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Postharvest application of calcium chloride and 1-methylcyclopropene for quality conservation on organic ripe fig

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Abstract: The postharvest phase is an important step in the fruit production chain. Fig is an especially perishable fruit, which has encouraged researchers to study the effects of various substances on the postharvest life of this commodity. The objective of the present work was to evaluate the effects of calcium chloride ($CaCl_2$) and 1-methylcyclopropene (1-MCP) on the postharvest quality of the 'Roxo-de-Valinhos' fig cultivar. This study aimed to verify the effects of applying a 4% solution of $CaCl_2$ and a 1% solution of 1-MCP to figs and evaluating at four different storage times (0, 2, 4, and 6 days). The results showed that a 4% solution of $CaCl_2$ promoted better firmness, and when $CaCl_2$ at 4% solution was applied in combination with 1-MCP at 10 µg l^{-1}, the maturation index increased. In contrast, the 1-MCP treatment alone did not improve the postharvest quality of 'Roxo-de-Valinhos' ripe fig. We conclude that application of 4% solution of $CaCl_2$ and 1-MCP at 10 µg l^{-1} promote firmness and increase maturation index of 'Roxo-deValinhos' figs.

1. Introduction

Fig (*Ficus carica* L.) is an important crop globally, particularly in Southeast Europe, the Middle East, North Africa, and the Americas, where the USA and Brazil are highlighted for fig production (Rosianski *et al*., 2016; Uzundumlu *et al*., 2018; Paolucci *et al*., 2020). In Brazil, 19.6 tonnes were harvested from 2.114 hectares in 2020 (IBGE, 2022), where fig trees are cultivated mainly in the southeast and south regions where ripe figs are harvested for consumption and unripe figs for industrialization (Tofanelli *et al*., 2018).

Although fig tree cultivation has the potential to escalate, some production bottlenecks have inhibited this expansion, such as lack of commercial cultivars, unfamiliarly about ripe fig as excellent fruit for food from consumers, relative high price of ripe figs in the market, inefficient fresh ripe fig distribution and difficult to conserve quality of ripe figs after harvested. Thus, one of the principal challenges is the high perishability of ripe fig after it has been harvested (Mirshekari *et al*., 2020), especially in Brazil where the main cultivar used is 'Roxo-de-Valinhos', also known as 'Brown Turkey', which produces highly-perishable fruit (Ferraz *et al*., 2020). 'Roxo-de-Valinhos' figs have a very short postharvest life of 1 to 2 days when stored under environmental conditions of market shelf, or 4 to 10 days under refrigerated storage (Lakshmi *et al*., 2018).

Several studies have been conducted on the effects of postharvest treatments on figs to extend their storage duration. The majority of research has focused on substances such as calcium chloride ($CaCl_2$), 1-methylcyclopropene (1-MCP), fungicides, and sodium hypochlorite (Irfan, 2013; Song *et al*., 2019; Jusoh *et al*., 2020).

$CaCl_2$ has been applied to fruits both in preharvest and postharvest to promote quality conservation (LI *et al*., 2014). This nutrient has been considered the most consequential for fruit quality promotion through its contributions to decreased softening, browning, and senescence of fruits, thereby extending their postharvest life (Suriati *et al*., 2021). 1-MCP has an unsaturated cyclic olefin that acts as a competitive ethylene antagonist and blocks ethylene receptors, consequently inhibiting maturation processes such as ethylene production, respiration rate, browning, and softening, and extending the shelf life of fruits (Sozzi *et al*., 2005; Watkins, 2008; Freiman *et al*., 2012).

The response of $CaCl_2$ or 1-MCP application on fruits varies according to the application form, fruit species, cultivar, maturation, and ripening stage of the fruit (Sozzi *et al*., 2005; Watkins, 2006; Irfan, 2013). Currently, there are few reports applying $CaCl_2$ and 1-MCP to figs to evaluate their effects on fruit quality.

Therefore, the objective of this study was to evaluate the effects of the application of $CaCl_2$ and 1-MCP on quality of fresh ripe fig 'Roxo-de-Valinhos' after harvested to extend their shelf life.

