Polyamine Response to External Mechanical Bruising in Two Mandarin Cultivars

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Abstract. The polyamines putrescine, spermidine, and spermine (free form) were measured during 48 hours at 20 °C after mechanical damage of 'Fortune' and 'Clementine' mandarins (*Citrus reticulata* Blanco.) with forces of 0, 10, 20, and 30 N. In control fruits (without damage), the three polyamine levels were higher in 'Fortune' than in 'Clementine', and putrescine and spermine levels tended to diminish during the experiment, while spermidine levels increased slightly in 'Fortune' and showed a prominent peak in 'Clementine' 6 hours after storage. In general, polyamine levels increased in the peel following bruising. The magnitude of this effect differed for both cultivars and applied forces. Putrescine levels were positively correlated with the applied force in 'Fortune', while in 'Clementine' only the 20 N force was effective. Spermidine levels also increased in 'Fortune' following injury, but there was no difference in effects among the three forces used. Only the 10 N force increased spermidine levels significantly in 'Clementine'. Spermine levels were slightly higher than control levels after 48 hours, but not higher than initial levels.

Bruising is a major cause of loss of fresh fruit quality, and most bruising occurs as a result of impact. Some factors that determine the differences in bruising susceptibility of fruits to a given force include packaging methods, transportation modes, ripening stage at harvest, storage conditions, and fruit characteristics (Ericsson and Tahir, 1996). Bruising is closely related to impact energy (Pang et al., 1992). Fruit temperature at impact may affect bruising susceptibility, but contradictory results have been reported. Thus, warm sweet cherries (*Prunus avium* L.) developed fewer bruises than cold ones (Lidster and Tung, 1980). Saltveit (1984) reported that bruising of apples (*Malus domestica* Borkh.) increased with increasing fruit temperature at impact, whereas Klein (1987) found no relationship between apple temperature at impact and bruise susceptibility.

Endogenous polyamine levels and fruit development are closely related. However, the polyamines involved vary with species (Kakkar and Rai, 1993), and there seem to be species-specific relationships. Information regarding endogenous levels of the various polyamines

in *Citrus*, their role in growth and development, and their biosynthesis and metabolism is limited (El-Otmani et al., 1995). An accumulation of putrescine occurs in response to several plant stresses, such as acid (Young and Galston, 1983), salinity (Das et al., 1995), osmotic (Galston, 1983), and mineral deficiency stress (Feng and Barker, 1993). In *Citrus* fruits, most of the work has focused on the role of polyamines in chilling injury (CI), especially the accumulation of putrescine during exposure to chilling stress. Thus, in lemon [*Citrus limon* (L.) Burm.] and grapefruit (*Citrus paradisi* Macf.) flavedo, a relationship was found between CI severity and the level of putrescine (McDonald and Kushad, 1986). Many researchers have proposed that putrescine is a plant senescence inhibitor and ameliorates CI (González-Aguilar et al., 1994; McDonald, 1989; Yuen et al., 1995).

Most published work deals with the influence of exogenous polyamines in retarding fruit softening and increasing fruit firmness during storage (Kramer et al., 1991; Ponappa et al., 1993). However, little is known about the effects of mechanical bruising of fruit on endogenous polyamine levels in general, and particularly in *Citrus*. Since mechanical injury by impact is a kind of stress, we investigated the effects of mechanical injury on endogenous polyamine levels in mandarin fruit. Our aim was to study the effects of mechanical bruising on the polyamine contents of the peel in two mandarin cultivars ('Fortune' and 'Clementine') with different peel characteristics related to thickness, sponginess, and adherence to the juice segments. These two cul-

tivars are widely distributed in the market and have different susceptibilities to mechanical damage during handling in commercial packaging houses, with 'Fortune' being more susceptible to bruising than is 'Clementine'.

Materials and Methods

Plant materials. Mandarin ('Fortune' and 'Clementine') fruit were harvested from commercial farms in Murcia at the commercial maturity stage. At the laboratory (2 h after harvest), 100 fruit of each variety were rinsed in distilled water. Four different forces were applied to the fruit (0, 10, 20, and 30 N), using a universal assay machine (Lloyd LR 5K; Lloyd Instruments, Segensworth, U.K.) interfaced to a computer. Force was applied with an 8-mm-diameter cylindrical probe at a rate of 40 mm·min⁻¹ until the desired force was achieved, then held for 10 s. For each fruit, three impact forces were applied at three different zones of the equatorial plane separated by 120°. The fruit deformation in mm was registered by the computer for each impact. For each cultivar, 25 fruit were treated with each force level. After wounding, the fruit were kept at 20 °C. Five fruit of each cultivar and force level were sampled after 3, 6, 24, and 48 h, and 10-mm-diameter discs of peel (flavedo plus albedo) were taken from the damaged surface or from three equidistant points on the equator of control fruit, and immediately frozen in liquid N₂ for polyamine analysis.

