Effects of Ripening Stage, Temperature, and Film Wrapping on the Elasticity Index as Determined by a Nondestructive Resonance Vibration Method and Fruit Quality in ‘Irwin’ Mango

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Abstract. We examined the effects of ripening stage, temperature, film wrapping, and storage period on the elasticity index (EI) and fruit quality of ‘Irwin’ mangos to develop a storage technique for improving the storage life of this fruit and to determine whether flesh firmness can be estimated using the EI. The second resonant frequency measured between the two sides of the fruit was nearly equal to measurements between the dorsal and ventral surfaces of the fruit. The EI declined as fruit ripening progressed and was not influenced by fruit size. Fruits harvested at the pre-natural fruit drop stage (pre-NFDS; 2 to 3 days before fruit drop) and the post-natural fruit drop stage (post-NFDS) were kept at 25 °C for 10 days. No significant differences were detected between fruits at these two stages in terms of the EI and flesh firmness, except that both variables at the pre-NFDS were higher than those at the post-NFDS after 0 and 4 days and after 0 day of storage, respectively. Similarly, no significant differences were found between fruits at these two stages in terms of total sugar and organic acid contents, regardless of the length of the storage period. Fruits harvested at pre-NFDS were individually wrapped in low-density polyethylene film (11 µm thick) and stored at 10, 15, 20, and 25 °C for 10 days. The EI of fruits kept at 10 °C was higher than that of fruits stored at 15 to 25 °C regardless of the presence of film wrapping. At 10 °C storage, the EI of film-wrapped fruits was higher than that of non-film-wrapped fruits. Film-wrapped fruits harvested at pre-NFDS were stored at 10 °C for 30 days. After 15 days in storage, relatively rapid and then gradual declines in both the EI and flesh firmness were observed. Sugar content was unaffected by the storage period, but organic acid content decreased after 30 days in storage. A strong positive correlation was found between the EI and flesh firmness. When the data set was limited to fruits less than 20 N·cm⁻² in flesh firmness and more than 350 g in weight, the correlation coefficient was higher compared with a data set with all fruit combined. The results of this study indicate that flesh firmness in ‘Irwin’ mangos can be estimated by measuring the EI. Moreover, the ripening stage, storage temperature, and film wrapping affected the retention of fruit quality.

Mango (*Mangifera indica* L.) cultivation in Japan has been increasing over recent years. In 2010, the total area dedicated to mango growth was 437 ha, and total production was 3200 t; these values represent increases of ≈40% and 60%, respectively, of those produced only 5 years ago (Ministry of Agriculture, Forestry and Fisheries, 2013). The early-maturing variety ‘Irwin’ mango has been the leading in Japan, because

consumers prefer its bright red skin, soft fleshy texture, and sweet scent. Given that the ‘Irwin’ fruit exhibits the characteristic natural fruit drop from the bearing branch with ripening, farmers cover the fruit with a bag-shaped net and harvest those that drop into the net. Fruits harvested in this way at the full-ripe stage are boxed and typically shipped to market. Originally, the ‘Irwin’ mango have a relatively short shelf life (Ishihata, 2000) because this method of harvesting can leave fruit in the net for any length of time at high temperatures in a greenhouse, which may result in increased fruit temperature and a reduced postharvest shelf life.

The development of a technology to improve the storage life of the mango could reduce the number of fruits discarded as a result of quality deterioration, thereby benefiting consumers as well as distributors and sellers. Furthermore, providing excellent fruit to consumers for extended periods would contribute to an enhanced popularity of the consumption of mango fruits.

Several studies have examined technologies to extend the storage life of mango fruits by investigating ripening (Medlicott et al., 1990), temperature and storage periods (Bustamante et al., 1997; Chaplin et al., 1991; Medlicott et al., 1990; Ueda et al., 2001), and packaging materials (Singh et al., 2001; Sornsirivichai et al., 1989). In terms of the ‘Irwin’ mango, previous studies have tested various storage techniques (e.g., Soe et al., 2005; Tajiri et al., 1996; Ueda et al., 1999, 2001), but information regarding the relationship between fruit quality and ripening remains scarce. Therefore, developing storage techniques for improving the storage life of mango fruits is necessary.

One of the most commonly used quality parameters for evaluating shelf life and storability in fruit is flesh firmness. Traditionally, this index is measured by destructive methods using a Magness-Taylor-type penetrometer. Because this technique involves penetrating the fruit flesh with a plunger, followed by recordings of maximum force, the fruit cannot be reused in subsequent measurements. A nondestructive method for estimating flesh firmness would allow the same fruit to be measured in succession, and the effect of treatment could be detected in real time. Such an approach would also improve research efficiency and minimize costs by reducing the number of fruits needed for investigation.