2. Materials and Methods

Plant materials

The experiment was conducted at the Federal University of Paraná State. Figs were harvested from plants grown in the orchard located in the Canguiri Experimental Farm (Pinhais County, Paraná State, Brazil, 25°26' S and 49°16' W, 947 m above sea level), considered Cfb by the Köppen climate classification, with annual maximum and minimum temperatures of approximately 24°C and 11°C, respectively, and annual average rainfall of 1,500 mm.

Fig trees used were 5-year-old Roxo-de-Valinhos cultivars, also known as 'Brown Turkey'. The first harvest was done when plants were 2 years old. Plants were cultivated in an organic plantation system, subjected to heavy annual pruning, and spaced at 1×2.5 m. Fertilization was done using cattle manure at 60 L per plant annually, separated into three 60 days intervals beginning in August. Phytosanitary management was performed by spraying a 2% lime sulfur solution after pruning and a 0.2% bordeaux mixture (biweekly in rainy period and monthly in dry period) from the vegetative and production stages of the fig trees to two weeks prior to harvest, mainly to prevent rust (*Cerotelium fici*). To control pests, such as tree borer (*Azochis gripusalis*) and borer beetle (*Coleobogaster cyanitarsis*), a 1% solution of oil extracted from neem (*Azadirachta indica* A. Juss) was sprayed on plants when these pests were detected damaging the fig trees. Mechanical weeding in crop rows and mowing between rows were used to control weeds, integrated with black oat (*Avena strigosa* L.) planted in July in 2012 and 2013.

Fruit materials

Figs were hand-harvested on April 1, 2014. Overall, this period is the late season for harvest of ripe fig in Brazil. Fruits were harvested when they showed at least a 50% change to their skin color, from green to reddish brown at stage 6 (Freiman *et al*., 2012) by visual evaluation. This maturation stage is usually better accepted by Brazilian market. After harvesting, figs were immediately transported to the Postharvest Laboratory and located in the same experimental farm for experimental proceedings. Figs were divided into lots containing 8 figs each lot for storage during 0, 2, 4, and 6 days, which were then placed in a paper box commonly used in ripe fig markets.

Postharvest treatments

After figs were harvested, they were treated with calcium chloride ($CaCl_2$) (Oliveira Junior *et al*., 2018) and 1-methylcyclopropene (1-MCP) (Tofanelli *et al*., 2018). Half of the selected figs were dipped in the solution of $CaCl_2$ at 4%, and the other half were dipped in distilled water and immersed for 15 min. After that, figs were taken from the treatments to dry their surface by natural air-drying under the laboratory bench for 30 min.

After drying, figs were placed in a specific commercial paper box that fit 8 figs each. The packed figs were placed into a plastic container (70 L) when they were ready to be treated with 1-MCP. SmartFresh^{Ò} powder (0.14% active ingredient) was used to prepare the 1-MCP solution. This powder was measured with a precision balance and dissolved in distilled water to create a concentration of 10 µg l^{-1} inside the container, where figs were kept after treatment for 24 h. Untreated figs (control) were compounded only with distilled water and stored under the same conditions. Thus, 1-MCP powder was introduced into a syringe and supplemented with water until a specific volume was reached. The 1-MCP solution and control were injected into the respective plastic containers, which were immediately blocked. Therefore, both untreated figs and those treated with $CaCl_2$ were also treated with 1-MCP or the control (distilled water), resulting in a combination of 2 levels of $CaCl_2$ (0 and 4%) *vs.* 2 concentrations of 1-MCP (0 and 10 µg l^{-1}).

After 1-MCP treatment, packed figs were removed from boxes and stored at 4±1°C and 90-95% RH for 6 days, when they were removed to procedure analyses.

Fruit quality parameters

Fig quality conservation was evaluated using firmness, total soluble solids (TSS) concentration, titratable acidity (TA), maturation index (MI), and weight loss.

The samples were withdrawn from the refrigerator and 2 figs per replicate were analyzed every two days. Fruit-peel firmness was determined on the equator of the fig using a manual penetrometer (PTR-100) with a 7.9-mm-diameter tip and expressed in terms of lb force.

