Evaluation of antioxidants and preharvest plant growth regulators to reduce physical damage and improve firmness in 'Manzanillo' olives in 2008

Kitren Glozer, Bill Krueger and Louise Ferguson, *Plant Sciences Department, U. C. Davis*

Summary

2008 trials included testing of firmness and color instrumentation used in other fruit industries (Mitcham et al., 1996; Slaughter et al., 2005; Valero et al., 2003). These technologies are being applied to judge results from the pre- and post-harvest trials for improved quality. Tests of agents to reduce browning in 2008 included ascorbic acid, salicylic acid, sodium ascorbate and sodium hydroxide (lye), reported to have benefit in reducing bruising in table olive (Ben-Shalom et al., 1978). Firmness in treated fruit was improved in most cases, however, the greatest reduction in browning of bruises was found with sodium hydroxide. In 2008 we visual vs colorimetric assessment of bruising using a colorimeter and determined that the colorimetric method will require significant modification of technique if adopted. We also implemented on a larger scale than in 2007 our use of firmness testing (FirmTech II) that has been adopted worldwide in many small fruit industries as well as research facilities.

Introduction

Enzymatic browning of fruit tissue damaged by rough handling, high pressure or machine harvest is caused by conversion of natural phenolics to quinones that are then oxidized to brown, red or black pigments (bruises). Thus, bruising results from cellular breakdown, which also often results in fruit softening. Exclusion of oxygen can reduce polymerization to colored pigments by immersion in brine or water alone. However, once fruit is removed to the air, oxidation continues.

Chemical additives can be used to reduce or prevent enzymatic browning if applied quickly enough and are appropriate to the particular type of enzymatic browning. Additives can include ascorbic acid and its analogs, sulfites (metabisulfites and bisulfites) that interact with quinones to form colorless products, and cysteine, a reducing agent. Further tests for optimum concentration, length of time prior to immersion after induced damage, temperature of lye solution, and length of immersion indicate some guidelines that may be implemented on a larger scale in future. Other treatments remain to be tested, including combinations of calcium products with bruise-inhibiting agents for improved firmness post-harvest. While Ben-Shalom et al. (1978) indicated that inhibition of enzymatic bruising in olive by polyphenol oxidase inhibitors or reducing agents is not possible due to impermeability of the whole fruit and that only dipping fruit in 0.4% NaOH (sodium hydroxide or lye) prevented bruising after mechanical injury, other procedures tested more recently on other crops show potential for reducing bruising, including calcium citrate, citric acid (Terdbaramee et al., 2003) and others. Recent research in olive characterizing the nature of the polyphenol oxidase enzyme from olive, its activity and response to enzyme inhibitors (Segovia-Bravo et al., 2007) provides specific information that aids understanding of our own trial results and future possibilities. These authors found that the browning reaction in olive has maximum activity at pH 6, is completely inhibited below pH 3, regardless of temperature and that pH inhibition at pH 9 is dependent on a temperature of 8EC, and at pH 11 at 25EC.

Development of instrumental techniques to assess olive fruit quality before and after processing, particularly with respect to bruising development and fruit firmness, would be beneficial to determine the effects of treatments and the comparative level of damage from different mechanical harvest techniques. In 2008 we moved from visual assessment alone of bruising to colorimetric assessment. We also implemented on a larger scale than in 2007 our use of firmness testing (FirmTech II) that has been adopted worldwide in many small fruit industries as well as research facilities. These instruments have allowed progress toward a better system of evaluation, but they are not yet optimal for use in immature olive, nor has the FirmTech been tested on processed fruit (hand- and machine-harvested) post-processing.