Several studies have attempted to non-destructively estimate the flesh firmness of fruit (Jha et al., 2006, 2010). One such approach involves the use of acoustic methods. Polderdijk et al. (2000), Shmulevich (2003), and Shmulevich et al. (1998) measured the first resonant frequency generated from fruits after being struck by a small pendulum or hammer, whereas Subedi and Walsh (2009) measured the sound velocity and Mizrach et al. (1997) measured the ultrasonic attenuation, revealing positive correlations between the numerical value (e.g., the EI) obtained using these nondestructive methods and the penetrometer reading. However, because a device that can detect and measure an effective clear resonance frequency has not yet been developed, these approaches could not accurately assess flesh firmness and were thus not put into practical use.

Conversely, additional research has shown that the nondestructive measurement of fruit resonance is possible using a laser Doppler vibrometer (LDV; Muramatsu et al., 1997, 1999), and LDVs have been applied to investigate the ripening of various fruits such as pears (Murayama et al., 2006), kiwifruit (Muramatsu et al., 1999; Terasaki et al., 2001), apples (Muramatsu et al., 1999), persimmons (Muramatsu et al., 1999), and

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nectarines (Muramatsu et al., 1996). In addition, Sakurai (2012) developed a smaller, more practical device that could measure the second resonant frequency (SRF) of fruits with accuracy similar to that of an LDV in the field. This device has clear practical use and has substantially advanced the field of non-destructive estimations of flesh firmness (Chino et al., 2011; Ohata and Sakurai, 2011; Takahashi et al., 2010). Currently, no studies have examined time-course changes in mango flesh firmness measured by resonance vibration, and no nondestructive evaluations of flesh firmness have been put to practical use.

The objectives of the present study were to identify the effects of ripening stage, temperature, film wrapping, and storage period on the EI and fruit quality to develop a storage technique for improving the storage life of the 'Irwin' mango and determine whether flesh firmness in mango can be estimated using the EI.

Materials and Methods

'Irwin' mango (*Mangifera indica* L.) fruits harvested from 14-year-old trees planted in the ground and 6-year-old trees potted in containers (32 cm in diameter, 35 cm in height) in a greenhouse were used for the following experiments. Expts. 1 and 2 to 5 were performed in 2012 and 2011, respectively. The full bloom dates of the trees used in these experiments were 25 Apr. 2011 and 22 Apr. 2012, respectively.

Effect of measurement position on the SRF (Expt. 1). Ninety fruits were harvested between 11 and 22 Aug., and the SRF was measured among three different portions of the fruit: 1) dorsal and ventral surfaces; 2) the two sides; and 3) the top and the base. For measurements 1) and 3), the vibrators were attached to the center of the equator on the dorsal surface and the top of the fruit, respectively, and the receivers were attached to the ventral surface and the base of fruit, respectively. Using data obtained in this experiment, correlations among these three measurement positions for the SRF were estimated. The maximum, minimum, and average fruit weights used for the three measurements were 516, 273, and 389 $\pm$ 57 g ($\pm$ SD), respectively.

Effects of fruit ripening stage and fruit size on the SRF, the EI, and fruit quality (Expt. 2). Fruits were harvested at three ripening stages: the mature green stage, the pre-natural fruit drop stage (pre-NFDS), and the post-natural fruit drop stage (post-NFDS). Fruits at the mature green stage were harvested between 5 and 10 Aug. Fruits at the pre-NFDS, during which peel color began to change from magenta to bright red and fruit drop was expected in 2 to 3 d, and fruits at the post-NFDS, which dropped into a net that day, were harvested between 15 and 17 Aug. Fruits were sorted into small (less than 350 g), medium (350 to 450 g), and large (greater than 450 g) size classes. Six fruits at each the mature green stage and the pre-NFDS and 10 fruits at the post-NFDS were

used for measurements of the SRF, the EI, and fruit quality.

Effects of harvest time on time-course changes of the EI and fruit quality (Expt. 3). Medium-sized fruits at the pre-NFDS and post-NFDS were harvested on 24 Aug. for use in this experiment. Fruits were laid on a plastic tray and placed in an incubator at 25 °C. The entire tray was wrapped in low-density polyethylene film (Pori-Rappu; Ube-Film, Sanyo-Onoda, Japan) that was 11 μ m thick and 30 cm wide to prevent water loss from the fruit. The oxygen gas permeability and moisture permeability of the film were 14,000 mL $\cdot$ m $^{-2}$ per 24 h per atm (at 23 °C) and 10 g $\cdot$ m $^{-2}$ per 24 h (at 25 °C and 90% relative humidity), respectively. Time-course changes in the SRF, the EI, and fruit quality using the same individual fruits were determined after 0, 2, 4, 6, 8, and 10 d of storage. The average weights of fruit harvested during pre-NFDS were as follows: after 0 d of storage: 396 $\pm$ 88 g (n = 10); after 2 d: 385 $\pm$ 38 g (n = 10); after 4 d: 409 $\pm$ 36 g (n = 10); after 6 d: 412 $\pm$ 21 g (n = 6); after 8 d: 416 $\pm$ 44 g (n = 6); and after 10 d: 411 $\pm$ 32 g (n = 6). Similarly, the average weights of fruit collected at post-NFDS were after 0 d: 430 $\pm$ 63 g (n = 10); after 2 d: 414 $\pm$ 35 g (n = 10); after 4 d: 395 $\pm$ 44 g (n = 10); after 6 d: 436 $\pm$ 54 g (n = 6); after 8 d: 385 $\pm$ 34 g (n = 6); and after 10 d: 403 $\pm$ 45 g (n = 6).

