Evaluation of antioxidants to reduce physical damage in 'Manzanillo' olives in 2006

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Summary:

Several antioxidants were tested for reduction of physical damage induced by containerized shaking of 'Manzanillo' olives in 2006. Antioxidants tested included glutathione (naturally-occurring free radical scavenger), ascorbic acid, a proprietary lactic/acetic acid solution('an organic low Ph solution'; M. Erickson, personal communication), and quercetin and luteolin, flavones found in olive that have demonstrated strong anti-oxidant properties. Antioxidants were applied either as sprays on single limb replicates prior to damage, or as immersion overnight after mechanical damage. Different methods of inducing mechanical damage were tested to reproduce as closely as possible the type of damage observed in mechanical harvest trials in 2006. Ascorbic acid applied prior to hand harvest and mechanical damage improved the appearance of fruit after overnight storage by reducing the severity of bruising; luteolin alone and in combination with ascorbic acid also decreased apparent damage.

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 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. Candidates for testing antioxidant potential included several chosen from naturally-occurring plant antioxidants. Glutathione is possibly the most abundant antioxidant scavenger found in plant and animal cells (Wonisch and Schaur, 2002) and glutathione is involved in quenching free radicals through the ascorbate/reduced glutathione cycle (Alscher, 1989; Smirnoff, 1995). L-ascorbic acid (vitamin C) is a well-known antioxidant and part of the same cycle. Luteolin and quercetin, flavones found in the polyphenolic fractions of olive oil (Andrikopoulos et al., 2002) are purported to have antioxidant activity two to four times greater than that of vitamin E (Cao et al., 1997). A mixture of lactic and acetic acids—a proprietary solution used for storage of freshly-harvested olives prior to processing to prevent degradation of color—was also tested. Both spray treatments and immersion tests were used to simulate preharvest and postharvest treatments that might have potential in olive cultivation and processing in combination with mechanical harvest.

Objectives:

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

2. Evaluate fruit appearance after treatment by spray vs immersion methods to determine whether pre- or postharvest application may be better.

3. Design a mechanical damage system that induces mechanical damage similar to that observed with Korvan harvester for testing purposes.

Plans and Procedures:

The experiment was carried out at the Nichols Soils Lab in Arbuckle on six year old 'Manzanillo' olive trees planted to a hedgerow system with a 12 ft. X 18 ft. spacing.and drip irrigated Spray treatments were laid out in a complete random design in which 5 shoots bearing numerous fruit were selected for each treatment among several similarly-cropped trees with fruit at similar stages of maturity (few fruit colored past green-mature stage). Fruit were treated with sprays of antioxidants + Helena Premium MSO (methylated seed oil; 0.05% v/v) on 16 October, hand-harvested on 18 October and treated to mechanical damage by shaking in containers. Controls included untreated, unshaken fruit, untreated, shaken fruit, adjuvant only spray (in the case of spray treatments) and water immersion (in the case of immersion treatments). Sprays were applied to drip by hand-held spray bottles. Antioxidants included L-ascorbic acid (vitamin C) and luteolin (Table 1); a combination treatment included ascorbic acid followed by luteolin. All antioxidant treatments were in aqueous solution although luteolin was solubilized initially in absolute ethanol (solubility is 5 mg luteolin per ml ethanol). Antioxidants were obtained either from Sigma-Aldrich or vitacost.com (quercetin and glutathione); the lactic/acetic acid solution was donated by its manufacturer.

All fruit from treated shoots were harvested and subjected to mechanical damage after transport to UC Davis. . Mechanical damage methods were tested to produce similar internal and external bruising as seen in fruit mechanically harvested by the Korvan harvester. Fruit damage was most similar when produced by shaking sampled fruit as a batch sample in a large covered plastic jar for 7 seconds. An overnight storage period at ambient temperature followed shaking. In the case of sprayed fruit, storage was in paper bags open to the air.