Objectives

1. Optimize quality measures that identify key maturity and quality parameters for table olive, using nondestructive and destructive measures, working in concert with quality researchers in the industry processors and the USDA standards investigators, on fruit before and after processing.
2. Investigate anti-browning and plant growth regulator (PGR) potential for reduction of damage due to simulated mechanical harvest in both pre- and postharvest applications.
3. 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.
4. 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

1) Firmness, bruising and color baseline data development (Rocky Hill) – Objective 1

Hand-harvested 'Manzanillo' fruit was obtained from the Rocky Hill commercial orchard (Exeter, CA) for testing for firmness and defect rating, to develop information on these quality measures and the instrumentation best suited to test the measures. Six replicate samples were evaluated, each a 2 lb sample from bins harvested from a 14-tree block within a tree row (each replicate was 14 consecutive trees, randomized among rows). A 50-fruit subsample was first graded by color (green-straw vs colored, showing any blush color development), then scored (yes/no) for cuts or punctures, compression (soft, flattened spots), light bruising or heavy bruising. Cuts were scored only if larger than 1 mm in length and/or 0.5 mm in width. All compressions were scored regardless of size. Bruising and overall darkening of the flesh due to oxidation were evaluated on a longitudinally cut surface, external to the pit axis. Light bruising consisted of less than 25% of the cut surface showing discoloration due to bruising, heavy bruising was 25% or more of the cut surface bruised. A second 50-fruit subsample of green-straw fruit only was tested for firmness using a FirmTech II firmness testing device (BioWorks, Inc.; http://www.bio-works.us/); this device is the standard for non-destructive firmness testing for the sweet cherry industry in California and Chile, and other fruit industries and researchers in various locations. Student's T tests were performed using SAS (SAS Institute Inc., Cary, NC) for differences in damage by color grade and means for firmness of green-straw fruit obtained for each replicate.

2) Color measurement methodology (used for bruise analysis), Objective 1

Bruising and oxidation response after mechanical damage and chemical treatment was measured on a longitudinally cut surface external to the pit with a Konica Minolta colorimeter CR-10 and expressed in *LCH* color space, in which *L** = lightness, C is chroma (saturation) and H is hue angle. *L** = 0 is equivalent to black, *L** = 100 is equivalent to white. Of these measures, L and H appeared to correspond best to visual evaluation of darkening overall of the flesh with oxidation (measured as L) and darkening (browning) of bruises best measured by H. Hue angle decreases with browning (Samim and Banks, 1993); measurement of browning by reflectance is not uncommon in food quality studies (Bates, 1968; Jamieson et al., 2002). The aperature size of the colorimeter should be reduced in future tests by masking in order to reduce 'read' of tissue outside of bruised areas so as to obtain greater accuracy in measuring the bruises without measuring unbruised flesh.

3) Antioxidant treatments: Objectives 2, 3

Fruit subjected to testing were bulk samples without replication, collected randomly at a uniform green-straw maturity from various mature 'Manzanillo' olive trees in the University of California, Davis, Pomology

orchard (Davis, California). Fruit were treated to simulated mechanical harvest as previous trial years by shaking a 20 to 50-fruit sample in a large closed plastic jar for 7 seconds, with external and internal damage induced in this manner similar to that of mechanically-harvested fruit under 'worse case' conditions. Fruit were evaluated after antioxidant treatment, in some cases after a several-hour delay period, simulating time delay between commercial harvest, grading at the receiving station and receipt at the processing plant. Those fruit held for an extended period were uncut and at ambient temperatures of ~75 ºF. In all trials, firmness was non-destructively measured prior to cutting fruit for bruise evaluation.

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; $P = 0.05$. PROC TTEST was used for comparison of firmness by harvest method (hand vs machine) within treatments for PGR applications in the preharvest trial.

a) *Antioxidants, survey group* -- Treatments (Table 2) included as controls: untreated and bruised only, as well as immersion in water with and without mechanical damage (bruising treatment). Although water immersion decreased browning initially, once fruit were allowed to air-dry, browning progressed as with bruised fruit that had not been immersed in water. Thus, water immersion gives a temporary benefit that ceases once exposure to oxygen occurs. Results from various water immersion results are omitted from the treatment lists and discussion as not being worthy of consideration.