Effects of temperature and film wrapping during short-term storage on time-course changes in the EI and fruit quality (Expt. 4). In total, 32 medium-sized fruits collected at pre-NFDS were harvested on 22 Aug. Half were individually wrapped in polyethylene film, whereas the other half was not wrapped. Four fruits of each group were placed into incubators and stored at 10, 15, 20, and 25 °C. Time-course changes in the EI, the rate of weight loss, and peel color of these fruits were determined using the same individual fruits after 0, 2, 4, 6, 8, and 10 d of storage. The average weights of wrapped fruit used in the experiment were as follows: 398 $\pm$ 21 g at 10 °C, 428 $\pm$ 34 g at 15 °C, 399 $\pm$ 30 g at 20 °C, and 446 $\pm$ 22 g at 25 °C. The average weights of unwrapped fruit were 397 $\pm$ 39 g at 10 °C, 411 $\pm$ 17 g at 15 °C, 418 $\pm$ 45 g at 20 °C, and 392 $\pm$ 49 g at 25 °C.

Effects of long-term storage on the EI and fruit quality (Expt. 5). In total, 32 medium-sized fruits were harvested at pre-NFDS on 26 Aug. Fruit were then wrapped in polyethylene film and stored in an incubator at 10 °C. Eight fruits each were stored for 0, 15, 20, and 30 d. Measurements of the SRF, the EI, and fruit quality were conducted at each of the four time points. However, because two of the eight fruits stored for 30 d were corrupted by anthracnose disease, measurements of the SRF and fruit quality were determined using the six remaining fruits. The average weights of the fruit used in this experiment were as follows: at 0 d: 445 $\pm$ 77 g; 15 d after harvest: 425 $\pm$ 61 g; 20 d after harvest: 429 $\pm$ 46 g; and 30 d after harvest: 434 $\pm$ 60 g.

Relationship between the EI and flesh firmness. Using data from the 224 fruits used in this study, including 18 fruits at the mature green stage, the correlation between the EI and flesh firmness was investigated. The maximum, minimum, and average fruit weights were 722 g, 232 g, and 401 $\pm$ 71 g, respectively. Simple linear regression analysis was performed with the EI as the explanatory variable (X) and flesh firmness as the target variable (Y). The analysis was performed using the data from all fruits and using only those fruits measuring less than 20 N $\cdot$ cm $^{-2}$ in flesh firmness, because all fruit at the ripening stage fall below this value. The analysis was also performed using only data from fruit less than 20 N $\cdot$ cm $^{-2}$ in flesh firmness and more than 350 g in weight because the EI of fruit less than 350 g tended to be high in Expt. 2; however, no significant difference was detected.

Measuring the SRF and the EI. The SRF was measured using portable vibration measurement equipment (Vp-2; Seibutsu-Sindo-Kenkyusyo, Hiroshima, Japan). The vibrator and the receiver were, respectively, attached at the center of the equator on the dorsal and ventral surfaces of the fruit by sandwiching them by hand. For measurements between the top and base of the fruit, the vibrator was attached to the top of the fruit. Vibrations (0- to 3-kHz swept sine wave signal, 10-s measurement time, 1.35-Hz resolution) generated by the PC were applied to the fruit from the vibrator, the vibration from the fruit was detected by the receiver, and the corresponding voltage signals were transferred to the PC. The resonant frequency of the fruit was determined by fast Fourier transformation of the acquired signal. The EI was calculated according to the following formula (Cooke, 1972; Terasaki et al., 2001):

$$EI = f_2^2 m^{2/3},$$

where f_2 (Hz) is the SRF and m (g) is the mass of a fruit. SRF measurements were conducted immediately after removing the fruit from the incubator to avoid a rise in fruit temperature. The room temperature at the time of measurement was $\approx$ 28 $\pm$ 1 °C.

