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Effect of short heat treatments with a sodium bicarbonate solution on storability of the yellow germoplasm plum 'Meloni'

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Key words: decay control, GRAS compounds, *Penicillium expansum*, quality.

Abstract: The behavior of a Sardinian plum, cv. Meloni, was investigated with regard to short-heat treatments at 20, 50, 55 or 60°C in water with 0 or 2% $NaHCO_3$ (SBC) for 0, 15, 30, 45 or 60 seconds. Fruits were stored for one month at 5°C and 95% RH followed by a simulated marketing period (SMP) at 20°C and 80% RH for six days. Quality and decay percentage were monitored. In addition, fruits were artificially inoculated with *P. expansum* and stored for 10 days at 25°C and 95% RH. Compared to the control, all short-heat dip treatments lowered the degree of decay, and the efficacy was positively correlated with temperature and treatment duration. The use of SBC increased the efficacy of decay control and the best results were attained at 55 and 60°C. Heat treatments increased levels of total flavonoids and antioxidant activity after SMP. None of the heat treatments induced rind damage (browning or discoloration), but the overall appearance decreased significantly when fruit was treated at 55 or 60°C for 60 s after SMP. Scanning electron microscopy (SEM) observations showed that treatments at 55 and 60°C with SBC cause damage and loss of cuticular wax on fruit surface.

1. Introduction

In the past century, the use of synthetic agro-chemicals has contributed to the exponential increase of food production, particularly in industrialized countries. However, it is more and more evident that safeguarding human health and the environment depends upon an appropriate use of synthetic chemicals in food and ecological chains (Frangenberg, 2000). As a result, new and more restrictive regulations have become mandatory, significantly limiting the use of agro-chemicals. Among fungicides, only a few are authorized in postharvest and the levels of residues are strongly restricted. In addition, European Union (EU) regulation establishes for some crops (*e.g.* stone fruits) that postharvest treatments with conventional agro-chemicals is not allowed (European Commission, 2008). Furthermore, the control of postharvest diseases may be jeopardised by the outbreak of resistant biotypes of postharvest fungal pathogens to fungicides (Holmes and Eckert, 1999; Ma *et al*., 2003). This is a real challenge for highly perishable crops such as stone fruits (peach, nectarine, plum and prune) where postharvest physio-pathological disorders can inflict severe economic losses. In recent years, alternative control of postharvest diseases of fresh commodities has become a chief field for research (González-León and Valenzuela, 2007; Palou *et al*., 2008). Among the investigated alternatives, having hardly any restriction, are some food additives, preservatives and generally recognized as safe (GRAS) compounds that provide an acceptable control of decay, although lower than synthetic fungicides (Palou *et al*., 2009; Molinu *et al*., 2010). The efficacy of these agents was shown to depend upon the commodity and the pathogen. Indeed, carbonic acid salts, especially sodium carbonate and bicarbonate, proved to be fungistatic and to control *in vivo Penicillium digitatum* Sacc., the agent of citrus green mould, whereas *P. italicum*, the agent of citrus blue mould, was much lesser affected, especially with mandarin fruit (Smilanick *et al*., 1999; Palou *et al*., 2002). Palou *et al*. (2009) evaluated, in a primary *in vivo* screening, the efficacy of several safe compounds against the major postharvest pathogens of stone fruits and found that sodium carbonate exerted an inhibitory activity on most pathogens. However, when applied at 20°C for 1 m in a small-scale *in vivo* trial, the efficacy and persistence resulted unacceptable, whereas when solutions were heated to 55 or 60°C an increased efficacy was attained, but it was not superior to that of the heated water alone. Brief hot water treatment was employed by Karabulut *et al*. (2010) to control *Monilinia fructicola* on artificially inoculated Californian plums and they observed that hot water applied at 60°C for 60 s completely inhibited the disease after five days of incubation at 20°C ad 90% RH.

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Conducting a comparative study between local prune and international plum varieties, Molinu *et al*. (2010) found that decay was significantly reduced on prunes but not plums when adding 2% SBC to water heated at 55°C. The results attained with plums, nectarines and peaches are in contrast with those attained with *Citrus* fruits where a synergic interaction increased the efficacy of heated salt solutions. In *Citrus* fruits, in addition to the direct fungistatic activity of carbonic acids, a clear induction of natural resistance contributed to increase decay control efficacy (Venditti *et al*., 2005). This aspect has received slight attention in pome and stone fruits treated with GRAS compounds. In addition, limited data are available on the effect of postharvest treatments with GRAS compounds on the quality of prune fruit and no literature is available concerning changes in bioactive components during storage. In order to improve our understanding on the effect of postharvest treatments with heated GRAS solutions, a factorial design experiment was carried out employing 2% SBC with immersion duration and water temperature as quantitative variables.