   Antioxidants included: 0.3% ascorbic acid (AA, pH 3), 4 mM salicylic acid (SA, pH 7), 1% sodium ascorbate (SASC, pH 7) and 0.4% sodium hydroxide (lye, pH 13). Other antioxidants intended for testing included calcium citrate alone and in combination with AA or AA + SA, sodium erythorbate alone and in combination with AA. These antioxidants were not tested on fruit because of solubility problems. All immersions were in room temperature solutions, for 1.25 hr after bruising treatment, after which fruit were stored in air and ambient temperature before firmness testing and evaluation of bruise development.

b) *Sodium hydroxide series* -- This series used the results generated in the 'Antioxidant, Survey Group' test to further develop best conditions for lye reduction of bruise development, adjusting time between bruising and lye treatment (Immersion delay test), time of immersion (Immersion duration test) and Lye concentration test. There was no delay in testing for firmness and degree of bruising after treatment (no storage), other than as noted. All immersions were in room temperature solutions except as noted.

   i) Immersion delay test (Table 3) – Controls included: no treatment and bruised only. Lye treatments were all at 0.4% with immersion delays of 0, 1, 2, 3, 4, and 5 hours.

   ii) Immersion duration test (Table 4) – Controls consisted of no treatment, bruised only, bruised with immediate immersion for 15, 30 or 60 minutes. Lye treatments were all at 0.4% for the same immersion durations.

   iii) Lye concentration test (Table 5) – Controls included untreated and bruised only. Lye treatments were all immersions for 30 minutes and varied as: 0.1% NaOH, 0.2% NaOH, 0.3% NaOH, 0.4% NaOH and 0.4% NaOH at 43EF (refrigerated).

4) <u>Preharvest plant growth regulator and mechanical harvest trial (Objectives 2 and 3)</u>

A single tree row of 'Manzanillo' olives spaced at 9' x 18' (269 trees per acre), running east-west, was pruned for mechanical harvest and utilized for this trial at the Erick Nielsen ranch, Orland, CA. Three treatments and an untreated control were randomized in a complete block design down the row in four replicate blocks. Treatments included ProGibb (30 g a.i. per acre of 4% GA_3; Valent BioSciences), Grow More LSE (4 pt/acre; seaweed foliar fertilizer; www.growmore.com), and Accel (30 g per acre of 1.8% 6-benzyladenine solution; Valent BioSciences). All treatments were applied on August 29 by handgun sprayer to runoff. No phytotoxicity was found with any treatment. Harvest occurred on October 6; fruit were sampled from each treated tree prior to harvest (~100 fruit sample) and fruit

were also sampled from the harvester bin immediately after fruit removal (~100 fruit sample). Mechanical harvest was with an ENE (Erick Nielsen Enterprises) trunk shaker. Fruit were then transported to UCDavis and a 25-fruit subsample of green-straw fruit evaluated for firmness, comparing hand-harvested and machine-harvested fruit, both treated and untreated (Table 6).

Results and Discussion

1) Firmness, bruising and color baseline data development (Rocky Hill) – Objective 1

Firmness of green-straw colored fruit ranged from 622 to 1456 g/cm^2, however, mean firmness was 915-1030 (Table 1). When green-straw fruit were compared to colored fruit that were commercially hand-harvested into half-ton bins, green-straw fruit developed significantly more light bruising than did colored fruit (Table 1); heavy bruising wasn't different between treatments. Green-straw fruit were also more susceptible to cuts and punctures than colored fruit (Table 1), with no significant difference in compression or soft spot development. The increased risk that green-straw fruit had toward light bruising and cuts might be due to higher tensile strength of the fruit skin than that of more mature fruit. We have not tested the firmness of colored fruit, however, it is likely that with maturity increase, fruit skin tensile strength may decrease and fruit be less prone to minor injury. Whether the same susceptibility is true under mechanical harvest conditions is not known, nor is it known what this level of damage, or the change in firmness with maturity change will affect fruit quality after processing.