Fruit quality measurements. Fruit weight, peel color, flesh firmness, total soluble solid (TSS) content, sugar content, and organic acid content were determined as measures of fruit quality. Peel color (Hunter's L, a, and b values) was measured using a color-difference meter (CR-400; Konica-Minolta, Tokyo, Japan) at the center of the equator on the side of the fruit. To measure flesh firmness, a 3-cm-diameter patch of peel was horizontally removed with a sharp knife, and firmness was determined using a Magness-Taylor-type fruit penetrometer (FT011, FT327; Effegi, Alfonsine, Italy) mounted to a plunger that was 11.3 (in fruits less than 49 N $\cdot$ cm $^{-2}$ in flesh firmness) or 8.0 mm in diameter. The plunger was penetrated into the flesh, and the maximum force was recorded when penetrating 7 mm into the flesh through a cut surface. Measurements were performed on both sides of the fruit, and the average value was

calculated. The flesh was collected from the center of the equator on both sides of the fruit. Next, the juice from the fruit was squeezed and filtered through gauze, and TSS, sugar content, and titratable acidity were determined. TSS was determined using a refractometer (PAL-1; Atago, Tokyo, Japan), and titratable acid levels were determined using the titration method with 0.1 N NaOH to a phenolphthalein endpoint and conversion to citric acid content. The juice was diluted 20-fold with distilled water and then filtered through a syringe filter with a 0.45- μ m pore size. Sugar content and composition were then determined using a sugar analyzer (SU300; TOA-DKK, Tokyo, Japan) by pulsed amperometry. The anion exchange column method was used to determine the contents of fructose, glucose, and sucrose.

Statistical analysis. Data obtained in this study were subjected to analysis of variance (ANOVA) followed by a Tukey–Kramer’s multiple range test. Two-way ANOVA was used to evaluate significant differences in the fruit ripening stage and fruit size in Expt. 2. The significance of correlation coefficients was determined using *t* tests.

Results

Effect of measurement position on the SRF (Expt. 1). From the regression analysis using data for the SRF measured between the dorsal and ventral surfaces of the fruit, and between both sides of the fruit, both the regression coefficient and the correlation coefficient (*r*) were ≈ 1 (Fig. 1A). The regression coefficients and the correlation coefficients (*r*) of the SRF measured between the top and the base of the fruit for those measured between the dorsal and ventral (Fig. 1B) or between both sides of the fruit were somewhat low (Fig. 1C).

Effects of fruit ripening stage and fruit size on the SRF, the EI, and fruit quality (Expt. 2). The SRF declined as fruit ripening progressed and as fruit size increased (Table 1). The EI decreased as the ripening stage progressed but was unaffected by fruit size. The EI was $\approx 450 \times 10^5$ at the mature green stage, $\approx 150 \times 10^5$ at pre-NFDS, and $\approx 80 \times 10^5$ at post-NFDS. The flesh firmness of fruit harvested at the mature green stage was higher than for those harvested at pre-NFDS or post-NFDS, but no significant difference was observed between fruit harvested at pre-NFDS and those harvested at post-NFDS. When evaluating significant differences between the two stages using the *t* test, the flesh firmness of fruit harvested during pre-NFDS was significantly higher than that for fruits harvested at post-NFDS ($P < 0.05$). Flesh firmness declined as fruit size increased for fruits harvested at the mature green stage, but flesh firmness was unaffected by fruit size at pre-NFDS and post-NFDS. Flesh firmness had a significant combined effect on both fruit ripening stage and fruit size. The *a* value of peel color increased as the fruit ripening stage progressed, but *a* values were unaffected

by fruit size at the same ripening stage. The total sugar content of fruit harvested at pre-NFDS and post-NFDS was higher than in fruit harvested at the mature green stage. The fructose content was $\approx 3\%$ at the mature green stage and 5% to 6% at pre-NFDS and post-NFDS; values at pre-NFDS and post-NFDS were significantly higher than those at the mature green stage. The sucrose content was $\approx 2.5\%$ at the mature green stage but significantly increased to 8% to 10% at pre-NFDS and post-NFDS. The glucose content was $\approx 1\%$ at all ripening stages. No significant differences were observed between fruits harvested at pre-NFDS and post-NFDS in terms of total sugar content or the contents of each sugar. The sugar contents were unaffected by fruit size at any ripening stage. The content of organic acid in fruits harvested during pre-NFDS and post-NFDS was lower than that at the mature green stage. No significant difference in organic acid content was observed between pre-NFDS and post-NFDS with the exception of medium-sized fruit at pre-NFDS.