Fruit juice was extracted from the stored figs using a centrifuge in order to assess TSS and TA. TSS was measured with a handheld refractometer using a drop of juice and expressed as a percentage while TA was expressed as % citric acid and measured using 100 ml of the solution (10 ml of juice + 90 ml distilled water) that was immediately titrated with 0.1 N NaOH using 3 drops of phenolphthalein as an indicator. MI was determined by dividing TSS by TA (TSS/TA ratio).

Weight loss of stored figs was obtained using the relative weight variation (%) of 2 figs per replicate during a storage period of 6 days. The figs were weighed at harvest time and at every storage period of 2 days, and weight loss was determined. Fig weights were assessed using an electronic balance.

Experimental design

Three replicates of samples with 8 figs per replicate for each treatment were used for physical and chemical quality analyses; 2 figs were analyzed immediately after 1-MCP proceedings and the other 6 figs were stored. Four storage periods (0, 2, 4, and 6 days) were used to evaluate postharvest quality conservation. Theses samples of figs were randomized selected avoiding to select damage figs by pests and mechanical injury.

The experimental design was completely randomized. Treatments included the application or absence of 1-MCP (10 µg l^{-1}) combined with the application or absence of $CaCl_2$ (4%) and with no storage (0) and three storage times (2, 4, and 6 days). Thus, the experiment was designed using a 2^3 factorial arrangement (2´2´4): (2) $CaCl_2$ at 2 levels (0 and 4%), (2) 1-MCP at 2 levels (0 and 10 µg l^{-1}), and (4) storage period at four levels (0, 2, 4, and 6 days).

Statistical procedures

Analyses were carried out with analysis of variance (ANOVA) using the Sisvar Statistical Program (version 5.3) (Ferreira, 2011). Treatments that showed a significant effect were subjected to multiple comparisons of the mean using the Tukey test at a 5% probability level.

3. Results

Results from ANOVA showed that $CaCl_2$ promoted significant effect on firmness and soluble solids of the 'Roxo-de-vlinhos' ripe figs, that storage periods had significant influence on soluble solids, titratable acidity, weight loss, and maturation index, whereas 1-MCP only affected significantly the maturation index (Table 1). The exclusive interaction that showed a significant effect was $CaCl_2$ x 1-MCP on the fig ratio. As expected, storage influenced the quality parameters evaluated in the ripe figs, except in terms of firmness. There was no significant effect of $CaCl_2$ and 1-MCP on all parameters evaluated in this present work after storage of 6 days (Table S1).

Table 1 - Analysis of variance (ANOVA) for parameters evaluated on storage of ripe figs treated with calcium chloride ($CaCl_2$) and 1-methylcyclopropene (1-MCP)

Source of Variation	df	FI (Lb)		SS (°Brix)		TA (%)		WL (%)		MI (Ratio)	
		MS	*F*	MS	*F*	MS	*F*	MS	*F*	MS	*F*
CC	1	25.37	17.06 **	6.31	9.84 **	0.02	0.09 NS	102.11	0.36 NS	404.26	1.70 NS
MCP	1	2.34	1.57 NS	0.08	0.13 NS	0.64	3.15 NS	28.29	0.11 NS	1029.53	4.33 *
SP	3	3.94	2.65 NS	4.57	7.14 **	1.04	5.09 **	97.99	104.25 **	765.64	3.22 *
CC×MCP	1	0.07	0.05 NS	0.61	0.95 NS	0.81	3.97 NS	2.53	1.69 NS	987.36	4.15 *
CC×SP	3	0.48	0.33 NS	1.01	1.57 NS	0.18	0.86 NS	0.04	0.14 NS	9.85	0.01 NS
MCP×SP	3	1.39	0.93 NS	0.86	1.35 NS	0.09	0.44 NS	0.09	0.05 NS	342.21	1.44 NS
CC×MCP×SP	3	3.90	2.62 NS	0.58	0.91 NS	0.46	2.27 NS	0.33	0.39 NS	84.59	0.36 NS
CV (%)		46.2		9.6		19.9		13.2		22.2	

CC= Calcium Chloride; MCP= Methylcyclopropene; SP= Storage Periods; CV= Coefficient of Variation; FI= Firmness; SS= Soluble Solids; TA = Titratable Acidity; WL= Weight Loss; MI= Maturation Index; MS= Mean Square; F= F test;
** Significant at P≤0.01; * Significant at P<0.05. NS = not significant.