2) Color measurement methodology (used for bruise analysis), Objective 1

While the colorimeter gave some indication of browning and darkening of flesh, the results were not as clear-cut as desired, possibly because the olive fruit is very hard at green-harvest stage, not allowing compression of the instrument against the flesh so as to exclude extraneous light, and because the aperature size is much larger than the typical diameter of bruises due to mechanical damage. While the second problem might be readily addressed, the first is inherent to the fruit. Other fruits that work well with the colorimeter have some 'give' to their flesh or skin, thus allowing complete contact with the instrument. Although results using the colorimeter are presented, the current system of judging visually the extent of the damage is sufficient for survey purposes, as is the case in these trials.

3) Antioxidant treatments: Objectives 2, 3

a) *Antioxidants, survey group* (Table 2) – Firmness was least in untreated control fruit and bruised fruit with no other treatment. All antioxidant treatments improved firmness with salicylic acid, sodium ascorbate and sodium hydroxide (lye) increasing firmness most. Any of these treatments would be acceptable for the purposes of improving post harvest fruit firmness, but sodium ascorbate showed the greatest improvement.

Lightness, or measure of darkening of cut flesh due to oxidation after cutting, was not greatly different in any treatment, especially when comparing the untreated control and the bruised control. Darkening of bruises was least in the lye-treated fruit, compared to the bruised only control, as indicated by the lowest value for hue angle. Because results for bruising were best for the lye-treated fruit, particularly as it corresponded very well to the visual assessment, lye was chosen for all other treatment series, concentrating on concentration, delay before immersion and length of immersion.

The benefit of immersion in weak solutions of lye immediately after mechanical harvest has been reported in the literature (Ben-shalom et al, 1978; Kailis and Harris, 2004). 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. Shomer et al. (1979) found that browning of bruised olives due to the enzyme catechol oxidase which is found

in chlorophyll-rich green olives. As the fruit ripens and turns black the enzyme is released once the chloroplasts degrade. Solutions of caustic materials such as lye inactivate the enzyme, inhibiting browning associated with bruised flesh. Exclusion of the oxygen required for oxidation changes in pigments is another method of decreasing bruising, as is cold storage as soon as possible after harvest (Kader et al., 1989).

In these preliminary tests, pH of antioxidant solutions was noted, but not adjusted to fit within the pH optima found by Segovia-Bravo et al. (2007). It is to be expected that lye (pH 13) would be effective, given the alkaline nature of the treatment, less expected where pH was 7 (salicylic acid and sodium ascorbate).

b) *Sodium hydroxide series*

i) Immersion delay test (Table 3) – Firmness was improved by immersion in 0.4% NaOH compared to the 'bruised only' control numerically by all lye treatments, however, a statistically significant difference was found only when lye immersion occurred at 1 hour after bruising treatment. All fruit discolored (reduced L or lightness) when cut compared to the untreated control, although a single lye treatment showed statistical equality to the control. This data, as well as that for bruising generated by the colorimeter, was inconclusive, however, visual inspection suggested that immersion within a short period of time after bruising treatment was better than longer delays.

ii) Immersion duration test (Table 4) – Firmness was significantly improved by immersion of 15 and 60 minutes in lye solution, compared to the bruised control. Lightness was significantly improved by immersion for 30 to 60 minutes compared to the bruised control, and browning was significantly less in the same immersion treatments.

iii) Concentration test (Table 5) – Firmness of bruised fruit was significantly improved by immersion for 30 minutes in room temperature lye at 0.3 and 0.4%, as well as 0.4% lye at 43EF, which also improved firmness compared to the untreated control. This firmness increase was not due to colder fruit at the time of measuring firmness as the fruit was allowed to come to room temperature. Thus, cold storage may provide a benefit in addition to that of lye, consistent with previous reports for table olive (Kader et al., 1989, 1990). Fruit treated in lye at 0.2 and 0.4% developed less overall flesh darkening compared to the bruised control; fruit treated with 0.4% lye in refrigeration had significantly less browning due to bruising than the bruised only control.

Results of the antioxidant tests indicate that immersion in 0.4% lye as soon as possible after mechanical harvest, for a duration of at least 30 minutes, preferably in refrigeration, would ameliorate bruising damage significantly. Other treatments that have potential for this purpose that should be tested include sodium benzoate and sodium chloride.