Effects of harvest time on time-course changes of the EI and fruit quality (Expt. 3). The EI of fruit harvested at pre-NFDS rapidly declined until 2 d of storage (Fig. 2A); thereafter, values gradually declined. However, the EI showed little change after 6 d in storage, stabilizing at a range of 45 to 51 $\times 10^5$. The EI in fruits at post-NFDS also gradually declined throughout storage but showed little change after 6 d, stabilizing at 36 to 38 $\times 10^5$. The EI values of fruit at pre-NFDS were higher than those at post-NFDS at 0 and 4 d of storage. Flesh firmness exhibited similar time-course changes as the EI (Fig. 2B). The flesh firmness of fruit harvested at pre-NFDS was higher than those harvested at post-NFDS at only 0 d of storage. The rate of weight loss increased with increased length of the storage period. No significant difference was observed between pre-NFDS and post-NFDS in rates of weight loss, which were 6.1% $\pm$ 4.2% (mean $\pm$ SD) and 7.1% $\pm$ 3.9%, respectively, after 10 d of storage. The *a* values gradually increased until 4 d of storage and gradually declined thereafter (Fig. 2C). The *a* values of fruit harvested at post-NFDS were higher than those harvested at pre-NFDS at 0 d of storage, but no difference was observed between these two stages after 2 d of storage. Furthermore, no significant differences were detected between pre-NFDS and post-NFDS in terms of sugar and organic acid contents, except for fructose content at 10 d of storage (Fig. 2D–H). Throughout the storage period, no symptoms of gas disorder or off-flavor were observed in fruit harvested at either pre-NFDS or post-NFDS.

Effects of temperature and film wrapping during short-term storage on time-course changes in the EI and fruit quality (Expt. 4). Overall, the EI rapidly declined until 2 to 4 d of storage, after which values gradually declined (Fig. 3A). In terms of film-wrapped fruit, the EI of fruit stored at 10 °C was higher than that for fruit stored at 15 to 25 °C

Fig. 1. Correlation in the second resonant frequency between measurement sites on ‘Irwin’ mango fruit ($n = 90$). ***Significance at $P = 0.001$. (A) Between dorsal and ventral surfaces of the fruit and between both sides of the fruit. (B) Between dorsal and ventral surfaces of the fruit and between the top and base of the fruit. (C) Between both sides of the fruit and between the top and base of the fruit.

between 4 and 8 d of storage. Similarly, for the non-film-wrapped fruit, the EI of fruit stored at 10 °C exhibited higher values than those of fruit stored at 15 to 25 °C. At 10 °C storage, the EI of film-wrapped fruit was higher than that for non-film-wrapped fruit after 6 d of storage. The rate of weight loss was lower for film-wrapped fruit than for non-film-wrapped fruit (Fig. 3B). Weight loss was positively correlated with temperature for non-film-wrapped fruit. However, no significant difference in weight loss was noted among storage temperatures for the film-wrapped fruit. The *a* values at 15 and 20 °C for both film-wrapped and non-film-wrapped fruit tended to be higher than those at other temperatures (Fig. 3C), gradually increasing until 4 d of storage after which *a* values stabilized. The *a* values at 25 °C in both wrapped and non-wrapped fruit increased after 2 d of storage and then gradually decreased. The *a* values at 10 °C in both groups were low early in storage and gradually

Table 1. Effects of ripening stage and fruit size on the second resonant frequency, the elasticity index, and fruit quality in mango cv. Irwin.

Fruit ripening stage	Fruit size	Fruit wt (g)	Second resonant frequency (Hz)	Elasticity index ($\times 10^5$)	Flesh firmness ($\text{N}\cdot\text{cm}^{-2}$)	Skin color a value	Total sugar (%)	Fructose (%)	Glucose (%)	Sucrose (%)	Organic acid (%)
Mature green	Small ^z	306 c ^y	1020 a	472 a	172 a	8.2 c	6.5 b	3.0 b	0.9 a	2.6 b	0.74 a
	Medium	395 b	904 ab	440 a	132 b	10.8 c	6.4 b	3.5 b	0.9 a	2.0 b	0.76 a
	Large	528 a	819 b	438 a	105 c	10.0 c	6.8 b	3.0 b	1.0 a	2.8 b	0.73 a
Pre-natural fruit drop	Small	297 c	591 c	171 b	14 d	17.5 b	15.3 a	5.9 a	1.3 a	8.1 a	0.42 c
	Medium	416 b	565 cd	160 b	15 d	17.9 b	14.7 a	4.8 a	1.1 a	8.8 a	0.45 b
	Large	535 a	444 de	133 bc	13 d	17.7 b	15.0 a	5.1 a	1.3 a	8.6 a	0.44 bc
Post-natural fruit drop	Small	312 c	438 e	88 c	13 d	25.4 a	15.9 a	5.1 a	1.0 a	9.8 a	0.30 c
	Medium	399 b	380 ef	78 c	11 d	25.1 a	15.9 a	5.2 a	1.0 a	9.7 a	0.35 c
	Large	557 a	337 f	77 c	9 d	24.4 a	16.2 a	5.2 a	1.2 a	9.8 a	0.37 c
Significance	Fruit ripening (A)	NS ^x	**	**	**	**	***	***	*	***	**
	Fruit size (B)	**	**	NS	**	NS	NS	NS	NS	NS	NS
	A $\times$ B	NS	NS	NS	**	NS	NS	NS	NS	NS	NS

^zEach 10 fruit was used. Small fruit: less than 350 g; medium one: 350 to 450 g; large one: over 450 g.