Except for effect of the interaction between $CaCl_2$ x 1-MCP on the maturation index of figs, we could also observe from ANOVA that there was no significant effect of interactions between the factors on parameters evaluated in this present work (Table 1).

When ripe figs were post-harvest treated with $CaCl_2$, they demonstrated more firmness than untreated figs (3.37 lb and 1.91 lb, respectively) (Table 2). $CaCl_2$ postharvest treatment also influenced TSS on ripe figs, where untreated fruits showed a higher value (8.70%) compared to the treated figs (7.98%).

Figs stored for 4 (8.74%) and 6 days (8.81%) showed higher TSS than non-stored fruits (7.47%) (Table 3). The titratable acidity was higher after 6 days of storage (2.58%), when compared with no storage (1.98%) and 2 days of storage (2.07%) (Table 3). The weight loss of ripe figs was significantly

Table 2 - Effects of calcium chloride ($CaCl_2$) treatments on quality parameters of ripe figs stored over different periods

Fruit parameter	$CaCl_2$	
	Firmness (Lb)	Soluble Solids (%)
Untreated	1.91 ± 1.34* b	8.70 ± 1.00 a
Treated	3.37 ± 1.29 a	7.98 ± 0.91 b
Coefficient of Variation (%)	46.2	9.6

* Standard deviation. Values followed by the different lowercase letter in a column are significantly different from each other (P<0.05).

Table 3 - Effects of storage periods on quality parameters of ripe figs

Storage (days)	Fruit parameters			
	Soluble solids (%)	Titratable acidity (%)	Weight loss (%)	Maturation index
0	7.47 ± 0.99* b	1.98 ± 0.35 b	0 ± 0.00 d	69.22 ± 12.56 ab
2	8.35 ± 0.68 ab	2.58 ± 0.73 b	7.67 ± 0.86 c	62.22 ± 16.49 b
4	8.74 ± 0.82 a	2.07 ± 0.47 ab	13.67 ± 2.84 b	80.47 ± 21.62 a
6	8.81 ± 1.02 a	2.46 ± 0.22 a	18.75 ± 3.96 a	65.23 ± 11.54 ab
Coefficient of Variation (%)	9.6	19.9	27.3	22.2

* Standard deviation. Values followed by the same lowercase letter in a column are not significantly different from each other (P<0.05).

enhanced as storage duration increased, as expected. The greatest weight loss was 18.75% after 6 days of cold storage.

The maturation index (TSS/TA ratio) was the parameter that showed a significant interaction effect (Table 1). When ripe figs were treated with $CaCl_2$ after with 1-MCP, they showed a higher ratio (75.55) than those treated only with $CaCl_2$ (57.22) (Table 4).

4. Discussion and Conclusions

$CaCl_2$ has been widely used for food conservation, mainly because it can maintain firmness during the shelf life of vegetables, especially in fruits immersed in calcium chloride solution after harvest (Li *et al*., 2014). Calcium plays an important role in maintaining the structure of the cell wall, since its cation binds to pectins producing calcium pectates that structure the cell wall, thereby inhibiting enzymes such as polygalacturonase and polymethylesterase.

Irfan *et al*. (2013) recorded the lowest sugar content when figs were treated with $CaCl_2$ at 4%, as reported before when we showed that figs treated with $CaCl_2$ obtained lower sugar content against untreated figs. These authors concluded that this likely occurred because calcium caused a delay in fruit ripening through the inhibition of certain events such as starch hydrolysis and formation of sugars, a response to the high amounts of calcium deposition in the fruit cell wall of the treated figs. These authors also mentioned that components such as pectic substances, calmodulin, and hemicellulose polysaccharides are the most likely binding sites for calcium, preserving the texture and stability of the treated fruit and promoting higher fruit resistance by maintaining high calcium concentration in the cytosol, as well as less free sugars released during storage.