2. Materials and Methods

Plant material and processing

Fruit. European plum or prune fruit (*Prunus domestica* L. cv. Meloni) was harvested at commercial maturity in an *ex situ* germplasm conservation orchard belonging to the ISPA-CNR located in Oristano (Sardinia - Italy). After harvest, fruits were selected and randomized in order to obtain homogeneous sets. The fruit surface was disinfected by 2 min. immersion in a 1% (v/v) commercial sodium hypochlorite solution, followed by a deionised water rinse and finally air drying.

Treatment and storage. The sets of fruit were arranged in a 3-factorial randomized complete block design (RCBD) (2x5x4) with two treatments (with 0 or 2% SBC), five immersion times (0, 15, 30, 45 or 60 s) and four solution temperatures (20, 50, 55 or 60°C). Each set was made up of three replicates of 30 fruits. After treatment, fruit was left to dry and then stored at 5°C and 95% RH for one month, followed by a six-day simulated marketing period (SMP) at 20°C and 80% RH.

Inoculation treatments. Artificial inoculation was performed with a wild isolate of *P. expansum* Link, obtained from a decayed plum ('Stanley') subjected to a two-month storage at 2°C. The inoculum was obtained from a 12-day-old sporulating culture by adding 5 mL of sterile water with 0.05% (v/v) Tween 80 and gently scrubbing the agar. After filtering, the spore concentration was determined using a haemacytometer and concentration adjusted to $1x10^4$ conidia/mL with sterile water.

The artificial inoculation experiment was performed by wounding, once, 1200 fruits at the equatorial area with a sterile stainless rod (2 mm wide by 2 mm deep) and by introducing, 1 h later, 15 µL (~ 150 conidia) of the inoculum into half of the wounds (20 sets of 30 fruits), and into the remaining fruits (20 sets of 30 fruits) 15 µL of distilled water (controls). One hour later, fruit was treated according to the experimental plan. When dry, each set was placed into a plastic container, covered and stored at 25°C and 95% RH in the dark. The percentage of decay was monitored after 10 days and treatment efficacy was expressed as inhibition percentage compared to control.

Weight loss and appearance. Mass change of prunes, expressed as % of weight loss compared to harvest weight, was determined on 30 fruits after storage and SMP.

Visual quality was evaluated by employing a rating scale composed of five categories (9, excellent; 7, very good; 5, good, limit of marketability; 3 fair, limit of usability; 1, very poor, unusable) (Chena and Zhub, 2011). The extent of quality loss was described as an index, which was determined by summing the products of prunes in each category by the value of each category, and then dividing this sum by the total number of prunes assessed.

Chemical analysis

pH, Titratable acidity and Total soluble solid content. All chemical analyses were performed on centrifuged and filtered juice obtained by homogenizing de-stoned prunes using a blender. pH, titrable acidity (TA) and total soluble solid content (TSS) were carried out in three replicates of ten fruits each at harvest, after cold storage, and after SMP. A 5 mL sample of juice was titrated with 0.1 N sodium hydroxide to an end point of pH 8.2 using a digital pH meter (ORION, model 420A) and the acidity was calculated as % of malic acid content (g/100 mL of juice). Soluble solids concentration was measured using an ATAGO 0-32°Brix temperature compensating refractometer (Atago Co., Japan). After storage and SMP fruit was rinsed in deionised water before analysis.

Antioxidant activity, Total flavonoids and Phenolics. The total antioxidant activity, total flavonoids and phenolics were measured at harvest, after storage, and SMP in fruit immersed for 60 s in water at 20 and 55°C, with or without SBC.

Antioxidant activity (AA) was measured using two different spectrophotometer methods: ABTS and DPPH assay according to Surveswaran *et al.* (2007). For each assay, 0.1 mL of diluted juice (1:10 in water) was used, a calibrated standard curve with Trolox (3-15 µM; R_2=0.992 for DPPH assay and R_2=0.998 for ABTS assay) was made and results expressed as TEAC units (mmol Trolox equivalents per 100 g of fruit). For both assays, absorbance was recorded with an Agilent spectrophotometer (8453 UV-Visible Spectrophotometer, Agilent Technologies, Palo Alto, CA, USA). Samples were analysed in triplicate.