Immersion tests with antioxidants after induced mechanical damage were also conducted to simulate postharvest submersion. Treatments included a water control, ascorbic acid, glutathione, ascorbic acid + glutathione, quercetin and a proprietary lactic/acetic acid solution (Table 1). Immersion was overnight. Each treatment was replicated on fruit collected from 5 shoots randomly distributed among the spray treatment shoots. Mechanical damage was induced immediately after harvest in samples collected for immersion tests. Harvested fruit were placed in net bags, immersed in the field and kept completely submerged in solutions overnight at ambient temperature.

Fruit evaluation was conducted 19 October on a subsample of 25 fruit from each treated shoot selected at random from each replicate. Each fruit was individually numbered and scores for the appearance of external and internal bruising was recorded for each fruit individually. Internal bruising was evaluated when fruit were cut longitudinally along the seed and both halves were evaluated as a total sample. Evaluation consisted of rating fruit visually as:

1. Externally bruised (1, 2, 3 scale with 3 most bruised)
2. Internally bruised (1, 2, 3 scale with 3 most bruised)

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; $P = 0.05$. Spray treatments were compared without immersion tests; immersion treatments were also compared without spray treatments. All treatments were also compared for significant differences.

Results:

Spray treatment comparison (Table 2)

External bruising was least in the untreated, unshaken control. Among treatments subjected to mechanical damage (shaking), the untreated, shaken control showed the most damage. All antioxidant treatments significantly decreased the appearance of external bruising to the some extent. Internal bruising was also least in the untreated, unshaken control. The appearance of internal bruising was greatest in the untreated, shaken control, adjuvant control and the luteolin treatment. Internal bruising was decreased significantly by ascorbic acid and ascorbic acid followed by luteolin.

Immersion treatment comparison (Table 3)

External bruising was least in the shaken, water control and the ascorbic acid + glutathione treatment. The lactic/acetic acid treatment showed the greatest amount of external bruising; every impact bruise was clearly marked after this treatment. All other treatments were significantly different and intermediate to the above treatments. Internal bruising was also least in the shaken, water control, greatest in the ascorbic acid treatment, and significantly different and intermediate to these treatments for other antioxidant immersion treatments.

Spray and immersion comparison (Table 4)

When all treatments were compared, external bruising was least in the untreated, unshaken control with all other treatments significantly greater. The lactic/acetic acid treatment showed the greatest external bruising. Sprays of antioxidants were all significantly better in reducing the effects of external bruising than were the immersion treatments, with the exception of the ascorbic acid + glutathione immersion treatment. This treatment, however, was not as good as sprays with luteolin or ascorbic acid + luteolin. Sprays of adjuvant, ascorbic acid, ascorbic acid + luteolin gave the best reduction in internal bruising and were better than all immersion treatments with the exception of the luteolin spray alone.

Conclusions and considerations for future work:

Spray treatments of antioxidants were generally better than immersion in reducing external and internal bruising due to mechanical damage, although not all antioxidants tested in sprays or immersions were duplicated in the other form of application. The treatments most effective in reducing both forms of bruising were sprays that included the methylated seed oil adjuvant alone and in combination with ascorbic acid and/or luteolin. Mechanical damage induced by shaking proved a viable substitute for mechanical harvest for testing purposes. While many other antioxidants may be tested, including those that are oil-based (such as α-tocopherol—vitamin E), those that were tested are readily solubilized in water. Solubility of native α-tocopherol was found to be extremely poor in aqueous solution; a water-soluble form exists for diagnostic purposes (Trolox), however, this form is prohibitively expensive and was not tested. Of those tested, ascorbic acid is very inexpensive and available as food-grade. This may prove to be a good treatment to test preharvest and prior to application of chemical loosening agents and mechanical harvest. Efficacy may be increased by other adjuvants that encourage better penetration.

Other antioxidant treatments that may be tested in future could include ethoxyquin or diphenylamine, used as postharvest antioxidant drenches or sprays in Canada with some apple varieties in which scald can be a problem (Kupferman and Guzwiler, 2003).

