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Effect of microwave mild heat treatment on postharvest quality of table grapes

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All relevant data are within the paper and its Supporting Information files.

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Abstract: Table grapes are characterized by high susceptibility to mold development during post-harvest, mostly due to *Botrytis cinerea*. Microwave application on ready-to-eat product can represent an alternative to antifungal treatment. With the aim of identifying the maximum energy that can be applied on grape without detrimental effects a Central Composite Design was developed testing the application of 10 combinations of treatment time (seconds) and microwave power (Watt). As a result, energies above 8000 kJ negatively affected the sensorial quality of fresh product, both in the presence and absence of *B. cinerea* inoculum (10^6 log CFU g^{-1}). The physico-chemical parameters did not show significant differences, but two time/power combinations improved sensory quality of table grape, being selected for the subsequent packaging trial: 14 s/100 W and 80 s/100 W. Treatments were applied before or after packaging in polypropylene bags. At the end of storage period, 100 W applied for 80 seconds before packaging led to a better external appearance of the product than the other treatments, maintaining an intermediate level of mesophilic bacterial load and no significant differences in terms of nutritional quality. 80 seconds at 100 Watt combined with packaging can represent a valuable starting point for further experiments.

1. Introduction

Table grapes are characterized by high susceptibility to mold and rot development during prolonged postharvest storage and commercialization, often leading to a general decrease in overall bunch quality. Among other factors, fungal decay, mainly caused by *Botrytis cinerea*, is the principal responsible for deterioration with grey mold development (Williamson *et al.*, 2007; Ahmed *et al.*, 2018). After harvest, a favorable environment for the germination of fungal spores is created on berries surface, mainly for damaged fruits (De Simone *et al.*, 2020). For this reason, during post-harvest life of fruits and vegetables, processing technologies and biotechnologies aimed to provide physical, chemical, and biological hurdles to limit the development of undesired microorganisms

(Capozzi *et al.*, 2009). Conventional thermal processes can result in the reduction of nutritional and sensory quality of the product, due to slow heat transmission within the plant tissue. Application of mild thermal treatments aimed to control postharvest disease by means of microwave heating (Dar *et al.*, 2020), could allow to avoid the use of chemical compounds and therefore residues in the treated product and ensuring at the same time minimal environmental impact, thus representing a valuable alternative to traditional thermal processing. Microwaving ensures instead a fast and effective heat treatment reducing risk of injuries and decrease of nutritional compounds. However, oversized intensity of the treatment can induce an excessive temperature increase, resulting in a damage to the fresh plant tissue. Moreover, high temperatures could affect grape biochemical characteristics, for example losses of aroma-related compounds and development of oxidative processes (Modesti *et al.*, 2020). To date, a small number of studies deals with the use of microwave treatment on fresh produce (Karabulut and Baykal, 2002; Zhang *et al.*, 2004, 2006; Sisquella *et al.*, 2013), showing its efficiency in prolonging postharvest life of peaches, in which microwave inhibited growth of inoculated pathogens after 2 minutes, being also effective in controlling endogenous microflora with a very low decay incidence. As for nectarines, brown rot incidence was significantly reduced by microwaving to less than 14% versus 45% of untreated product. Treatment caused a delay of softening and internal damage. Zhang *et al*. (2006) observed that in pears treated for 2 or 3 min *Penicillium expansum* population was significantly lower than control samples without impairing quality of the fruits. Fresh-cut carrots, apples, and minimally processed bok choy were subjected to high power/short time treatments showing promising results. Microwave treatments maintained physical, chemical and sensory quality of fresh-cut carrots over storage period, reducing surface whitening and avoiding firmness modification, also enhancing bioactive compounds concentration. However, microbial growth was greater than control samples during shelf-life (Martínez-Hernández *et al.*, 2016). Application of 454 W for 5 s on bok-choy decreased respiration rate, decay occurrence and etiolation, while improving integrity of cell membrane with a final better quality of product (Song *et al.*, 2018). As for fresh cut apples, a significant microbial reduction was observed for treatment at 300 W for 35 s, with no detrimental effect on nutritional parameters and a slight decrease of visual quality (Colelli *et al.*, 2021). However, there is the need to deeply study this technology and its effect on the species of interest with the aim to keep intact the fresh-like characteristics of the product. Moreover, microwave application as a part of a hurdle technology and its application in combination with packaging could be recommended to avoid recontamination, thus the objective of this paper is to provide preliminary information concerning the effect of microwave heating on table grape quality, in terms of efficacy in maintaining physical and microbiological quality.