^yValues in a column followed by the same letter are not significantly different ($P < 0.05$) by Tukey-Kramer's multiple range.

^xNS Nonsignificant; *, **, *** significant at $P = 0.05, 0.01, 0.001$, respectively.

Fig. 2. Effects of harvest time on time-course changes in the elasticity index (A), flesh firmness (B), a value (C), total sugar (D), fructose (E), glucose (F), sucrose (G), and organic acid (H) during various lengths of storage period in 'Irwin' mango fruit. The number of fruit used for each period was six with the exception of 10 fruits at 0, 2, and 4 d of storage. Vertical bars represent $\pm$ SE. NS, **, and ***Nonsignificance and significance at $P = 0.01$ and 0.001 , respectively, using a t test.

increased thereafter. Neither the L nor b values were affected by storage temperature or film wrapping (data not shown). Throughout the storage period, no symptoms

of gas disorder or off-flavor were observed regardless of temperature and film wrapping.

Effects of long-term storage on the EI and fruit quality (Expt. 5). The EI and flesh

firmness rapidly declined after 15 d in storage; thereafter, values of both variables gradually declined (Fig. 4A–B). The rate of weight loss increased with increased length of storage (Fig. 4C). The a values increased after 15 d of storage and then stabilized (Fig. 4D). The contents of total sugar, fructose, and sucrose were unaffected by the storage period (Figs. 4E–F and 4H), although the glucose content decreased with increased length of the storage period (Fig. 4G). Organic acid content decreased after 30 d in storage (Fig. 4I). Regardless of the length of storage, neither chilling injury (CI) nor symptoms of gas disorder and off-flavor developed.

Relationships between the EI and flesh firmness. A strong positive correlation was detected between the EI and flesh firmness. When the data set included only those fruits less than $20 \text{ N}\cdot\text{cm}^{-2}$ in flesh firmness, the correlation coefficient between the EI and flesh firmness was $r = 0.738$ (Fig. 5B). When the data set was limited to fruits less than 350 g in weight, the correlation coefficient between the EI and flesh firmness was $r = 0.788$ (Fig. 5C). When all fruits were included in the analysis, the two regression lines crossed at $\approx 150 \times 10^5$ and did not follow the linear regression equation (Fig. 5A).

Discussion

Effect of measurement position on the SRF (Expt. 1). Previous studies have reported that when evaluating flesh firmness using acoustic methods, measurement values are influenced by factors such as measurement position and fruit shape (Al-Haq and Sugiyama, 2004; Shmulevich et al., 2003). Because mango fruits contain a disk-shaped hard stone and exhibit an asymmetrical shape, the SRF can vary greatly depending on the measurement position. In Expt. 1, the SRF was measured at three different positions on the same fruit. For measurements between the dorsal and ventral surfaces of the fruit, the vibrator was attached to the dorsal surface because this surface is relatively flat

Fig. 3. Effects of temperature and film wrapping during short-term storage on time-course changes of the elasticity index (A), weight loss (B), and a value (C) in 'Irwin' mango fruit. Four each of wrapped and non-wrapped fruit were stored at each temperature. Vertical bars represent $\pm$ SE. Values followed by the same letter and NS indicate nonsignificantly differ ($P < 0.05$) according to the Tukey-Kramer's multiple range test.

and the vibration direction was stable compared with attachment on the ventral surface; as a result, the reproducibility of values was very high. This experiment demonstrated that SRF values measured between the dorsal and ventral surfaces of the fruit were nearly equal to measurements made between the two sides of the fruit (Fig. 1A). Furthermore, the shape of the stone did not affect values of the SRF. In contrast, SRF values measured between the top and base of the fruit were higher (by ≈ 100) than measurements taken at the other positions (Fig. 1B–C); these higher values may reflect, to some extent, the particularly high flesh firmness at the base of the fruit, because the maturation of flesh at the base of the fruit is delayed compared with the top of the fruit. Therefore, we suggest that the most appropriate strategy is to measure the SRF between the dorsal and ventral surfaces or between the two sides of the fruit, and the former is preferable to the latter as a result of high reproducibility and ease of measure-

Fig. 4. Effects of long-term storage on the elasticity index (A), flesh firmness (B), weight loss (C), a value (D), total sugar (E), fructose (F), glucose (G), sucrose (H), and organic acid (I) in 'Irwin' mango fruit. Four fruits were kept at each storage period. Vertical bars represent $\pm$ SE. Values followed by the same letter did not significantly differ ($P < 0.05$) based on the Tukey-Kramer's multiple range test.

ment in 'Irwin' mango fruit. Thus, the SRF was measured between the dorsal and ventral surfaces of fruit in Expts. 2 to 5; however, when the peak of the SRF was not clear as a result of overripening, the measurement was confirmed by a second measurement between the two sides of the fruit.