Storage having no influence on firmness likely relates to the figs being harvested at a relatively advanced ripening stage, when they showed at least a 50% change in skin color, from green to reddish brown at stage 6. In Brazil, the late season to harvest ripe figs for table takes place from late March to early April, when they usually get better prices in the market. Thereby, it would be interesting whether growers had a technique that was able to extend shelf life of ripe figs in order to add value to their product. Thus, at this harvest time, figs already displayed preharvest firmness reduction; therefore, the collected data of firmness from harvest to last storage period did not show a significant difference. According to Freiman *et al*. (2012), postharvest treatment on figs at the maturation stage can be ineffective for their quality conservation or may have a slight effect on softening, promoting its retardation, especially when figs are collected at an advanced stage. These authors mentioned that marketable ripe figs are defined as commercially mature when they have changing skin coloration from 20 to 70% of their surface.

Tofanelli *et al*. (2018) also showed TSS increasing during storage in 'Roxo-de-Valinhos' figs collected at stage 5 (Freiman *et al*., 2012) and treated with 1-MCP. According to those authors, this TSS increase likely occurs either due to the hydrolysis of several polysaccharides, such as pectins, starch, and other oligosaccharides in the cell wall, which become part of the cellular juice when they are solubilized in the aqueous phase, or due to starch accumulation during fruit maturation, which is degraded into sugars by the action of enzymes such as α-amylase, β-amylase, and starch phosphorylase, consequently increasing the TSS concentration.

Although the acidity of figs tends to decrease during the storage period (Irfan *et al*., 2013; Song *et al*., 2019), it has been observed that in the initial storage time, acidity tends to increase for a short period in the first few days. Gözlekçi *et al*. (2008) evaluated the effect of 1-MCP on the quality of figs stored during three different periods (5, 10, and 15 days) and

Table 4 - Effects of calcium chloride ($CaCl_2$) and 1-methylcyclopropene (1-MCP) treatments on the ratio (%) of ripe figs

$CaCl_2$	1-MCP	
	Untreated	Treated
Untreated	72.09 ± 11.65* aA	72.28 ± 18.67 aA
Treated	57.22 ± 11.10 bB	75.55 ± 20.28 aA
Coefficient of Variation (%)	22.2	

* Standard deviation. Values followed by the same uppercase letter in a column and lowercase letter in a row are not significantly different from each other (P<0.05).

observed that fruit acidity slightly increased until the first storage period. In addition, Álvarez-Herrera *et al*. (2016) studied the effect of 1-MCP on the postharvest conservation of pitahaya (*Selenicereus megalanthus*) during seven storage periods (4, 8, 12, 16, 20, 24, and 28 days), and discovered an increase in acidity in the treated pitahayas until the second storage period (8 days). In the present work, the last evaluated storage period was 6 days; therefore, it was expected that the acidity of ripe figs would increase during the entire experimental period.

Fruit transpiration and several physiological disorders of figs, such as skin side cracking and ostiole-end cracking/splitting occur during storage, and these could promote increasing levels of TA and TSS, as well as the maintenance of several organic acids during the storage period (Byeon and Lee, 2020).

In our study, 1-MCP treatments only affected on maturation index (ratio). Cantín *et al*. (2020) also did not obtain improvement on shelf life of 'Cuello Dama Negro' dark-skin commercial figs treated with 1-MCP at 1 μL l^{-1} and according to these authors the advance maturity stage of the figs at the moment of harvest could be promoted a high increase in ethylene production even under 1-MCP treatment. They also highlighted that, although the best moment to harvest fresh-market figs is variable according to each cultivar or variety, it is usually done as early as possible. In our work, studied figs were harvested at maturation stage 6 (Fig. 1B), thus an early harvest (Fig. 1C) could be promoted different results, what we would release here as a challenge for future studies, as well as evaluate how these figs harvested on early maturation stage would be taken up by the market. However, figs may be a climacteric or non-climacteric fruit due to their maturation shows rapid changes of compositional features that are typical of climacteric fruit, whereas figs are not capable to keep ripening after harvested (D'Aquino *et al*., 2015). Thus, that is a good question when would be the better time to harvest ripe figs for post treatment in order to enlarge their shelf life as well as promote their quality conservation.