2. Materials and Methods

A preliminary test to select maximum microwave energy output to be applied on fresh table grape was carried out by means of a Central Composite Design (CCD). Treatment time (seconds) and microwave power levels (Watt) were considered as CCD factors, ten combinations were identified using the software StatGraphics Centurion XVI.I (StatPoint Technologies, Inc., USA) (Table 1). Table grape was divided into 100 g-batches and treated according to the experimental design, using a solid-state microwave oven at a laboratory scale (2450 MHz, maximum power 1000 W). Processed products were stored in air at 5±1°C and 95% RH up to 14 days after treatment. Each combination was performed once, being the statistical variability already considered during designing of the experimental CCD plan. The experiment was also conducted on samples previously inoculated by

Table 1 - Treatment time (seconds) and microwave power levels (Watt) according to an experimental plan based on Central Composite Design 2^{2+} star with two central points

Run	Treatment time (seconds)	Microwave power levels (Watt)
1	14	100
2	47	32
3	94	265
4	47	265
5	47	498
6	0	265
7	14	430
8	47	265
9	80	430
10	80	100

immersion in a 1x10^6 spores/mL solution of *Botrytis cinerea* CECT 20973 purchased from the Spanish Type Culture Collection (CECT, Paterna, Spain), and stored at low temperature for 7 days+7 days of room temperature shelf life. For not inoculated samples, immediately after the treatment and after 7 and 14 days, the main physicochemical and microbiological parameters were evaluated, while inoculated samples were evaluated only for external aspect due to the massive presence of *B. cinerea* that could affect in a non-realistic way the quality of these latter samples. Obtained results were subjected to the specific statistical analysis for CCD using the software StatGraphics, to create estimated response surface plots. The second trial was subsequently performed on uninoculated samples using the two most effective combinations to understand the best moment for microwave heating application throughout the minimal processing. Ready-to-eat table grape bunches (100 g) were subjected to treatments before or after packaging within polypropylene (PP) bags (10x15 cm), and subsequently stored at 5°C up to 14 days. During storage (at initial time and after 7 and 14 days), physicochemical, microbiological, and organoleptic evaluations were carried out and results were subjected to statistical analysis. The treatments were as follows: CTRL: samples not treated; *LowMW*: microwave treatment at 14 s/100 W and subsequent application of the packaging; *HighMW*: microwave treatment at 80 s/100 W and subsequent application of the packaging; *LowMW PP*: microwave treatment at 14 s/100 W on packed samples; *HighMW PP*: microwave treatment at 80 s/100 W on packed samples. All the treatments were performed in triplicate.

Ranking test

First of all, ranking test was performed on samples treated as described for CCD to select the maximum microwave output energy to be applied on the products without detrimental effect. Seven trained panelists were asked to rank the samples according to their preference, evaluating the fresh-like appearance of the product and its organoleptic properties and the presence of typical fresh-like flavor, by assigning a score (from 1 to 10 considering each sample as a whole, based on the following characteristics: fresh-like appearance and organoleptic properties and the presence of typical fresh-like flavor). The sum of the score given by each panelist was compared with preset values to statistically evaluate the differences between the samples in terms of the tested parameters.