To check the maturity of each cultivar, titratable acidity and total soluble solids of the juice were analyzed. Titratable acidity was determined by potentiometric titration with 0.1 N NaOH up to pH 8.1, using 1 mL of juice diluted in 25 mL H₂O. The results were expressed as g of citric acid per 100 g of FW. Two measurements were made from each fruit. Total soluble solids were analyzed by a P20 RL2 refractometer (PZO, Warsaw, Poland) and the concentration was expressed as °Brix at 20 °C. Three determinations were made from each fruit.

Polyamine analysis. Free polyamines were analyzed according to Flores and Galston (1982). One g of peel (flavedo plus albedo) was extracted with 10 mL of 5% cold perchloric acid, and 1,6-hexanediamine (100 nmol·g⁻¹) was added as an internal standard. The homogenate was then centrifuged for 30 min at 20,000 g. Free polyamines left in the supernatant were benzoylated as previously described (Serrano et al., 1995). Derivatives were analyzed by high-performance liquid chromatography (HPLC). The elution system consisted of MeOH-H₂O (64:36) as solvent, running isocratically with a flow rate at 0.8 mL·min⁻¹. The benzoyl-polyamines were eluted through a reversed-phase column (LiChroCart 250-4, 25 cm, 5 µm, Hewlett Packard) and detected by absorbance at 254 nm. A relative calibration procedure was used to determine the concentrations of the polyamines in the samples, using 1,6-hexanediamine as the internal standard and standard curves for putrescine, spermidine, and spermine. Two extractions of

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polyamines were made from each fruit.

Statistical analysis. A one-way analysis of variance (ANOVA) was conducted to determine the effect of force level on fruit deformation. Trend comparisons based on polynomials were made to examine the functional relationship between fruit deformation and the treatment applied.

A two-factor ANOVA was used to determine the effects of force treatments and storage time on polyamine levels in each cultivar. Mean comparisons were performed using HSD (Tukey's test) to examine differences between storage time at given force treatments. All analyses were performed with the SAS statistical software package (SAS Institute, 1988).

Results and Discussion

Effect of mechanical forces on fruit deformation. Two mandarin cultivars with different peel properties were used. The peel of 'Clementine' is thicker and more spongy and adheres lightly to the segments, while that of 'Fortune' is thinner and adheres tightly. The cultivars differed significantly in fruit deformation (Fig. 1). For both, fruit deformation increased with the external force applied, but this increase was greater in 'Clementine' than in 'Fortune'. However, inspection of the damaged sections under a magnifying glass (10×) revealed that mechanical damage due to the force applied occurred only in 'Fortune'. Break-down and disruption of the albedo was apparent 24 h after treatment with the 30 N force. This did not occur in 'Clementine' at any of the forces applied.

The levels of acidity and soluble solids in recently harvested fruits were 2.29 ± 0.14 g of citric acid per 100 g fresh tissue and 13.65 ± 0.13 °Brix, respectively, in 'Fortune', and 2.17 ± 0.11 g of citric acid and 13.24 ± 0.11 °Brix in 'Clementine' mandarin, respectively. These values indicate that both cultivars were of similar maturity at the beginning of the experiment. The differences in fruit deformation by the 10, 20, and 30 N forces were not associated with differences in maturity stage, but rather with different peel properties, such as thickness, sponginess, and adherence to segments, as previously described.

Effect of mechanical forces on polyamine content. The level of spermine was not affected by the force applied in either cultivar. Putrescine and spermidine content were affected by both the force applied and the storage time, as well as their interaction, in both 'Fortune' and 'Clementine' (Table 1). In both cultivars, putrescine and spermine levels decreased in control fruit during 48 h of storage at 20 °C (Figs. 2 and 4), while spermidine levels remained unchanged in 'Fortune' and increased in 'Clementine' fruit (Fig. 3). A reduction of putrescine and spermine concentrations in harvested fruits has been reported for pear (*Pyrus communis* L.) and avocado (*Persea americana* Mill.) (Toumadje and Richardson, 1988; Winer and Apelbaum, 1986), and could be a consequence of the ripening process. Also in tomato (*Lycopersicon esculentum* Mill.), putrescine and spermine levels

Fig. 1. Relationship between fruit deformation and external force applied in (a) 'Fortune' and (b) 'Clementine' mandarins.