Fig fruits are highly perishable products in which physiological events such as transpiration, respiration, and degradation occur quickly (Gözlekçi *et al*., 2008; Ozkaya *et al*., 2014; Byeon and Lee, 2020). It is interesting to consider the unique morphological structure of figs, as they have a thin skin over the entire fruit with a distal end orifice called the ostiole where water loss through evaporation occurs by exudation of a syrupy liquid (D'Aquino *et al*., 2003; Freiman *et al*., 2012).

$CaCl_2$ combined with 1-MCP applied on postharvest ripe figs likely promoted sugar content accumulation and was not favorable to acidity oscillation of fruits during storage.

Some structural effects on figs have been observed when they are treated with calcium, such as mechanical strength improvement and increased resistance of fruit tissue due to absorbed calcium in the apoplast primarily complexing with the cell wall, where the plasma membrane works as a cementing material. Calcium is also involved in the maintenance of the cell wall structure by interacting with pectic acid and forming calcium pectate (Irfan *et al*., 2013). Thus, in the present study, $CaCl_2$ application likely promoted these events in figs, resulting in sugar content accumulation and acidity stabilization, consequently enhancing the maturation index (ratio).

In addition, when 1-MCP was applied after calcium on the ripe figs, the MI ratios increased, potentially due to ethylene inhibition, decreased fruit respiration, and glucose and fructose stabilization during cold storage (Ozkaya *et al*., 2014; Song *et al*., 2019).

Overall, the results showed that a 4% $CaCl_2$ solution was capable of promoting higher firmness and sugar content stabilization, whereas when $CaCl_2$ was applied in combination with 1-MCP at 10 μg l^{-1}, the maturation index increased. These results reinforce

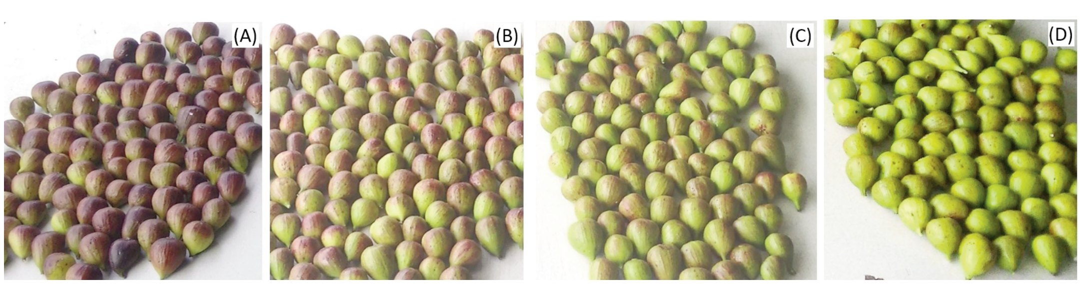

Fig. 1 - Figs of Roxo-de-Valinhos harvested at different stages of ripening. A - Advanced maturation; B - Maturation at 50% change skin color; C - Maturation at 25% change skin color, D - Maturation at 10% change skin color.

that calcium really may maintain more stable the structure of the cell wall, seeing that when it was combined with 1-MCP there was no effect on firmness. An explanation in this case, may be because humidity into the container increased softly due to 1-MCP solution applied indoor, what could be sufficient to promote moisture saturation from $CaCl_2$ reducing its absorbing capacity.

The present work encourages the development of further investigation in order to verify the effects of both $CaCl_2$ and 1-MCP applied at numerous different concentrations on quality conservation of 'Roxo-de-Valinhos' ripe figs harvested at earlier maturation stage and during a longer storage period than studied here.

In conclusion, this study showed the effect of calcium chloride and 1-methylcyclopropene on conserve quality of 'Roxo-de-Valinhos' ripe figs to market for table. The calcium chloride solution applied at 4% improves firmness and promotes soluble solid stabilization of ripe figs stored for 6 days in a refrigerator, whereas 1-MCP treatment at a dose of 10 µg l^{-1} does not contribute for maintaining postharvest quality of ripe fig, nor its shelf life. However, treatment with 4% $CaCl_2$ combined with 1-MCP 10 µg l^{-1} increases the maturation index (ratio) of fresh ripe figs. Finally, a better understanding of harvest seasons, maturation stages and postharvest treatments on ripe figs is essential for crop fig, grower and market.

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