Evaluation of antioxidants to reduce physical damage in 'Manzanillo' olives in 2007

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Summary:

Table olive quality must be maintained while need for mechanized harvest increases. Antioxidant tests made in 2006 on 'Manzanillo' olives subjected to physical damage simulating mechanical harvest evaluated glutathione, ascorbic acid, a lactic/acetic acid solution, quercetin and luteolin. Several of these are naturally-occurring in olive or other fruits. Ascorbic acid appeared the most promising as a drench in 2006. In 2007 trials we tested drenches of ascorbic acid, a 'cocktail' of ascorbic acid, calcium lactate and cysteine (Gorny et al., 2002), and two commercial treatments for specific postharvest applications (ethoxyquin and diphenylamine; ETOX and DPA, respectively); a preharvest spray application of ascorbic acid was also tested. Repeated trials tested antioxidant concentration and length of drench time to find the best combination for reducing bruising and softening in fruit mechanically damaged by machine harvest and by shaking in a closed container to simulate mechanical harvest. Replicated and non-replicated trials were conducted in commercial and trial orchards in California; results were similar regardless of location or orchard conditions. Ascorbic acid, ETOX and DPA showed significant reduction of bruise development over time and improved firmness compared to mechanically damaged, untreated fruit; effectiveness for antioxidants was improved as concentration and time of exposure were increased. Optimum results were gained with ETOX and DPA, however, the optima were at immersion times or concentrations in excess of what could be expected for commercial purposes. These extended periods of exposure at high concentrations were used to gain information on what damage mitigation may be expected of an ideal treatment. We also tested the ethylene binding inhibitor, 1-MCP (1-methylcyclopropene), in controlled atmosphere for improvement in firmness after mechanical damage in a pilot test.

Problem and its significance:

Mechanical harvest of olives results in varying levels of visible damage within hours of fruit removal. Damage becomes more visible over time as the fruit is exposed to air and ambient temperatures after harvest. Bruising consists of a local degradation of tissue combined with intracellular water exit (free water) and browning (oxidation) of phenolic compounds from released intracellular water. As fruit sit in bins in the field, at the receiving station, in transit, or at the processing plant, prior to processing, oxidative processes continue and fruit damaged in harvest deteriorates noticeably; high ambient temperatures exacerbate fruit damage due to mechanical injury. Significant advances have been made in 2006-2007 toward reducing mechanical damage to olives that are mechanically harvested. These advances have addressed the process of olive removal from the tree and reduction of cuts and bruises, however, optimal quality similar to that of hand-harvested fruit is a goal that may be facilitated with preharvest or postharvest treatments.

L-ascorbic acid (vitamin C) is a well-known antioxidant that may be used in food applications as a 'reduced risk' chemical. Other viable candidates for maintaining fruit quality could be postharvest applications for pome or stone fruits, such as postharvest chilling, controlled atmosphere and drenches. Our tests in 2007 explored some of these options, building on results obtained in 2006.

Objectives:

1. Investigate antioxidant potential for reduction of damage due to simulated mechanical harvest.

2. Test treatments varying application method (preharvest spray, postharvest drench), exposure time and concentration to maximize benefit and obtain baseline information about maximum damage reduction that might be expected of an ideal treatment. An ethylene-binding inhibitor was also tested in a controlled atmosphere pilot study.

3. Develop a strategy that would be consistent with mechanized harvest, postharvest transportation and short-term storage, and the goals of fruit quality necessary for a high-quality processed product.

Plans and Procedures:

Trials included:

1. Fruit hand-harvested and subjected to mechanical damage from:
    a. UC Riverside's Lindcove Research and Extension Center (trees planted 1989, spacing 25' x 25') – replicated and non-replicated trials
    b. UC Davis Nickels Soil Lab in Arbuckle (trees planted 2001 at 12' x 18' in hedgerow) – replicated and non-replicated trials
    c. UC Davis Pomology orchard (Davis; trees of variable ages and spacings)
2. Hand-harvested and machine-harvested 'Manzanillo' fruit from one of the Rocky Hill commercial orchard (Exeter) machine harvest trials and from a demonstration of mechanical harvester at Nickels Soil Lab, Arbuckle.