Fungal strain and growth condition

Botrytis cinerea CECT 20973, purchased from the Spanish Type Culture Collection (CECT, Paterna, Spain), was used to inoculate samples. Cryopreserved cultures were plated on Potato Dextrose Agar (PDA, Oxoid), and incubated at 25°C for 5 days. Fungal spore suspension was prepared by brushing the plates surface with saline solution (8.6 g L^{-1} NaCl) supplemented with 0.01% Tween 80 using a sterile swab and stored at 4°C for short-term uses. Fungal spores concentration was determined by plating serial dilution on PDA plates and adjusted to approximately 1 x 10^6 spores/mL.

Microbial load determination

Grape berries from each replicate were diluted (1:10) with NaCl 8.6 g L^{-1} solution, and homogenized in a blender (Bag Mixer, Interscience, Saint-Nom-la-Bretéche, France) for 2 min. Then, samples were submitted to tenfold serial dilution. Mesophilic microorganisms were enumerated by plate counting on Plate Count Agar (PCA) and incubated at 25 for 48 h. Yeasts and molds were plated on Potato Dextrose Agar (PDA) (Oxoid) added with chloramphenicol (100 mg L^{-1}) and incubated at 30°C for 48 h.

Ascorbic acid, dehydroascorbic acid and total vitamin C determination

Ascorbic acid, dehydroascorbic acid and total vitamin C amounts were assessed homogenizing 5 grams of fruit tissue with 5 ml of extraction medium (MeOH: H_2O (5:95) plus citric acid (21 g L^{-1}) with EDTA (0.5 g L^{-1}) and NaF (0.168 g L^{-1}). The homogenate was filtered, centrifuged, and the supernatant was recovered. Ascorbic acid (AA) and dehydroascorbic acid (DHAA) contents were determined through HPLC analysis (Agilent Technologies 1200 Series; Agilent, Waldbronn, Germany) as described by Zapata and Dufour (1992) with some modifications. AA and DHAA contents were expressed as mg of ascorbic or dehydroascorbic acid per 100 g of fresh weight. Vitamin C content corresponds to the sum AA+DHAA and was expressed as mg 100 g fw^{-1}.

Color analysis and determination of the berries' temperature

Color of berries surface was measured on 5 berries per each replicate using a spectrophotometer (CM 2600d, Konica Minolta, Japan) in the reflectance mode with the CIE L*a*b* color scale. Immediately after the treatment, berries surface temperature was acquired using thermal imaging camera Flir C5 (Teledyne Technologies, Wilsonville, Oregon, USA).

Texture

Berries firmness evaluation on 5 berries from each replicate was performed using a TA-XT2 Texture Analyzer (Stable Micro Systems Ltd., England, UK) by measuring the maximum compression force at a rate of 1.5 mm s^{-1}.

Sensorial quality

A panel of six trained people evaluated external appearance and overall rating of bunches, berries, and rachis of the stored product from each replicate at each sampling day. It was used a hedonic scale associated to a brief description corresponding to a score from 1 to 5, where 1= really poor and 5=excellent, being 3 the limit of marketability and 2 the limit of edibilit.

Gas analysis

Oxygen (O_2) and carbon dioxide (CO_2) concentrations, expressed as kPA, inside plastic bags containing table grapes were monitored over storage time by using a hand-held gas analyzer (CheckPoint, PBI Dansensor) to measure gases concentration in 15 ml of headspace.

Statistical analysis

Data were subjected to a two-way ANOVA (for treatment and sampling time), and means were separated by Tukey's test at P<0.05 (5% significance level) using Stat Graphics Centurion XVI.I software. Mean values within each sampling were separated applying Tukey's test with significant difference when P≤0.05.

