Relationship between the firmness of Golden Delicious apples and the physicochemical characteristics of the fruits and their pectin during development and ripening

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Abstract Firmness at harvest determines the postharvest storability and quality of apples. The climate change has altered the physiological processes of fruits and the reliability of ripening indicators typically used to determine the harvest time, compromising fruit firmness. In this study, ‘Golden Delicious’ apples were harvested at several developmental/ripening stages (107, 122, 137, 152 and 167 days after full bloom, DAFB) and evaluated for physicochemical attributes, which were correlated with fruit firmness. The 167 DAFB fruit corresponded to fruit at the commercial harvest. Fruit harvested at 107 and 122 DAFB did not develop the characteristics of ripe fruit while fruit harvested after 137 DAFB ripened normally. Fruit at commercial harvest showed low firmness. The changes of fruit weight, diameter, height, tristimulus color (L^* values) as well as the content of total soluble solids and moisture in

fruit correlated well with fruit firmness (r values from -0.76 to -0.97). The changes of pectin content, degree of esterification, molecular weight and content of glucose and galactose in pectin showed a positive relationship with firmness ($r = 0.62$ – 0.94). The content of protein, galacturonic acid and mineral elements in pectin correlated negatively with firmness ($r -0.66$ to -0.99). The results demonstrated that commercial harvest was delayed 30 days, compromising fruit firmness. Some underestimated ripening indicators may help in determining the harvest time of apples.

Keywords Fruit development and ripening · Physicochemical characteristics · Pectin · Texture · Firmness

Introduction

The storability and postharvest quality of apples are highly influenced by firmness at harvest time, with several changes of fruit being widely employed to determine the harvesting time of apples (de Castro et al. 2007; Hoehn et al. 2003). The target values for these changes were established long time ago for specific producing areas and they have remained unchanged for many years. However, the climate change and global warming have altered the physiological processes of apple trees and fruits (sprouting, flowering, fruit setting, and fruit development and ripening) and, consequently, the reliability of ripening indicators typically used for the determination of the harvest time and compromised the firmness of apples (Atkinson et al. 1998; Ramírez-Legarreta et al. 2011). This is particularly true for the largest apple producing area in northern Mexico, where the negative effects of climate change have affected the

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apple industry since 1992 and where the values of ripening indicators have remained without changes for many years (Ramírez-Legarreta et al. 2011). This might explain the very low firmness of Mexican ‘Golden Delicious’ apples as compared with that of apples from other geographical origins (Salas et al. 2011). The reliability of ripening indicators currently used to determine the harvest time must be determined.

Several physical and chemical attributes of apples have been related to firmness, but the results are conflicting and disperse. It has been proposed that large fruits are firmer than small fruits; however, this relationship seems to be dependent on fruit variety and geographical origin (Saei et al. 2011; Volz et al. 2004). The water and dry matter content have also been related to apple firmness in a cultivar-dependent manner, but the studies in this regard are scarce. Fruits with higher dry matter content tend to be firmer (Saei et al. 2011). The pH of apples can also influence the firmness (Johnston et al. 2002). However, the apple softening has mainly been explained in terms of the pectin modification in the primary cell wall during development and ripening. During these physiological stages, the pectin is slightly depolymerized, increasing its solubility (Siddiqui et al. 1996). However, the role of pectin depolymerization on apple firmness is still inconclusive because some studies with several apple varieties, including ‘Golden Delicious’ apples, have demonstrated that only in some cases pectin depolymerization and softening are related to each other (Gwanpua et al. 2016; Yoshioka et al. 1992). The degree of methyl esterification of pectin is also altered during apple development and ripening, diminishing the force of the binding between pectin chains by ions and reducing the integrity of the cell wall and apple firmness; however, the involvement of this pectin property on apple firmness is not completely clear (Gwanpua et al. 2016; Yoshioka et al. 1992). The apple softening occurs simultaneously with the liberation of galactose and arabinose residues from pectin (Redgwell et al. 1997); however, the changes in the composition of neutral sugars in pectin and their association with apple softening seems to be variable for the same apple variety. The content of calcium ions in the pectin is reduced during ripening, causing the dissociation of pectin chains and reducing the cohesion between cells and fruit firmness (Ortiz et al. 2011). The effect of the content of other ions in pectin that could potentially be related to apple firmness has not been studied yet. The objective of the present study was to assess the relationship between several physicochemical changes of ‘Golden Delicious’ apples and their pectin with firmness during development and ripening.