Although sites varied geographically, in age, and management practices, all trees used had moderate to heavy crops and fruit of similar sizes and maturity (by color). The trial period extended from 20 September through 8 November. Only the trial on 8 November at the UCDavis orchard included a few fruit that were not hard green or yellow-green (Olive Maturity Index Calculator, 2006; http://cesonoma.ucdavis.edu/SpecialtyCrops/Olives.htm). The IOOC (1990; International Olive Oil Council) recognizes 'green' as '...full size, and the color of the fruit may vary from green to straw yellow.' (Colmagro et al., 2001). Those fruit in the last trial included a few that were 'turning color' in order to compare firmness changes with maturation and the effect of antioxidants and an ethylene-binding inhibitor on firmness loss due to mechanical damage..

When fruit were hand-harvested and treated to simulated mechanical harvest, that treatment initially consisted of shaking a 20 to 50-fruit sample in a large closed glass jar or 5 gallon plastic bucket with lid for 7 seconds, as in 2006, with external and internal damage induced in this manner similar to that of mechanically-harvested fruit, in trials up to 4 October. Fruit quality of mechanically-harvested fruit was greatly improved in 2007 trials with Dave Smith Enterprises harvester and other harvesters observed in a demonstration at Nickels Soil Lab on 11 October. Thus with trials beginning 4 October shaking was reduced to 3-4 seconds to more closely match 2007 machine-harvested fruit. Mechanical damage was induced within 30 to 60 minutes of hand harvest in all cases. Antioxidant treatments were either by preharvest spray application (handspray bottle to drip, ascorbic acid + Regulaid) or by immersion (drench, aqueous solutions) for varying lengths of time from 5 minutes to overnight. All antioxidant drench treatments were applied within 1 hour of mechanical damage, whether machine-harvested or simulated treatment. Drenches included ascorbic acid, ethoxyquin (ETOX; Deccoquin®

305), diphenylamine (NoScald 283 DPA) or a 'cocktail' of ascorbic acid, calcium lactate and cysteine (Gorny et al., 2002). The concentrations of each treatment tested was within experimental or label ranges that were effective in trials on other fruits for reduction of oxidative damage. Duration of drenches in commercial products were generally in excess of label instructions, however, since one of the goals of this project was to test the potential for reduction of bruising and softening by postharvest treatments, test materials were applied under a broad range of conditions. Ultimately, criteria for evaluation of fruit quality and treatment potential will be developed in cooperation with University researchers and industry food quality technologists.

Depending on the particular trial, fruit subjected to testing were either a bulk sample without replication, such as those collected from harvest bins in the machine harvest/hand harvest trial, or were from complete randomized block design applications made to fruit from replicate shoots. In each trial fruit were evaluated after antioxidant treatment, followed by an 'overnight' period of at least 8 hours, uncut and at ambient temperatures of ~75 ºF. Fruit were then evaluated visually for surface bruising and internal bruising on a median cut surface, rating bruising on a 0-4 scale, with 0 = no visible damage, and 4 = severe damage, combining evaluation of external and internal bruising together (in 2006 internal and external damage were rated separately). In the final trial on 8 November, fruit treated with antioxidant drenches were evaluated for change in firmness using a FirmTech II firmness testing device (BioWorks, Inc.; http://www.bio-works.us/); this device is the standard for firmness testing for the sweet cherry industry in California and Chile, and other fruit industries and researchers in various locations. In that last trial, a 'shaken' and 'unshaken' sample of fruit were also treated overnight to a controlled atmosphere application of 1 ppm 1-MCP, followed by firmness testing.

Analyses of variance were performed with Proc GLM procedure of SAS (SAS Institute Inc., Cary, NC) and mean separations were tested by Duncan=s Multiple Range Test; $P = 0.05$.

Results and Discussion:

The initial test of antioxidants in 2007 used an overnight drench of ascorbic acid compared to 5 minute drenches of three other antioxidant treatments (Table 1, Fig.1). The overnight drench was used in 2006 and proved somewhat effective in reducing the severity of bruising, however, when comparing this treatment to the short duration treatments of antioxidant 'cocktail', ETOX and DPA, it was evident that long soaking created halos of water-damaged flesh surrounding bruises that was unacceptable. Although the ascorbic acid treatment significantly reduced the severity of bruising, it was rated lower than other treatments that also reduced bruising development based on the water-logged appearance. All antioxidant treatments were significantly better than the untreated, shaken control either with respect to reduction of external bruising, internal bruising or both; the 'cocktail' improved bruising internally, but external bruising (as with overnight ascorbic acid drench) was not improved to the same extent as other treatments.