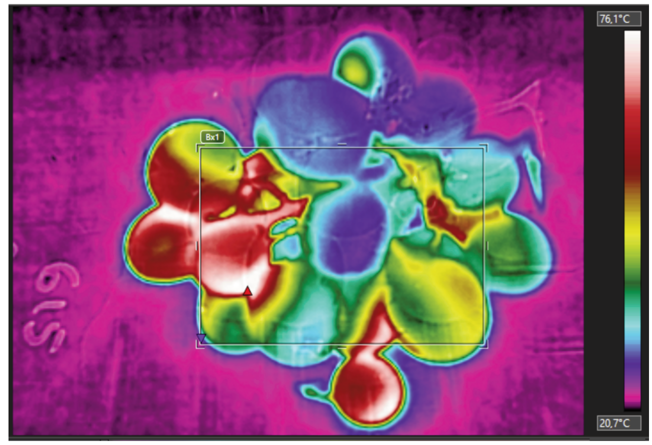

Fig. 1 - Example of temperature distribution on the bunch after microwave heating.

Table 2 - Minimum, maximum, and average temperature for samples treated as described by the experimental plan of the CCD

Run	Maximum temperature (°C)	Minimum temperature (°C)	Average temperature (°C)
Initial T	20.3	15.2	17.5
1	29.7	20.4	22.6
2	47.3	20.9	23.3
3	65.9	22.9	37.7
4	68.7	21.7	30.9
5	68.0	23.1	37.7
6	22.2	17.4	19.1
7	42.5	19.9	24.3
8	70.8	21.3	29.5
9	81.1	23.2	44.7
10	60.5	21.1	27.3

3. Results and Discussion

First of all, as from ranking test results, it was observed that samples from treatment 1 and 9, treated with 14 s/100 W and 80 s/430 W, respectively, were significantly different from the other, showing better and worse characteristics than the other treatments, respectively. Specifically, sample number 9 reached, after treatment, a maximum temperature of 81°C, with an average of 44.7°C, consequently the panelists evaluated this sample negatively, highlighting the significant presence of cooked flavor. In general, grape temperature during treatment increased very heterogeneously among all samples (Fig. 1, Table 2). In fact, as observed by many researchers, microwave heating often leads to the creation of hot spots in several product zones, depending on its geometry, thickness and dielectric properties, which are in turn dependent on the moisture content, and starting temperature of the food (Ho and Yam, 1992; Buffler, 1992; Zhou *et al.,* 1995; Campanone and Zaritzky, 2005; Vadivambal and Jayas, 2010). The increase in temperature was progressive, even if not proportional, to the increase in the total energy supplied to the product as expected by the treatment. Consequently, the results of the CCD showed that the more the microwave energy, the more the damage to the fresh product, leading to a worsening of the organoleptic quality of ready-to-eat table grapes for energies above 8000 kJ, both in the presence and absence of *B. cinerea* inoculum (10^6 log CFU g^{-1}). However, the chemical and physical parameters of uninoculated samples, did not show significant differences (data not shown), for this reason the following best treatments from a sensory point of view, as reported in figure 2, were selected for the subsequent packaging trials: 14 s/100 W and 80 s/100 W, respectively, treatment 1 and 10. Moreover, this latter evidence was associated with a slight reduction of the total mesophilic bacterial load of the selected samples treated with combination 1 and 10 (14/100 and 80/100), equal to 0.2 log and 0.6 log respectively, compared to the untreated sample, even if not statistically significant (data not shown). The selected treatments allowed to maintain a high visual quality score, above the limit of marketability, up to 14 days of storage. It is possible to observe that treatments 3, 5 and 9 caused instead a severe deterioration of table grape appearance, resulting not to be suitably

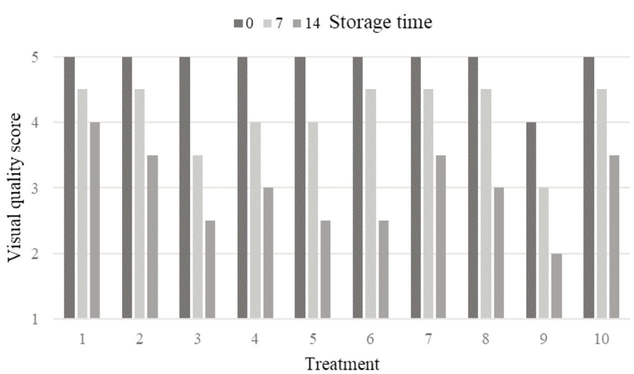

Fig. 2 - Visual quality score evolution during storage time for uninoculated table grape bunches treated as described in Table 1.