Effects of fruit ripening stage and fruit size on the SRF, the EI, and fruit quality (Expt. 2). The EI decreased as fruit ripening progressed and was unaffected by fruit size (Table 1). Moreover, the EI was not correlated with the SRF (Table 1). The flesh firmness decreased as fruit ripening progressed; its pattern was nearly consistent with the EI. Ohata and Sakurai (2011) and Takahashi

et al. (2010) measured the EI of grapes on the vine and prunes on the tree, respectively, and reported that values decreased with fruit maturation. For fruit at the mature green stage or later, the EI and flesh firmness decreased in parallel with ripening, therefore indicating that seasonal changes in flesh firmness can be nondestructively estimated using the EI. Furthermore, no significant differences in flesh firmness were observed between fruit at pre-NFDS and post-NFDS. However, the significant differences between these two stages in the EI suggest that non-destructively measured values of the EI more sensitively reflect flesh firmness compared with values of flesh firmness measured using

Fig. 5. Relationships between the elasticity index and flesh firmness in 'Irwin' mango fruit. ***Significance at $P = 0.001$ by t test. (A) All fruit ($n = 224$). (B) Fruit limited to less than 20 $\text{N}\cdot\text{cm}^{-2}$ in flesh firmness ($n = 204$). (C) Fruit limited to less than 20 $\text{N}\cdot\text{cm}^{-2}$ in flesh firmness and over 350 g in fruit weight ($n = 157$).

the penetrometer. In terms of sugar composition, the sucrose content rapidly increased after the mature green stage during pre- and post-NFDS. The proportion of sucrose to total sugar was highest, at $\approx 61\%$, during post-NFDS followed by a fructose concentration of 32% to 33%. Glucose was lowest at 6% to 7%. These sugar compositions and the time-course changes in the 'Irwin' mangos were nearly consistent with the results of Ueda et al. (1999, 2001).

Effects of harvest time on time-course changes of the EI and fruit quality (Expt. 3). Storage at 25 °C was based on ambient room temperature for fruits harvested from June to August. When mangos were kept at 25 °C for 10 d, EI values of the fruit harvested during pre-NFDS were higher than for those harvested at post-NFDS at 0 and 4 d of storage (Fig. 2A). Flesh firmness was also higher at 0 d of storage (Fig. 2B), suggesting that storage for 1 to 2 d was effective at improving the storage life of fruit harvested during pre-NFDS in contrast to those harvested at post-NFDS. After storage, the EI of the fruit harvested during pre-NFDS decreased exponentially with the progression of fruit ripening (Fig. 2A); however, the EI of

fruit harvested at post-NFDS showed a pattern of mild decline, which was presumably the result of having already begun ripening. Similar exponential declines in the EI with fruit ripening have been reported for fruits such as pears (Murayama et al., 2006), kiwifruit (Terasaki et al., 2001), and grapes (Takahashi et al., 2010). The L, a, and b values indicate brightness and the color direction of red and yellow, respectively. Peel color is a significant predictor of fruit quality, especially for 'Irwin' mangos, because fruits for which the entire surface is red have a higher commercial value. The values of fruit harvested during pre-NFDS were lower than those collected at post-NFDS at 0 d of storage (Fig. 2C), and no difference was observed between these two stages after 2 d of storage. These results indicate that the progress of color change was unaffected even when fruit were harvested at pre-NFDS. The total sugar content was also unaffected by storage period (Fig. 2D), although the glucose content tended to decline after storage (Fig. 2F). Ueda et al. (2001) reported that glucose content declined in fully ripened fruit stored for 3 d at 25 °C. Our data indicated only a slight quality retention effect even if fruit were harvested before natural fruit drop. However, preventing stress caused by natural fruit drop to a net and the subsequent increase in fruit temperature when kept in the net provide benefits in terms of both fruit appearance and improvement of shelf life as well as enhancing the harvesting efficiency.