Total flavonoids (TF) were determined according to the colorimetric assay described by Kim *et al.* (2003). An aliquot of diluted juice was used for the assay and the TF in samples were quantified by catechin calibration curve (2.5-20 µg/ml, R_2=0.999). The absorbance was measured at 510 nm with an Agilent spectrophotometer (8453 UV-

Visible Spectrophotometer, Agilent Technologies, Palo Alto, CA, USA). The results were expressed as mg of catechin equivalent (CE) per 100 g of fruit. Analyses were performed in triplicate on each sample.

Total phenolics (TP) levels were measured in juice using the Folin-Ciocalteu assay (Singleton and Rossi, 1965). Aliquots of diluited samples were mixed with Folin-Ciocalteu reagent (1:1) and 10 mL of 7.5% sodium carbonate in a 25-mL volumetric flask. Reaction mixture was incubated for 120 min at room temperature and the absorbance measured at 750 nm with an Agilent spectrophotometer (8453 UV-Visible Spectrophotometer, Agilent Technologies, Palo Alto, CA, USA). Results were expressed as mg of gallic acid equivalents (GAE) per 100 g of fruit by means of a calibration curve of gallic acid (10-100 mg/L, R_2= 0.989). Samples were analysed in triplicate.

Scanning Electron Microscopy (SEM)

Prunes subjected to the different treatments were used to study the effect of the treatment on the cuticle and epicuticular waxes by means of SEM. Observations were performed, on replica of the fruit rind, adapting the methodology of Dore *et al.* (2010). Replicas of the same marked area were made on dry fruit before and after the treatment (1 and 30 d post-treatment). Samples were observed with a DSM 962 SEM (ZEISS, Oberkochen, Germany) at 20 kV.

Statistical analysis

A one-way or three-way analysis of variance was applied to data using OpenStat (2007). Data from disease incidence were transformed to the arcsine of the square root of the proportion of decayed fruit. When appropriate, means were separated by Fisher's protected least significant difference test with a significance level of $P = 0.05$. Synergy testing of the combined treatments was performed according to Plascencia-Jatomea *et al.* (2003), calculating the expected and registered efficacy by Limpel's equation: $E_e = X + Y - (XY/100)$, where E_e was the expected additive response to hot water treatment and BCS immersion, and X and Y were the percentages of inhibition relative to each factor (heat or SBC) used alone.

3. Results

Decays

Concerning the development of molds during the storage trial (Table 1), over 90% of natural infections were caused mainly by *P. expansum*, while the remaining 10% consisted of *M. fruticola*, *Botrytis cinerea,* and *Rhizopus stolonifer*. It is possible to observe that salt, temperatures, and immersion times significantly influenced decay control. As a general rule, decay control was improved by increasing the immersion duration and by heating the SBC solution. After storage, the lowest degree of decayed fruit was attained in the sets dipped in the SBC solution at 55 or 60°C for 45 s and at 60°C for 60 s. As a result, compared to untreated fruit (28.5% decay) those treatments significantly reduced the natural infection to 4.1, 3.8 and 7.1%, respectively. After the SMP decay augmented in all sets of fruit, however, the best control remained that attained by dipping the fruit for 45 s in the heated salt solution at 55 or 60°C (7 and 6.7% of rots) with about 80% reduction of decay compared to control (35.4% of rots).

Table 1 - 'Meloni' prunes with decay (%) after 1 month of storage at 5°C and 95% RH and 6 days at 20°C and 80% RH (SMP) when treated with (+) or without (-) a 2% $NaHCO_3$ solution at 20, 50, 55 or 60°C for 15, 30, 45 or 60 s or left untreated (Control) [z]