applied on fresh product. There is a great lack of existing literature regarding the application of microwaves on grapes intended for fresh consumption, therefore these preliminary data were used as starting point for the next experiment, and it is difficult to compare them with other data for the same product. However, similar results were observed for different products such as fresh cut carrots and apples as reported by Martínez-Hernández *et al.* (2016) and Colelli *et al.* (2021). Authors stated that high energy treatments could detrimentally affect quality of fresh product. For this reason, it became crucial to carry out the reported preliminary screening, preferably based on a statistical approach such as that of the CCD which allows to test a large number of treatments at different energies to obtain a complete picture of the effects of microwaving on the product.

As for the second trial, Table 3 shows the results of the two-way analysis of variance, pointing out how storage time significantly influenced all the evaluated parameters while microwave intensity, combined or not with packaging, affected the sensory aspects described as visual appearance of bunches, berries and rachis, and some of the physico-chemical parameters. Moreover, the interaction between the two factors influenced several quality aspects of table grape subjected to microwave heating and stored up to two weeks. On the other hand, however, as described below, the significant effects of the treatment on the qualitative aspects of the product are lost during storage and, for most of the evaluated

Table 3 - Effect of microwave treatment, storage time and their interaction on physico-chemical, sensory and microbiological attributes of table grape stored up to 14 days

	Control	LowMW	LowMW PP	HighMW	HighMW PP	Treatment (A)	Storage time (B)	Interaction (A X B)
Total mesophilic load (logCFU g^{-1})	3.05±0.57	3.02±0.48	3.02±0.56	3.16±0.30	2.97±0.29	NS	****	NS
Yeasts and molds (logCFU g^{-1})	3.25±0.36 b	3.32±0.23 b	3.40±0.35 ab	3.55±0.24a	3.23±0.41 b	**	****	*
Ascorbic acid (mg 100g^{-1})	0.62±0.39	0.58±0.22	0.48±0.35	0.62±0.43	0.72±0.39	NS	****	NS
Dehydroascorbic acid (mg 100 g^{-1})	2.04±0.32	1.95±0.17	1.74±0.24	1.98±0.20	1.75±0.41	*	*	*
Vitamin C (mg 100g^{-1})	2.66±0.64	2.54±0.32	2.23±0.47	2.60±0.50	2.48±0.73	NS	****	NS
O_2 (kPa)	11.02±8.38	10.14±8.31	10.40±8.52	10.62±7.95	10.83±7.98	NS	****	***
CO_2 (kPa)	6.00±4.79 bc	6.72±5.07 a	6.65±5.39 ab	6.60±5.08 abc	5.95±4.56c	**	****	***
Bunch appearance (Score)	4.17±0.48 b	4.50±0.43 a	4.50±0.41 a	4.50±0.28 a	4.33±0.57 ab	**	****	***
Berries appearance (Score)	4.08±0.61 b	4.58±0.47 a	4.58±0.33 a	4.58±0.35 a	4.25±0.48 b	***	****	**
Rachis appearance (Score)	4.42±0.56 ab	4.50±0.43 a	4.42±0.35 ab	4.50±0.43 a	4.33±0.66 b	**	****	****
Firmness (N)	0.47±0.11 ab	0.57±0.17 a	0.47±0.09 b	0.50±0.12 ab	0.45±0.05 b	*	****	**

Mean values ± standard deviations of 9 samples are reported (3 replicates x 3 storage times). ****= P≤0.0001; *** = P≤0.001; **= P≤0.01; *= P≤0.05; NS, not significant.
LowMW = 14 s/ 100 W; HighMW = 80 s/100 W; PP= polypropylene bags.

parameters, the differences resulted to be not significant after 14 days.