4) Preharvest plant growth regulator and mechanical harvest trial (Objectives 2 and 3)

Harvest method effects on firmness: Firmness was significantly reduced in machine-harvested fruit compared to hand-harvested fruit when untreated fruit were compared; the change in firmness due to harvest method was highly significant (0.1% level), however, fruit were still very firm after machine harvest (more than 1 kg/cm^2), and while a greater loss of firmness may occur in processing and storage with machine-harvested fruit that were initially this firm, that remains to be tested. Firmness was also significantly different by harvest method in fruit treated with ProGibb and Accel (0.1% and 1%, respectively), but no significant difference was found due to harvest method in the Goëmar-treated fruit. All PGR treatments significantly increased firmness compared to the untreated controls, regardless of harvest method. These results indicate that a more extensive trial of these PGRs should be made with and without machine harvest.

Table 1. Fruit quality measures of hand-harvested fruit, Rocky Hill orchard (Exeter, CA) in 2008.

Color (fruit skin)	%Light bruise	%Heavy bruise	%Cuts/punctures	%Compression, soft spot
Green-straw	35.4 a	0.6 a	23.0 a	0.6 a
Colored (exhibiting any red-purple development)	7.6 b	3.3 a	3.9 b	4.0 a

Green-straw fruit, range of firmness (g/cm^2; FirmTech II, BioWorks Inc.) minimum 622, maximum 1456; average fruit firmness 915-1030

[x] Means separation by Student's T test, $P = 0.05$.

Table 2. Bruising reduction chemicals: firmness and color change of 'Manzanillo' olives after bruising mechanical damage and antioxidant treatments in 2008. Immersion was for 1.25 hr; evaluation after 20 hr in air and ambient temperatures (high of ~75 ºF). Bruising measured as browning of bruises and change in lightness of cut flesh. Firmness was tested nondestructively on a FirmTech II (BioWorks, Inc.).

Treatment	Firmness (g/cm^2)	L (lightness)	Hue angle [y]
Untreated	967.3 c [x]	59.6 ab	92.3 ab
Bruised only	1002.0 c	60.4 a	92.7 a
0.3% ascorbic acid (pH 3)	1078.1 b	58.6 bc	91.5 ab
4 mM salicylic acid (pH 7)	1103.2 ab	56.9 b	91.4 ab
1% sodium ascorbate (pH 7)	1171.6 a	59.3 ab	92.8 a
0.4% sodium hydroxide (pH 11)	1108.5 ab	58.3 bc	90.4 b

[x]Means within a column followed by the same letter do not differ at $P = 0.05$ by Duncan's Multiple Range Test.

[y]Hue is a color value in LCH color space as measured by Konica Minolta CR-10 colorimeter which decreases with browning of bruises (Samim and Banks, 1993). L = 0 is equivalent to black; L = 100 is equivalent to white. L change for cut flesh overall.

Table 3. Immersion delay test: firmness and color change of 'Manzanillo' olives after bruising mechanical damage and immersion in 0.4% NaOH in 2008. Immersion in NaOH followed a time course of 0-5 hours post-bruising, at hourly intervals; evaluation after ~30 min in air and ambient temperatures. Bruising measured as browning of bruises and change in lightness of cut flesh. Firmness was tested nondestructively on a FirmTech II (BioWorks, Inc.).

Treatment	Interval (hr) between bruising and immersion treatment	Firmness (g/cm^2)	L (lightness)	Hue angle [y]
Untreated		1175.1 ab[x]	70.2 a	104.7 ab
Bruised only		1125.8 b	66.0 d	104.3 ab
NaOH	0	1165.8 ab	68.2 bc	105.0 ab
	1	1201.7 a	67.0 cd	104.5 ab
	2	1158.3 ab	67.3 bcd	104.1 b
	3	1198.5 ab	68.2 bc	105.8 a
	4	1160.0 ab	68.9 ab	105.6 a
	5	1174.3 ab	66.4 d	105.6 a

[x]Means within a column followed by the same letter do not differ at $P = 0.05$ by Duncan's Multiple Range Test.
[y]Hue is a color value in LCH color space as measured by Konica Minolta CR-10 colorimeter which decreases with browning of bruises (Samim and Banks, 1993). L = 0 is equivalent to black; L = 100 is equivalent to white. L change for cut flesh overall.

