000	(23.6±2.0) ^b	28
	Eug-2000	(22.8±2.5) ^b	28
	Eug-3000	(24.1±5.6) ^b	35

No-dip-1=undipped sample of batch 1; Cnt-20=sample dipped in 20 % ethanol; Tra-1000=sample dipped in *trans*-2-hexenal 1000 mg/L and 20 % ethanol; Tra-2000=sample dipped in *trans*-2-hexenal 2000 mg/L and 20 % ethanol; Tra-3000=sample dipped in *trans*-2-hexenal 3000 mg/L and 20 % ethanol; KSor-1=sample dipped in 1 % potassium sorbate and 20 % ethanol; KSor-2=sample dipped in 2 % potassium sorbate and 20 % ethanol; KSor-3=sample dipped in 3 % potassium sorbate and 20 % ethanol; No-dip-2=undipped sample of batch 2; Cnt-50=sample dipped in 50 % ethanol; Cin-1000=sample dipped in cinnamon bark oil 1000 mg/L and 50 % ethanol; Cin-2000=sample dipped in cinnamon bark oil 2000 mg/L and 50 % ethanol; Cin-3000=sample dipped in cinnamon bark oil 3000 mg/L and 50 % ethanol; Eug-1000=sample dipped in eugenol 1000 mg/L and 50 % ethanol; Eug-2000=sample dipped in eugenol 2000 mg/L and 50 % ethanol; Eug-3000=sample dipped in eugenol 3000 mg/L and 50 % ethanol. ^{a-b}Mean values followed by a different lower case superscript letter are significantly different $p < 0.05$)

of these compounds is attributed to their hydrophobicity, which enables them to partition in the lipids of the cell membrane to disturb the integrity and ion equilibrium (34).

As regards sensory properties, Fig. 3 reports some examples of overall quality decay during storage; the solid horizontal line in the figure represents the overall quality threshold. The curves shown in the graph were obtained by fitting the Eq. 1 to sensory data, whereas the values of SAL^{OQ} are listed in Table 2. As can be seen in Fig. 3, No-dip-1, Cnt-20 and Tra-3000 show a similar behaviour. The sensory attributes of these samples, and in particular the fruit firmness, were judged negatively after about two weeks of storage so the overall quality fell down to the threshold value. Differently, KSor-3 showed an overall quality above the threshold for about 25 days, thus demonstrating that the lack of visible moulds near the detachment point of the pedicel allowed a generally better fruit preservation. For batch 2, the undipped samples rapidly fell down below the threshold. Also in this case the lack of a proper washing treatment provoked the unacceptability of fruit within 2 weeks. On the contrary, dipped samples were monitored for a longer time. Dipping in 50 % ethanol highlighted that the treatment was effective not only to prevent mould proli-

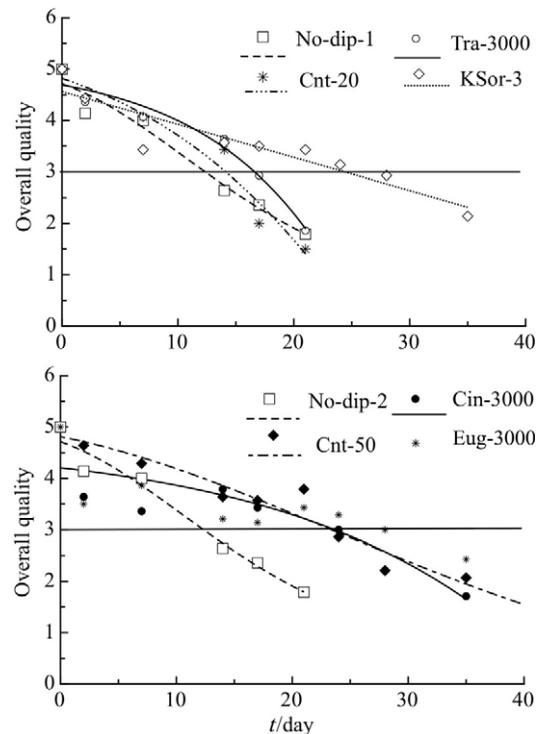


Fig. 3. Grape berry overall quality during storage time for some samples of: a) batch 1 and b) batch 2. The curves are the best fit of Eq. 1 to the experimental data; horizontal line represents overall quality threshold

No-dip-1=undipped sample of batch 1; No-dip-2=undipped sample of batch 2; Cnt-20=sample dipped in 20 % ethanol; Cnt-50=sample dipped in 50 % ethanol; Tra-3000=sample dipped in *trans*-2-hexenal 3000 mg/L and 20 % ethanol; KSor-3=sample dipped in 3 % potassium sorbate and 20 % ethanol; Cin-3000=sample dipped in cinnamon bark oil 3000 mg/L and 50 % ethanol; Eug-3000=sample dipped in eugenol 3000 mg/L and 50 % ethanol

feration but also to maintain product sensory acceptability. The addition of other compounds to the 50 % ethanol solution did not improve significantly the overall quality of grapes. In fact, similar effects were recorded at all tested concentrations and very comparable SAL^{0Q} values were calculated.

Given the above results, shelf life can be easily inferred. Since the microbial threshold was never reached, shelf life was assumed as the lowest value between the sensory quality (SAL) and visible mould appearance (VMT). As can be observed in Table 2, visible moulds affected the acceptability of grape berries; therefore, sensory properties affected the shelf life. The two detrimental phenomena are strictly related. In fact, the overall quality of table grapes fell down, above all changes in the product firmness and the decay were more pronounced when mould proliferation occurred (5,32). This is the case with the undipped samples. The sole 20 % ethanol (Cnt-20) did not improve significantly the shelf life if compared to the undipped grape. Similar results were also reported by Karabulut *et al.* (19). A slightly better effect was observed by combining 20 % ethanol with *trans*-2-hexenal, without any difference between volume fractions. On the contrary, a significant difference in respect to the undipped samples was recorded with potassium sorbate treatments. The efficacy was higher when the volume fraction of sorbate increased, thus reaching more than three weeks of shelf life. In the case of batch 2 the use of 50 % ethanol combined with cinnamon bark oil or eugenol promoted further control of moulds compared to the sole ethanol treatment but all samples exerted a similar trend in terms of sensory decay. Among odour, colour and firmness, product consistency and browning of the stem point were the two most compromised attributes. Therefore, shelf life values of all dipped samples of batch 2 were very comparable with each other and confirmed that the shelf life of table grapes can be prolonged to about one month.

Conclusions

Different antimicrobial compounds were tested against microbial and fungal proliferation in packaged minimally processed grape berries. From a microbiological point of view, the product maintained low cell loads but visible moulds proliferated on the surface in times that were different depending on the washing treatments adopted prior to packaging. In fact, the worst samples were the undipped grape berries. Ethanol 20 % and its combination with *trans*-2-hexenal slightly preserved the product. To the contrary, potassium sorbate and 20 % ethanol exerted a better control of mould development and sensory characteristics, thus promoting a significant shelf life prolongation (from 12 days for undipped to 24 days for dipped samples). Ethanol 50 % was effective, even alone, in maintaining product quality. The addition of essential oils seemed to further increase the shelf life, but the differences were not statistically significant. To sum up, the shelf life of ready-to-eat grape berries can be significantly prolonged by means of proper treatments in antimicrobial solutions and packaging in an OPP 60 film.

Acknowledgements

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