The most interesting results were reached in terms of maintenance of the sensorial quality of the fresh product. Specifically, all the samples, including not treated one, were characterized by a very high sensorial quality even after two weeks of storage within passive modified atmosphere packaging. However, HighMW sample, treated at 100 W for 80 sec and then packed, showed the highest rating due to the very fresh-like appearance of its berries and globally, its bunches, at the end of storage time. It is widely recognized that ready-to-eat and fresh-cut products should be visually free from defects, clean, with no presence of soil or off odor up to the end of the storage time, moreover, the entire bag content should be edible without no further requirement before consumption (Barrett *et al.*, 2010). Consequently, visual appearance of fresh table grapes and fresh product in general, represents the first aspect influencing the consumers decision, and size, color, visual quality, and external appearance in general are used to describe it (Musacchi and Serra, 2018). In this context, it was observed in the present work that microwave treatment was able to better maintain the visual appearance of table grape during storage when packed and stored as ready-to-eat product, representing a starting point for subsequent applications and optimization of this technique on fresh produce. From a microbiological point of view, no statistically differences were observed between samples at the end of storage time, neither due to the different intensity of the treatments nor to the presence/absence of the packaging film during microwave treatment. In figure 3 it is possible to observe how, concerning mesophilic microorganisms, a lower load, even if not significant, was maintained after 14 days of storage for all the treated samples, compared to untreated, reaching the latter the highest value equal to 3.59 log CFU g^{-1}. Final values for LowMW, LowMW PP, HighMW, HighMW PP were 3.29, 3.42, 3.34 and 3.06, respectively, with HighMW PP sample showing the highest difference compared to control (0.54 log CFU g^{-1}). Low efficacy of the treatment in terms of reduction of the microbial content can be related to non-uniform heating process, thus leading to the incomplete action on to the microorganisms due to uneven distribution of temperature (Vadivambal and Jayas, 2010). Little literature existing on ready-to-eat samples in order to compare results. Colelli *et al.* (2021) observed that 35 s/300 W

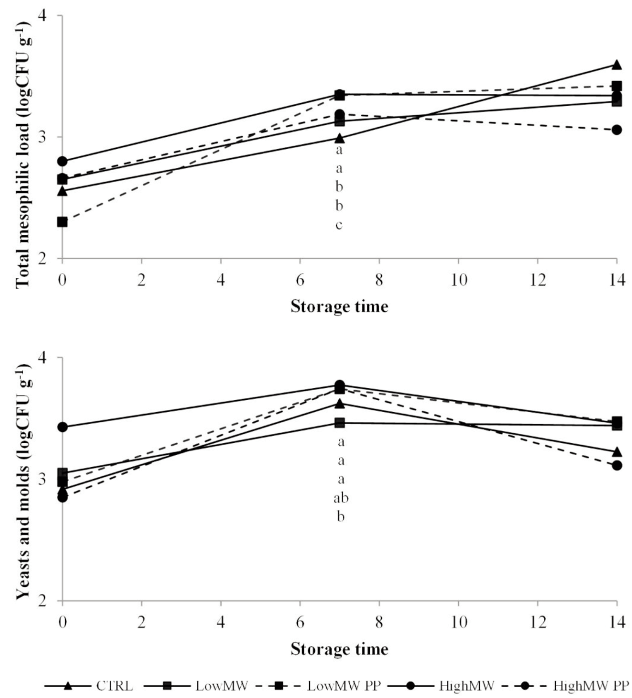

Fig. 3 - Total mesophilic and yeasts and moulds loads evolution over time on table grape samples untreated (CTRL) and subjected to low (14 s/100 W) and high (80 s/100 W) microwave treatment before (LowMW, HighMW) and after polypropylene packaging application (LowMW PP, HighMW PP). Means with different letters at the same time of storage are significantly different according to Tukey's test (P≤0.05).