Temperature (°C)		Immersion duration (s)							
	Storage	15	15	30	30	45	45	60	60
	Control	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)
	28.5 aA								
20		21.0 bB	20.8 bB	22.1 bB	19.1 bB	22.1 bB	18.1 bB	21.1 bB	23.4 bB
50		20.4 bB	19.1 bB	22.4 bB	19.4 bB	18.8 bB	11.4 cC	15.1 cB	13.4 cC
55		22.4 bB	18.1 bB	19.4 bB	17.1 bB	10.4 cC	4.1 dD	15.8 cB	12.4 cC
60		19.4 bB	16.1 bcC	15.4 cC	10.4 cD	7.4 cD	3.8 dE	8.8 dC	7.1 dD
	SMP	15	15	30	30	45	45	60	60
	Control	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)
	35.4 aA								
20		26.7 bB	26.7 bB	26.0 bB	22.0 bC	26.0 bB	21.3 bC	25.0 bB	20.3 bC
50		26.3 bB	23.0 bB	25.3 bB	24.3 bB	22.7 bB	15.3 cC	20.0 dB	16.3 cC
55		26.3 bB	22.0 bB	23.3 bB	23.0 bB	15.3 cC	7.0d D	19.7 dB	15.3 cC
60		26.3 bB	19.0 bB	19.3 bB	15.3 cC	11.3 cC	6.7 dD	11.7 cC	10.0 cC

[z] Values are means with N=90 each time. Capital letters relate to comparisons within rows, lower case letters to comparisons within columns, different letters indicate differences at $P \leq 0.05$ according to Newman-Keuls test.

Inoculation treatments

The *in vivo* experiment with artificially inoculated fruit (Fig. 1) evidenced that the immersion for 15, 30 or 45 s in a 2% SBC solution at 20 or 50°C did not improve decay control compared to water dips. The same occurred when fruit was treated for 15 s at 55 or 60°C, indicating that with immersions up to 45 s no additive effects took place by heating the solution up to 50°C; we observed the same behavior at higher temperatures with 15 sec dips. It is interesting to note that synergistic interactions took place at 55°C for 30, 45, 60 s while no such interaction occurred at 60°C, except for the 30-s dip. The best result was obtained when fruits were immersed in SBC solutions at 55°C for 45 and 60 s with an increase of decay inhibition by 49 and 53% respectively compared to the heat treatment only (20 and 25% respectively).

Weight loss and appearance

Weight loss during storage and SMP ranged between 2 and 4% for control fruit. The immersion of fruit at 20°C, with or without SBC, had no effect on weight loss, while it increased when fruit was immersed in heated water, reaching a maximum of 9.5% after SMP with 60 °C for 60 s. A significant increase of weight loss took place when fruit was immersed in the SBC solution at 55 and 60°C for 45 or 60 s (8.7, 9.7, 11.3 and 13%, respectively).

The visual quality of fruit in the storage experiment evidenced a harmful effect on fruit when immersed in water at 55 and 60°C for 60 s (visual quality score between 2 and 3). The reason for this result was related to fruit shrivelling, which was particularly evident after SMP. The SBC solution was not the cause of shrivelling but contributed to increasing the impairment when employed at 55 or 60°C. Indeed, at 50°C no shrivelling was observed, while at 55°C it appeared when fruit was immersed for more than 30 s, and it resulted slightly more visible with the SBC solution.

pH, Titratable acidity and Total soluble solid content

At harvest the pH, TA and TSS (°Brix) resulted 3.69, 0.69 and 19.2, respectively. During storage the values changed slightly in untreated fruit, while after SMP the pH and TSS increased (3.84 and 21.6 °Brix, respectively) and TA decreased (0.56). With respect to untreated fruit (control), the different treatments did not affect the pH value, both during storage and SMP. Total acidity decreased during the SMP except for a notable increase observed in fruit of the set treated at 55 and 60°C for 60 s. Consistent increases of TSS occurred during SMP in fruit from sets immersed at 55°C for 45 or 60 s without SBC (24.2 and 23.1, respectively) or with SBC (22.3 and 23.7, respectively). On the other hand, fruit treated at 60°C, with or without SBC (20.2 and 20.1, respectively), had lower TSS compared to untreated fruit (control) or that treated at 55°C.

Antioxidant activity, Total flavonoids and Phenolics

Figure 2 shows the data regarding total antioxidant activity measured by the DPPH assay after storage and SMP in fruit immersed for 60 s in water at 20 and 55°C, with or without SBC.

With respect to harvest, the antioxidant activity decreased during storage and treatment temperature did not affect this trend nor did the SBC solution. Compared to the end of storage, an increase of the AA occurred in all sets of fruit after the SMP. The increase in heat treated fruit with or without SBC reached values higher than the AA at harvest. Data relating to Abts assay were well correlated with DPPH (r^2=0.70).