Table 4. Immersion duration test: firmness and color change of 'Manzanillo' olives after bruising mechanical damage and immersion in 0.4% NaOH in 2008. Immersion in NaOH or water (control) followed a time course of 0-1 hours ost-bruising, at 15 minute increments; evaluation after ~30 min in air and ambient temperatures. Bruising measured as browning of bruises and change in lightness of cut flesh. Firmness was tested nondestructively on a FirmTech II (BioWorks, Inc.).

Treatment	Duration of immersion	Firmness (g/cm^2)	L (lightness)	Hue angle [y]
Untreated	0	1175.1 ab[x]	70.2 a	105.3 ab
Bruised	0	1125.8 b	66.0 d	104.7 b
NaOH	15 min	1220.5 a	67.1 cd	105.1 ab
	30 min	1151.2 ab	69.5 ab	106.4 a
	60 min	1223.4 a	68.4 bc	106.5 a

[x]Means within a column followed by the same letter do not differ at $P = 0.05$ by Duncan's Multiple Range Test.
[y]Hue is a color value in LCH color space as measured by Konica Minolta CR-10 colorimeter which decreases with browning of bruises (Samim and Banks, 1993). L = 0 is equivalent to black; L = 100 is equivalent to white. L change for cut flesh overall.

Table 5. Concentration test: firmness and color change of 'Manzanillo' olives after bruising mechanical damage and immersion in varying concentrations of NaOH in 2008. Immersion in NaOH was for 30 min; evaluation after ~30 min in air and ambient temperatures. A single treatment was included a 'cold' (43EF) treatment. Bruising measured as browning of bruises and change in lightness of cut flesh. Firmness was tested nondestructively on a FirmTech II (BioWorks, Inc.).

Treatment	Concentration	Firmness (g/cm^2)	L (lightness)	Hue angle [y]
Untreated		1175.1 bc[x]	70.2 a	105.3 ab
Bruised only		1125.8 c	66.0 d	104.7 b
NaOH	0.1%	1198.9 bc	67.3 cd	104.6 c
	0.2%	1206.4 bc	68.5 abc	105.5 bc
	0.3%	1216.3 b	67.7 bcd	104.7 c
	0.4%	1151.2 ab	69.5 ab	106.4 b
	0.4%, cold	1307.8 a	67.7 bcd	108.7 a

[x]Means within a column followed by the same letter do not differ at *P* = 0.05 by Duncan's Multiple Range Test.

[y]Hue is a color value in LCH color space as measured by Konica Minolta CR-10 colorimeter which decreases with browning of bruises (Samim and Banks, 1993). L = 0 is equivalent to black; L = 100 is equivalent to white. L change for cut flesh overall.

Table 6. Fruit firmness after plant growth regulator treatment preharvest, comparing hand-harvested and machine-harvested fruit in 2008. Trial location was the Erick Nielsen Ranch, Orland, California. Preharvest treatments were applied August 29 and harvest was October 6. Firmness was tested nondestructively on a FirmTech II (g/cm^2; BioWorks, Inc.).

Treatment	Hand-harvested fruit firmness	Machine-harvested fruit firmness	Significance by harvest method within treatment[y]
Untreated control	1057.8 b	1014.8 b	***
ProGibb (30 g a.i. per acre of 4% GA_3)	1088.3 a	1126.8 a	***
Grow More (4 pt/acre; seaweed foliar fertilizer)	1104.6 a	1103.4 a	ns
6-BA (30 g per acre of 1.8% solution)	1113.7 a	1145.1 a	**

[x]Means within a column followed by the same letter do not differ at *P* = 0.05 by Duncan's multiple range test.

[y]Significant differences by Student's ttest by harvest method (hand vs machine) for a given PGR treatment; ns, *, **, *** = non-significant, significantly different at 5%, 1% or 0.1% level, respectively.

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