on fresh-cut apples allowed to reach a 2-log reduction in mesophilic load at the end of storage time, however, the higher microwave intensity resulted in the appearance of side effects on nutritional quality. Otherwise, as reported by Martínez-Hernández *et al.* (2016) 60 s/900 W microwave treatment applied on fresh-cut carrots led to an initial microbial reduction, followed by an increment during storage mainly due to the detrimental effect on plant tissue caused by an excessive temperature increase. As demonstrated by the present experiment, using a well-modulated microwave energy, allowed at least to maintain a good visual quality of ready-to-eat table grape (Fig. 4), without significant reduction of nutritive compounds and firmness. Moreover, it was not possible to correlate the differences in the visual appearance with the different gaseous concentration within the plastic bags, in fact about 3 kPa of oxygen and 11 kPa of CO_2 were reached at the end of storage time for all the samples. This probably contribute to the maintenance of grape quality, also Cefola and Pace (2016) reported the beneficial effect of high CO_2 concentra-

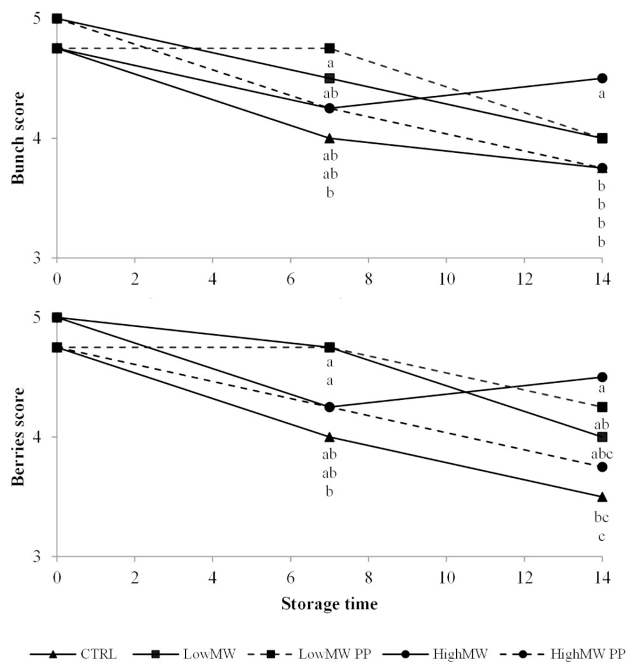

Fig. 4 - Bunches and berries sensorially evaluated score over storage time for table grape samples untreated (CTRL) and subjected to low (14 s/100 W) and high (80 s/100 W) microwave treatment before (LowMW, HighMW) and after polypropylene packaging application (LowMW PP, HighMW PP). Means with different letters at the same time of storage are significantly different according to Tukey's test (P≤0.05).

tion during storage on 'Italia' table grapes, both in terms of sensory quality preservation and decay control. It is therefore possible to state that the treatments, at the applied intensities, did not influence the respiratory rate of table grape and consequently there are no differences concerning metabolic activity and physiological aging. As for ascorbic and dehydroascorbic acid, it was observed a physiologically slightly decreasing trend, without however differences due to the intensity of the treatment (data not shown). Similar results were also observed for different table grape varieties when stored as minimally processed products (Nicolosi *et al.*, 2018).

4. Conclusions

At the end of the storage period, sample subjected to 100 W microwave power for a treatment time of 80 seconds and subsequently packed, showed a better external appearance than the other treatment and the control samples, however maintaining an intermediate level of mesophilic bacterial load. No significant differences in terms of nutritional quality were observed. The time/power combination identified with this preliminary experiment and its combination as hurdle technology with packaging can represent a valuable starting point for further experiments aiming at identifying a mild microwave treatment to be applied to improve table grapes quality and safety. Their combination with other treatments aimed to maximize the antifungal activity should be better investigated.

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