We propose testing ascorbic acid spray applications as a preharvest treatment in fall, 2007, incorporating this treatment into our mechanical harvest trial. Fruit would be collected for evaluation postharvest and subsampled for processing and evaluation after processing.

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Table 1. Anitoxidant treatments applied to 'Manzanillo' olive in 2006: effects on mechanical damage induced by shaking[x].

Spray treatments, 16 Oct	Immersion treatments, 18 Oct
Untreated, unshaken control	
Untreated, shaken control	Water control
0.05% Helena Premium MSO control	1000 ppm ascorbic acid
1000 ppm L-ascorbic acid + Premium MSO	10 ppm glutathione
10 ppm luteolin + Premium MSO	Ascorbic acid + glutathione
Ascorbic acid, followed by luteolin (both with Premium MSO)	10 ppm quercetin
	Lactic/acetic acid (proprietary solution, concentrations unknown)

[x] Fruit subjected to shaking as a mass sample in a large plastic jar to simulate damage received by mechanical harvester. Treatments applied by hand-held spray bottle or by immersion. Fruit treated with sprays were harvested 2 days post-treatment, shaken after harvest and evaluated 24 hr after storage at ambient temperature. Fruit treated by immersion were harvested, shaken prior to immersion overnight, then evaluated. All fruit evaluated 19 Oct.

Table 2. Comparison of spray treatments with antioxidants and their effect on mechanical damage appearance in 'Manzanillo' olive in 2006.

Treatment	Bruising[y]	
	External	Internal
Untreated, unshaken control	0.02 c[x]	0.03 d
Untreated, shaken control	1.20 a	1.76 a
0.05% Helena Premium MSO control	1.06 b	1.58 b
1000 ppm L-ascorbic acid + Premium MSO	1.05 b	1.46 c
10 ppm luteolin + Premium MSO	0.97 b	1.66 ab
Ascorbic acid, followed by luteolin (both with Premium MSO)	1.00 b	1.45 c

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

[y] External and internal bruising rating system (1, 2, 3 scale with 3 most bruised).

Table 3. Comparison of immersion treatments with antioxidants and their effect on mechanical damage appearance in 'Manzanillo' olive in 2006.

Treatment	Bruising [y]	
	External	Internal
Water control	1.22 d[x]	1.73 c
1000 ppm L-ascorbic acid	1.50 c	2.32 a
10 ppm glutathione	1.78 b	2.01 b
Ascorbic acid + glutathione	1.18 d	1.97 b
10 ppm quercetin	1.46 c	2.03 b
Lactic/acetic acid (proprietary solution, concentrations unknown)	2.34 a	1.90 b

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

[y] External and internal bruising rating system (1, 2, 3 scale with 3 most bruised).

Table 4. Comparison of spray and immersion treatments with antioxidants and their effect on mechanical damage appearance in 'Manzanillo' olive in 2006.

Treatment	Bruising [y]	
	External	Internal
Untreated, unshaken control	0.02 g [x]	0.03 g
Untreated, shaken control	1.20 d	1.76 cd
0.05% Helena Premium MSO control	1.06 ef	1.58 ef
1000 ppm L-ascorbic acid + Premium MSO	1.05 ef	1.46 f
10 ppm luteolin + Premium MSO	0.97 f	1.66 de
Ascorbic acid, followed by luteolin (both with Premium MSO)	1.00 f	1.45 f
Water control	1.22 d	1.73 d
1000 ppm L-ascorbic acid	1.50 c	2.32 a
10 ppm glutathione	1.78 b	2.01 b
Ascorbic acid + glutathione	1.18 de	1.97 b
10 ppm quercetin	1.46 c	2.03 b
Lactic/acetic acid (proprietary solution, concentrations unknown)	2.34 a	1.90 bc

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

[y] External and internal bruising rating system (1, 2, 3 scale with 3 most bruised).