The second and third trials represented in Table 2 were identically applied at 2 locations, at slightly different times, but on fruit of similar maturity. These results were not different by location and are shown combine. In these cases, ascorbic acid was also tested as a preharvest spray treatment, and compared to 5 minute drenches of ascorbic acid, ETOX and DPA, and controls. Although fruit treated in antioxidant drenches showed some reduction of bruising severity, none was acceptable. Preharvest application of ascorbic acid did not control bruising.

Drench duration of ascorbic acid, ETOX and DPA were tested at intervals for efficacy; increased time from 5 minutes, to 15 and 30 minutes, showed significant improvement with increase in exposure of fruit (Table 3). Fruit exposed for 30 minutes showed very little development of bruising with all treatments. These same treatments were used to test effects on bruising in fruit obtained from mechanical harvesting by DSE at Rocky Hill (Table 4). In this trial fruit damage was less than that in the 2006 mechanical harvest trial and less than that imposed by shaking to-date. Amelioration of bruising injury was similar to that in previous 30 minute drenches with these materials, with respect to relative reduction of injury among treatments; bruising was low overall and decreased further by antioxidant treatments with DPA and ETOX slightly better than ascorbic acid. A comparison of antioxidants at varying concentration and duration of drench following simulated mechanical harvest at lower injury induced (Table 5) found that treatment with 2000 ppm DPA for 30 min. resulted in fruit damage statistically identical to that of untreated, unshaken fruit. Slightly less effective was 2000 DPA for 15 min. and 3000 ppm ascorbic acid for 30 min. Other treatments were intermediate to these and the untreated, shaken control in bruising. When fruit that had been harvested by a Coe harvester was evaluated (Table 6), damage was less than that from the DSE harvester, and the slight bruising was not changed noticeably by antioxidant treatment.

A final trial to test treatment effects of antioxidant drenches and 1-MCP on firmness of damaged green fruit found a slight numeric but non-significant improvement in firmness compared to the untreated, shaken control by DPA and ETOX (Table 7). 1-MCP decreased firmness of unshaken fruit compared to the untreated, unshaken control, and of shaken fruit, compared to the untreated, shaken control. Firmness in colored fruit that had been shaken was significantly improved by treatment with DPA; a numeric but non-significant improvement was found with ETOX as well. Again, 1-MCP decreased fruit firmness in the more mature fruit. Fruit numbers were small in this trial, thus results should be considered very preliminary, however, tests with larger sample sizes should be conducted in future.

Conclusions and considerations for future work:

Drenches were more effective than the single spray treatment and DPA drenches appeared to be more effective, generally, than those with other antioxidants. Increasing concentration and duration of drench reduced bruising relative to those increases. Despite the promising results, however, the most effective treatments are in excess of recommendations on the label for other fruits. Efficacy may be increased by other adjuvants that encourage better penetration, reducing the need for long exposure times or high concentrations, however, adjuvants would also have to be approved for fruit use. In any treatments in future, residue testing before and after processing will be indicated. Antioxidant tests were more effective in reducing bruising than in 2006, although the perceived need for such treatments may be less due to better mechanically-harvested fruit quality.

Fruit from mechanical harvests that were evaluated by the processors show far more damage than those in these trials. This is probably due to longer time intervals for fruit in transport, high heat conditions, and holding time prior to evaluation. This fact alone, makes clear the need to work with the processors to address all sources for fruit damage, including postharvest.

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Table 1. Comparison of drench treatments with antioxidants and their effect on mechanical damage appearance in 'Manzanillo' olive in 2007; non-replicated trial 17 September at Exeter, Lindcove Research and Extension Center. Test for length of drench, concentration, antioxidant type for further trials.

<table>
<tr><th>Antioxidant treatment [z]</th><th>Mechanical damage</th><th>Exposure to antioxidant drench</th><th>Appearance rating [y]</th></tr>
<tr><td>Untreated</td><td>Unshaken</td><td rowspan="2">none</td><td>0.0 c [x]</td></tr>
<tr><td>Untreated</td><td rowspan="5">Shaken</td><td>3.8 a</td></tr>
<tr><td>1000 ppm L-ascorbic acid</td><td>~8 hours</td><td>2.5 ab</td></tr>
<tr><td>'cocktail' w/v 2% ascorbic acid, 1% calcium lactate, 0.5% cysteine, pH 7</td><td rowspan="3">5 min</td><td>2.5 ab</td></tr>
<tr><td>2700 ppm ethoxyquin (ETOX)</td><td>2.0 b</td></tr>
<tr><td>2000 ppm diphenylamine (DPA)</td><td>2.0 b</td></tr>
</table>

[x] Mean separation within columns by Duncan's Multiple Range Test, *P* = 0.05.