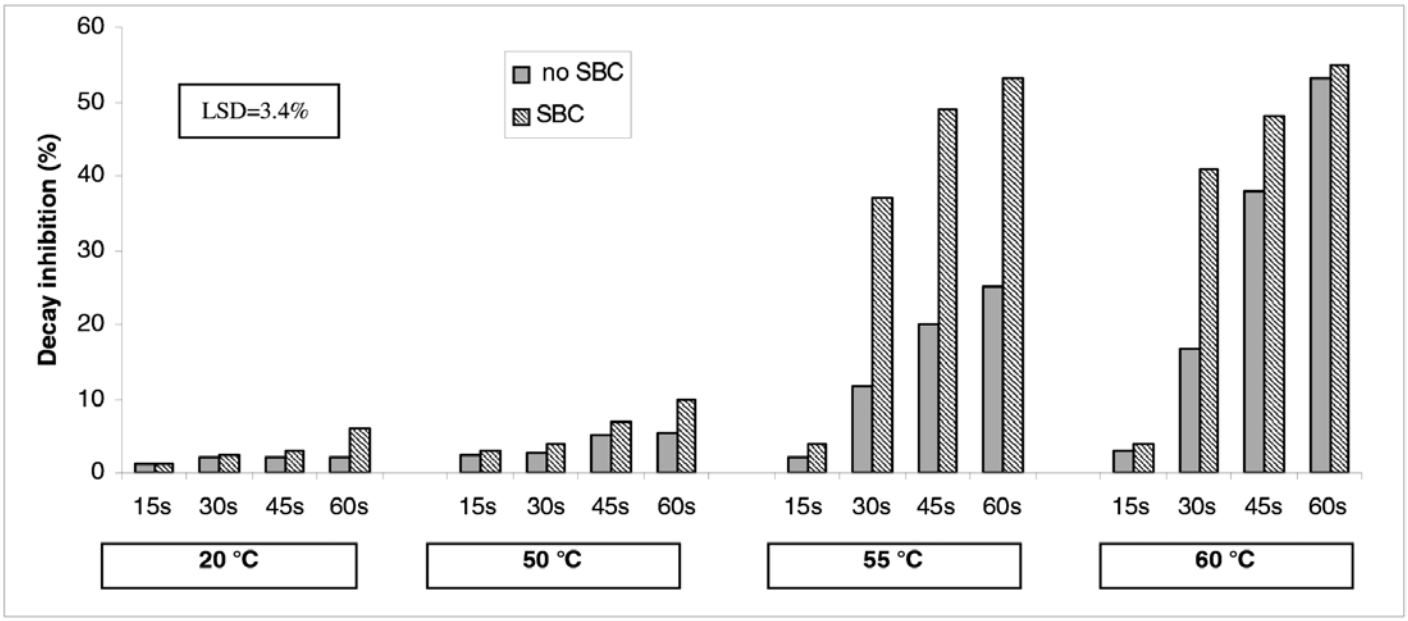

Fig. 1 - *P. expansum* inhibition (%) in 'Meloni' plum artificially inoculated 24 h before immersion at 15, 30, 45 or 60 s in water at 20, 50, 55, or 60°C with (SBC) or without (no SBC) 2% $NaHCO_3$ after 10 days at 25°C and 95% RH. Mean decay control= 90.5%.

The correlation between total polyphenols and total flavonoids resulted good (r^2= 0.70) and showed a similar trend with the AA values, thus increasing during the SMP and reporting higher values for heat treated fruit (data not shown). Following SMP the TF of fruit dipped at 55°C for 60 s, with or without SBC, resulted significantly higher (81.9 and 76.2 CE/100 g, respectively) compared to cold-water treated fruit (59.4 CE/100 g). Heat treatment increased both the TF and the AA values, measured by DPPH assay, attaining a high correlation (r^2= 0.80) between them, while the TP and DPPH values resulted less correlated (r^2= 0.63).

Scanning Electron Microscopy (SEM)

SEM observations of the fruit surface at harvest evidenced a constant layer of epicuticular wax with several wax plates (Fig. 3, micrograph A). After immersion for 45 or 60 s in water, at 50 and 55°C, the epicuticular wax layer was melted (B), while when immersed under the same conditions in the 2% SBC solution, new wax with a different crystalline structure appeared on the surface (micrographs C-D). When the immersion was performed for 45 or 60 s at 60 °C the cuticle was damaged by evident cracks near the stomata (E). When those conditions were applied with the 2% SBC solution, an erosion of the epicuticular wax was observed in most samples (micrograph F).