Effects of temperature and film wrapping during short-term storage on time-course changes in the EI and fruit quality (Expt. 4). Given that the EI values of fruit stored at 10 °C were higher than those of fruit stored at other temperatures between 2 and 8 d of storage (Fig. 3A) and cool storage inhibited weight loss (Fig. 3B), a quality retention effect was recognized under conditions of storage at 10 °C. Medlicott et al. (1990) reported that immature fruit showed superior storage capacity under low temperatures compared with fruit harvested at more advanced stages of physiological maturity; however, immature fruit failed to develop full-ripeness characteristics at storage temperatures of 8 and 10 °C. The fruits used in this experiment differed from immature fruits, because our fruits were harvested at pre-NFDS and maturation had already begun. Consequently, progression of maturation was observed during 10 d of storage at 10 °C.

When fruit weight declines during storage, the luster of the skin is lost and fine wrinkles occur, substantially degrading the appearance of the fruit. Film packaging keeps humidity high, preventing fruit weight loss and lengthening the storage capacity (Kubo, 2002). For mango fruit, the combination of film wrapping and cold temperature has been recognized as an effective means of enhancing storage capacity (Singh et al., 2001; Soe et al., 2005; Sornsriwichai et al., 1989). In this experiment, the EI values of film-wrapped fruits stored at 10 °C were higher than those of non-film-wrapped fruits from 6 d of stor-

age onward (Fig. 3A), suggesting that film wrapping effectively inhibited the overripeness of 'Irwin' mangos. Chino et al. (2011) demonstrated that film packaging inhibits water loss from the fruit, which prevents declines in the EI. This effect may also hold true for mangos. For film-wrapped fruit, no significant differences in weight loss were noted among storage temperatures; the rate of weight loss was only $\approx 2\%$ after 10 d of storage at 25 °C (Fig. 3B), which was the most extreme condition measured, and no surface wrinkles were observed at any storage temperature.

Effects of long-term storage on the EI and fruit quality (Expt. 5). In general, when fruit is stored at an optimal low temperature that does not cause CI, respiration and consumption of nutrients are inhibited and fruit quality can be maintained over long periods (Kubo, 2002). The minimum temperature for mango fruit storage that enables ripening and at which no CI occurs was estimated to be ≈ 10 °C. This temperature has been reported as ideal for the storage of the mango varieties 'Tommy Atkins' (Medlicott et al., 1990) and 'Keaw Sawoe' (Sornsriwichai et al., 1989) for ≈ 1 month, 'Azucar' for 15 to 45 d (Bustamante et al., 1997), and 'Kensington' for 3 weeks (Chaplin et al., 1991). In the present study, even when 'Irwin' mangos were stored for 30 d at 10 °C, symptoms of CI were not observed, and the internal fruit quality was well maintained (Fig. 4B–I). Soe et al. (2005) reported a quality retention effect for 20 d in 'Irwin' fruit at 10 °C storage using polyethylene bags. The temperature at which CI occurs is relatively low for 'Irwin' mangos; indeed, previous studies have reported that CI symptoms were not present at 4 to 5 °C in fully ripened fruit or in fruit that were stored for 3 weeks (Al-Haq and Sugiyama, 2004; Tajiri et al., 1996; Ueda et al., 1999). In future studies, we aim to determine the minimum optimal temperature for long-term storage of 'Irwin' mangos.

The results of this experiment suggest that the storage life of fruit harvested during pre-NFDS is slightly longer than for fruit harvested at post-NFDS. Furthermore, the combination of low temperature and individual wrapping using polyethylene film are effective for quality retention, and fruit wrapped in polyethylene film at 10 °C can be stored for ≈ 10 d.

Relationship between the EI and flesh firmness. Positive correlations between the EI determined by nondestructive resonance vibration and flesh firmness determined by a destructive method have been reported previously in fruits such as pears (Murayama et al., 2006), apples (Motomura et al., 2004), and kiwifruit (Muramatsu et al., 1999; Terasaki et al., 2001). Similarly, we observed a positive correlation between the EI and flesh firmness in mango fruits. The result of this experiment indicated that it is possible to estimate the flesh firmness of mango with relatively high accuracy from the EI, because the correlation coefficient between the EI and flesh firmness increased to ≈ 0.79 when the

analysis was limited to fruits less than 20 N·cm⁻² in flesh firmness and more than 350 g in weight (Fig. 5C). The flesh firmness of mature 'Irwin' mangos ranged from 7 to 15 N·cm⁻² throughout the storage period (Fig. 4B); these values are low, and variation with postharvest ripening was small. Because it is necessary to be able to detect slight changes in flesh firmness during ripening to determine the shelf life of mangos, we suggest that the most appropriate strategy is to estimate flesh firmness using the EI as determined by the resonant frequency method.

These results demonstrate the high use of the EI for testing optimal storage conditions for mangos, because this method is nondestructive and can be used to conduct ongoing measurements on the same individual fruits during ripening.

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