[y] Visual evaluation for surface bruising and internal bruising on a median cut surface, rating bruising on a 0-4 scale, with 0 = no visible damage, and 4 = severe damage, combining evaluation of external and internal bruising together.

[z] Chemical treatment concentrations based either on previous study efficacy or on upper label recommendation. All fruit evaluated ~8 hr after damage imposed. All treatments at ambient temperature.

Table 2. Comparison of spray and drench treatments with antioxidants and their effect on mechanical damage appearance in 'Manzanillo' olive in 2007; replicated trials at Exeter, Lindcove Research and Extension Center and Arbuckle, Nickels Soil Lab, September 17-21 and 26-28, respectively, 3 replicate fruiting shoots per treatment. Analysis of results showed no difference by location; results shown are compiled across locations.

Antioxidant treatment	Mechanical damage	Exposure to antioxidant [z]	Appearance rating [y]
Untreated	Unshaken	none	0.0 c [x]
Untreated	Shaken		3.8 a
0.15% Regulaid spray (control)		3 days prior to harvest	3.8 a
1000 ppm L-ascorbic acid + 0.15% Regulaid spray			3.4 ab
1000 ppm L-ascorbic acid drench		5 min drench	2.8 ab
2700 ppm ethoxyquin (ETOX)			2.0 b
2000 ppm diphenylamine (DPA)			2.0 b

[x] Mean separation within columns by Duncan's Multiple Range Test, P = 0.05; experimental design CRBD, 3 replicate branches per treatment.

[y] Visual evaluation for surface bruising and internal bruising on a median cut surface, rating bruising on a 0-4 scale, with 0 = no visible damage, and 4 = severe damage, combining evaluation of external and internal bruising together.

[z] All fruit evaluated ~8 hr after damage imposed and antioxidant treatment.

Table 3. Comparison of drench treatments with antioxidants testing duration of drench on mechanical damage appearance in 'Manzanillo' olive in 2007; non-replicated trial 1 October at Exeter, Lindcove Research and Extension Center.

Antioxidant treatment [z]	Mechanical damage	Exposure to antioxidant drench	Appearance rating [y]		
			5 min	15 min	30 min
Untreated	Unshaken	none	0.0 c [x]		
Untreated	Shaken	none	3.6 a		
1000 ppm L-ascorbic acid	Shaken	5, 15, 30 min intervals	3.2 a	2.5 ab	1.0 bc
2700 ppm ethoxyquin (ETOX)	Shaken	5, 15, 30 min intervals	2.8 ab	1.5 b	0.8 bc
2000 ppm diphenylamine (DPA)	Shaken	5, 15, 30 min intervals	2.6 ab	1.3 b	0.9 bc

[x] Mean separation within columns by Duncan's Multiple Range Test, $P = 0.05$.

[y] Visual evaluation for surface bruising and internal bruising on a median cut surface, rating bruising on a 0-4 scale, with 0 = no visible damage, and 4 = severe damage, combining evaluation of external and internal bruising together.

[z] All fruit evaluated ~8 hr after damage imposed. All treatments at ambient temperature.

Table 4. Comparison of drench treatments with antioxidants and their effect on mechanical damage appearance in ‘Manzanillo’ olive in 2007; non-replicated trial in Exeter, Rocky Hill commercial orchard, October 4. Fruit sampled from Dave Smith Enterprises machine-harvested or hand-harvested trees, held at ambient temperature (~75 ºF) 1 hr prior to antioxidant treatment.

Antioxidant treatment	Harvest method	Exposure to antioxidant [z]	Appearance rating [y]
Untreated	Hand	none	0.0 c [x]
Untreated	Machine	none	2.0 a
1000 ppm L-ascorbic acid	Machine	30 min drench	1.7 ab
2700 ppm ethoxyquin (ETOX)	Machine	30 min drench	1.2 b
2000 ppm diphenylamine (DPA)	Machine	30 min drench	1.3 b

[x] Mean separation within columns by Duncan’s Multiple Range Test, $P = 0.05$.