Table 1. Significance of F values for force level and storage times following ANOVA of dependent variables of polyamine levels from two mandarin cultivars.

	Putrescine	Spermidine	Spermine
'Clementine'			
Time	***	***	NS
Force	***	***	NS
Time x Force	***	***	NS
'Fortune'			
Time	***	***	***
Force	***	***	NS
Time x Force	***	***	***

NS, **, *** Nonsignificant or significant at $P \leq 0.01$ or 0.001, respectively.

Fig. 2. Effect of fruit deformation, by applying external forces of 0, 10, 20, and 30 N, on putrescine levels in (a) 'Fortune' and (b) 'Clementine' mandarins during 48 h at 20 °C. Vertical bars indicate the s.e.

decreased during the climacteric, but putrescine increased in two long-keeping varieties (Martínez-Madrid et al., 1996; Saftner and Baldi, 1990). These reports deal with climacteric fruits, for which ethylene biosynthesis during the climacteric may compete with polyamine synthesis. However, little is known about polyamines in the ripening of nonclimacteric fruit.

In 'Fortune' mandarins, 20 and 30 N forces induced an increase in putrescine concentrations (Fig. 2a) that was detectable after 3 h at 20 °C. At 6 h, all the forces induced higher values of putrescine, with the magnitude of the increase apparently force-dependent. After 24 h at 20 °C, putrescine levels in the fruit treated with 10 and 20 N had decreased to the level found in the control. However, in mandarins treated with 30 N, the levels remained high. After 48 h, putrescine levels were similar in both control and treated fruit. In 'Clementine' mandarins, mechanical damage resulted in only a small increase in putrescine (Fig. 2b), and only in the 20 N treatment. This effect was observed beginning 6 h after the force was applied and tended to decrease over time at 20 °C.

In 'Fortune' mandarins, mechanical damage also resulted in increased spermidine levels (Fig. 3a). After 3 and 6 h, spermidine concentrations in treated fruit were 40% and 80% higher, respectively, than in control fruit, but no significant differences were found between the 10, 20, and 30 N treatments. At 24 and 48 h, spermidine levels had fallen to the levels found in the control. Spermidine levels (Fig. 3b) in both control and treated 'Clementine' fruit increased between 3 and 6 h of storage and then decreased; the concentration was greater in the 10 N treatment and lower in the 30 N treatment than in the control fruit throughout the 48-h storage period, peaking 6 h after the force was applied.

During the first 24 h after mechanical damage spermine levels decreased in both control and treated 'Fortune' mandarins, then increased in treated fruit during the next 24 h; the increases were proportional to the force applied (Fig. 4a). In 'Clementine', the effects of mechanical damage on spermine levels (Fig. 4b) were small, with only slightly higher levels in treated than in control fruit beginning 6 h after mechanical damage.

The reduction in putrescine and spermidine levels in damaged fruit 24 h after the treatment coincided with a slight increase in spermine levels for the two cultivars between 24 and 48 h. This could reflect the conversion of putrescine and spermidine to spermine, or the formation of polyamine conjugates.

The results obtained show a relationship between the severity of mechanical damage and the increase in putrescine levels. As noted above, there was considerable damage to the peel of 'Fortune' mandarins treated with 30 N, and high levels of putrescine were present in these fruits. The 10 N and 20 N forces also increased this polyamine in 'Fortune' fruit, which indicates that a physiological modification occurred despite the lack of visible damage. Although the treatments caused high fruit

Fig. 3. Effect of fruit deformation, by applying external forces of 0, 10, 20, and 30 N, on spermidine levels in (a) 'Fortune' and (b) 'Clementine' mandarins during 48 h at 20 °C. Vertical bars indicate the SE.

Fig. 4. Effect of fruit deformation, by applying external forces of 0, 10, 20, and 30 N, on spermine levels in (a) 'Fortune' and (b) 'Clementine' mandarins during 48 h at 20 °C. Vertical bars indicate the SE.

deformation in 'Clementine', they did not result in visible mechanical damage and this may explain the small increase in putrescine. In other *Citrus* species, such as lemon and grapefruit, putrescine levels rise in response to other kinds of stresses, such as storage temperature that induces CI (McDonald and Kushad, 1986). This increase could ameliorate

the CI effects of cold storage (McDonald, 1989; Yuen et al., 1995).

However, our data may also indicate that the increase in putrescine is the result of mechanical stress rather than a defense mechanism against its effects, such as has been suggested to occur in chill-injured peppers (Serrano et al., 1997). Nevertheless, further

studies are needed with other *Citrus* species and storage conditions, as well as application of exogenous polyamines, to gain a better understanding of the physiological role of polyamines in mechanically damaged fruit.

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