4. Discussion and Conclusions

The hot water treatments at 55 and 60°C with 2% of SBC were effective in controlling *P. expansum* decay during one month of storage at 5°C and the subsequent SMP in both experiments.

The most effective treatments remained those of dipping the fruit for 45 s at 55 or 60°C in SBC solution; this result confirms the findings reported by Molinu *et al.* (2010) for other *cvs* of sardinian germplasm.

Karabulut *et al.* (2010) indicated 55 and 60°C as effective temperatures for decay control of "Casselman" plums inoculated with *M. fructicola* 12-16 h before treatments. The authors observed a total rot control at 60°C for 1 min, while with 'Meloni' prunes, inoculated 24 h pre-treatment, we obtained under the same conditions an inhibition percentage of about 53%, which rose to 55% with the SBC solution.

Also Palou *et al.* (2009) reported a significant increase of treatment efficacy by heating the solutions to 55 or 60°C with several GRAS compounds in stone fruits, inoculated *in vivo* with seven major postharvest pathogens.

The trends at 55 °C with SBC are similar to those attained with *Citrus* fruit, but with a much lower efficacy (Smilanick *et al.*, 1999). This difference was also observed by Palou *et al.* (2009) in stone fruits. The observed diversities between species is probably related to notable differences in fruit tissue texture, rind structure, cuticle thickness and chemical composition within the potential infection courts. Indeed, in wounded *Citrus* fruits, alkaline hydrolysis of pectins is promoted by carbonic acid salts along with an increased pH of the albedo and a remarkable induction of phytoalexin biosynthesis (Dore *et al.*, 2010). These additional factors affecting pathogenicity have not yet been investigated in stone fruits and may contribute to explaining the higher or lower efficacy according to species.

Concerning overall appearance, a significantly lower score was attributed to fruit immersed in water at 55 and