[y] Visual evaluation for surface bruising and internal bruising on a median cut surface, rating bruising on a 0-4 scale, with 0 = no visible damage, and 4 = severe damage, combining evaluation of external and internal bruising together.

[z] All fruit evaluated ~8 hr after damage imposed and antioxidant treatment.

Table 5. Comparison of drench treatments with antioxidants at different concentrations: effect on mechanical damage appearance in 'Manzanillo' olive in 2007; replicated trial in Arbuckle, Nickels Soil Lab on October 8. All fruit mechanically damaged except as indicated; duration of drench indicated. Damage induced by shaking was decreased in duration to match improved fruit quality of mechanical harvest observed with best machine harvest results at Rocky Hill.

Antioxidant treatment	Appearance rating[y]
Untreated, unshaken	0.1 d [x]
Untreated	2.6 a
1500 ppm L-ascorbic acid, 30 min	1.6 bc
3000 ppm L-ascorbic acid, 30 min	1.3 cd
1350 ppm ethoxyquin (ETOX), 30 min	2.2 ab
2700 ppm ETOX, 5 min	2.5 ab
2700 ppm ETOX, 15 min	2.0 ab
2700 ppm ETOX, 30 min	1.8 bc
1000 ppm diphenylamine (DPA), 30 min	2.0 ab
2000 ppm DPA, 5 min	2.0 ab
2000 ppm DPA, 15 min	1.2 cd
2000 ppm DPA, 30 min	0.4 d

[x] Mean separation within columns by Duncan's Multiple Range Test, *P* = 0.05. Experimental design was a CRBD with 5 fruiting shoot replicates per treatment .

[y] Visual evaluation for surface bruising and internal bruising on a median cut surface, rating bruising on a 0-4 scale, with 0 = no visible damage, and 4 = severe damage, combining evaluation of external and internal bruising together.

Table 6. Comparison of 30 minute drench treatments with antioxidants and their effect on mechanical damage appearance in 'Manzanillo' olive in 2007; non-replicated trial in Arbuckle, Nickels Soil Lab on October 11 testing antioxidants on machine-harvested fruit (Coe Harvester).

Antioxidant treatment	Appearance rating[y]
Untreated	0.7 a [x]
3000 ppm L-ascorbic acid	0.7 a
2700 ppm ethoxyquin (ETOX)	0.5 a
2000 ppm diphenylamine (DPA)	0.7 a
3000 ppm ascorbic acid + 2700 ppm ETOX	0.5 a
3000 ppm ascorbic acid + 2000 ppm DPA	0.7 a

[x] Mean separation within columns by Duncan's Multiple Range Test, $P = 0.05$. 15 fruit per treatment.

[y] Visual evaluation for surface bruising and internal bruising on a median cut surface, rating bruising on a 0-4 scale, with 0 = no visible damage, and 4 = severe damage, combining evaluation of external and internal bruising together.

Table 7. Effect on fruit firmness of mechanically-damaged fruit by 30 minute drench treatments with antioxidants and controlled atmosphere application of ethylene-binding inhibitor 1-MCP (methylcyclopropene) in ‘Manzanillo’ olive in 2007; non-replicated trial in UCDavis Pomology Orchard (applications made 7 November, firmness evaluated 8 November). Fruit maturity (color) considered in treatment effects.

Antioxidant treatment	Mechanical damage	Firmness (g)[y]				
		Green fruit	Colored fruit	All fruit		
Untreated	unshaken	855 a	622 a	816 a	816 a	
Untreated	shaken	651 b	384 cd	607 c	607 b	
3000 ppm L-ascorbic acid	shaken	Data on firmness lost due to computer problem				
2700 ppm ethoxyquin (ETOX)	shaken	701 b	442 bcd	658 bc	658 b	
2000 ppm diphenylamine (DPA)	shaken	711 b	462 bc	670 bc	670 b	
1-MCP, 1 ppm	unshaken	708 b	580 ab	686 b		686 a
1-MCP, 1 ppm	shaken	498 c	296 d	464 d		464 b

[x] Mean separation within columns by Duncan’s Multiple Range Test, $P = 0.05$. 25 green fruit and 5 ‘colored’ fruit per treatment tested. ‘Colored’ fruit showing 50% or less of skin not green.

[y] Firmness measured by FirmTech II Firmness device (BioWorks, Inc).

Figure 1. Appearance of fruit treated to mechanical damage, followed by antioxidant drenches and overnight ambient storage.