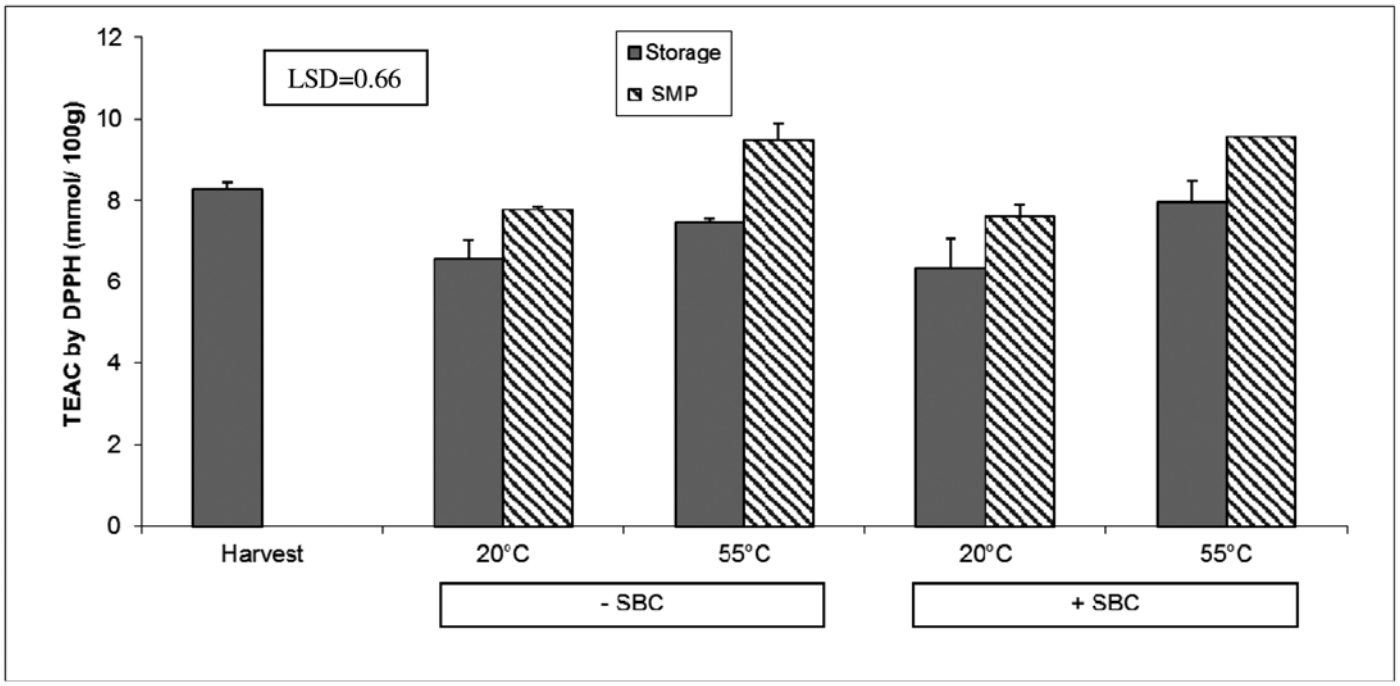

Fig. 2 - Total antioxidant activity in 'Meloni' plum immersed for 60 s in water at 20 and 55°C, with (+) or without (-) BCS, expressed as TEAC units (mmol Trolox equivalents per 100 g of fruit) by DPPH assay, after 1 month of storage at 5°C and 95% RH and 6 days at 20°C and 80% RH (SMP).

60°C for 60 s. This as the result of fruit shriveling, particularly evident after SMP in heat treatments with the SBC solution. Also fruit weight loss was affected by the heat-SBC treatments and the same trend was reported for other crops even though to a lesser degree.

In *Citrus* fruit, the greater weight loss was attributed to an increase of fruit transpiration following alterations on the cuticular permeability (Dore *et al.,* 2010). Peel-impairments, especially at the cuticle level near stomata, were evidenced by SEM on 'Meloni' fruit immersed in the SBC solution at 60°C for 45 and 60 s. As reported for

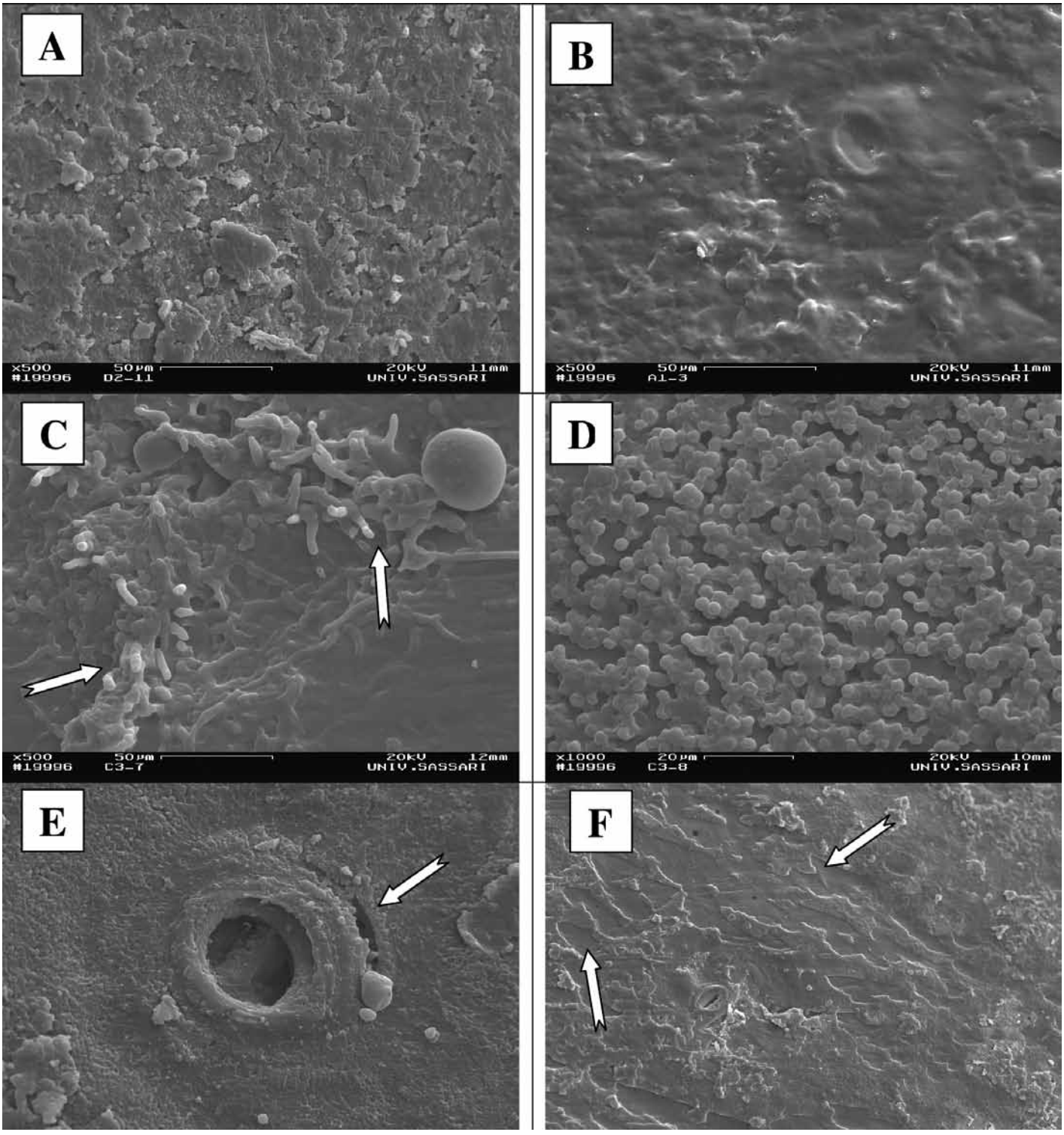

Fig. 3 - *Micrographs A-F*. Scanning electron microscopy micrographs of 'Meloni' prune epicuticular wax at harvest (A), following immersion at 55°C for 60 s in water (B) and in a 2% $NaHCO_3$ solution (C) with arrows indicating crystalline wax (C), and a magnification of the new wax layer (D). In micrograph 'E' damage to the cuticle and wax erosion (F) following immersion at 60°C for 60 s in the 2% SBC solution are evidenced (arrows).

Citrus fruits, it is likely that also for stone fruits harming of the cuticle produces the observed greater fruit weight loss and wilting.

With regard to chemical analysis, the variations occuring during storage and SMP in untreated and in 20°C treated fruit are consistent with literature. In fact, Díaz-Mula *et al.* (2009) reported a 40-45% decrease of total acidity detected in eight plum cultivars after 35 days of storage at 2°C and a significant increase of TSS in almost all the cultivars studied. Compared to treatments at 20°C, fruit dipped at 55°C had higher TSS and TA contents. According to Serrano *et al.* (2004) this trend could be related to a reduction of the respiration rate caused by the heat treatment. Somewhat less clear is the significant drop of TSS in fruit dipped at 60°C. A similar phenomenon was reported for *Citrus* fruit when the treatment temperature reached the fruit-damage threshold, and this drop was attributed to fruit physiology connected to stress-induced metabolic responses (Schirra and d'Hallewin, 1997). With respect to harvest, AA, TP and TF decreased during cold storage, while an increase was observed after SMP and, in heat treated fruit, values were higher compared to harvest. Díaz-Mula *et al*. (2009) monitored the bioactive compounds in several plum cultivars during cold storage and reported rising and dropping trends according to cv. and ripening stage at harvest. The increase of AA, TP and TF following heat treatments has been reported also for pomegranate and strawberry (Mirdehghan *et al.*, 2006; Vicente *et al.*, 2006), supporting our findings that heat affects nutraceutical properties in a positive way. The results attained with the heated SBC solution suggest that only heat and not SBC plays a role inducing AC, TP and TF.

As far as we are aware, no literature is available on the changes of bioactive compounds and antioxidant activity during cold storage and subsequent SMP of stone fruits according to treatment with SBC solution.

From the results attained with the European plum 'Meloni' it appears clearly that decay is furthermore reduced, compared to water at 55°C, when 2% of SBC is added and immersion is performed for 45 or 60 s. In addition, as for C*itrus* fruits, we observed that SBC induces the production of crystalline wax and that treatments at 60°C induce cuticular damage and loss of epicuticular wax from the fruit surface. These effects on the cuticle and epicuticular waxes can increase fruit transpiration, as demonstrated by the greater weight loss and shrivelling. After SMP, postharvest immersion of 'Meloni' prunes in hot water resulted in an increase of bioactive compounds and SBC did not contribute to this increase. Thus, heating a SBC solution seems not to affect nutraceutical properties but from our results it significantly improves the efficacy of this GRAS compound in containing *P. expansum* infection during storage. It is also important to evidence that a synergic anti-penicillium effect took place by employing the heated SBC solution.

Epicuticular damage highlighted by SEM undermines or hide the effects on fruit quality and probably further research is needed in order to establish the most appropriate concentration of the salt aimed at reducing the epicuticular damage and at clarifing the salt influence on the nutraceutical properties of fruit.

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