Experimental

Plant material and aim

‘Golden Delicious’ apples were harvested at several developmental stages (107, 122, 137, 152 and 167 days after full bloom, DAFB) from a commercial orchard in Cuauhtémoc, Chihuahua, Mexico. The last sample corresponded to fruit at the commercial harvest date. Each sample was composed of 50 apples. Twenty fruits were immediately evaluated for size (weight, diameter, and height), tristimulus color, firmness, moisture, and total soluble solids content (TSS%). Ten fruits were used to subjectively determine the capacity of the fruits to ripen at room temperature. The remainder fruits of each sample were subjected to pectin extraction. The obtained pectins were evaluated for molecular weight (MW) distribution, thermal stability, degree of methyl esterification (DM), monosaccharide composition, and protein and mineral element content. The relationship between the firmness and the physicochemical characteristics of the fruits and their pectin was determined.

Physicochemical characteristics of fruits

Twenty fruits were evaluated for physicochemical attributes. Weight (in g) was determined using an analytical balance while height and diameter (in mm) were measured using a Vernier caliper. Tristimulus color (L^* , C^* and h°) was measured on four opposite sides of the outer surface of each fruit (equatorial region) using a Minolta colorimeter (Minolta, Co. Ltd., Osaka, Japan). Firmness (peak force in N) was determined on four opposite sides of the surface of each fruit (equatorial region), using a TA-XT2i texture analyzer (Stable Micro System Ltd; Godalming, Surrey, England). After that, two slices (2 mm thick) were obtained from opposite sides of each fruit and dried (105 °C for 3 h) in order to gravimetrically determine the moisture content. Finally, the remainder tissue from the 20 apples was individually juiced using a commercial juice extractor and TSS% was directly measured in the obtained juice using a Pal-1 hand refractometer (ATAGO, Co. Ltd., Osaka, Japan).

Pectin extraction procedure

Twenty fruits were cut in 2 cm cubes and distributed in three groups, which were individually subjected to pectin extraction. The cubes were immediately blanched in boiling water for 5 min. The cubes were homogenized to puree using a kitchen blender and then mixed with 6.2% citric acid at a rate of puree to citric acid solution of 1:5 (w:v).

The pH of the mixture was adjusted at 2.5 using HCl. The mixture was heated and kept at 98 °C for 150 min. After cooling, the mixture was filtered through a Whatman paper No. 4, discarding the retained solids. The volume of the liquid extract was reduced to 1L by lyophilization. The extract was mixed with ethanol (3L) and kept in repose for 3 h to allow pectin precipitation. The pectin was recovered by filtration, using a Whatman paper No. 4. Retained pectin was washed four times with ethanol (500 mL) and acetone (200 mL), previous to be freeze-dried. The yield was gravimetrically calculated and expressed as % (dried weight basis). The protein content of pectin was immediately determined according to Bradford (1976).

Molecular-weight distribution of pectin

Pectins were solubilized in water (5 g/L). The solutions were filtered through a polyethylene membrane of 0.45 µm of pore size (Millipore Corp., Bedford, MA., USA), previous to be manually injected (20 µL) to a HPLC system (Varian Inc., Walnut Creek, CA., USA), which was equipped with a Refractive Index detector (Model Star 9040). Pectin fractions were separated at 40 °C using a series of TSK-GEL columns (GMPW_{XL}, G5000PW_{XL}, and G4000PW_{XL}; 7.8 × 300 mm each) (Tosoh Bioscience; Tokyo, Japan). Phosphate buffer (0.2 M; pH = 6.9) was used as mobile phase at a flow rate of 0.4 mL/min. The peak MWs were determined relative to dextrans after calibration with 10 DIN-accredited standards of known MW (1000–670000 Da) (Sigma-Aldrich., St. Louis, MO., USA).

Degree of methyl esterification of pectin

The galacturonic acid (GalA) was liberated from pectin by acid hydrolysis using H₂SO₄ and quantified colorimetrically according to Ramos-Aguilar et al. (2015). The colorimetric reaction was monitored at $\lambda = 525$ nm using a 6405 Jenway UV/Vis spectrophotometer (Jenway Ltd., Essex, UK). The GalA content was determined using a calibration curve constructed with three independent sets of dilutions of pure GalA.

The methanol was liberated from pectins by alkaline hydrolysis (7 M NaOH) according to Voragen et al. (1986). The extract was filtered through a polyethylene membrane with a pore size of 0.2 µm (Millipore Corp., Bedford, MA, USA) and manually injected (20 µL) to the HPLC system described above. The chromatographic system included a TSKGel SCX H⁺ (7.8 × 300 mm, 5 µm) ion exchange column (Tosoh Bioscience LLC, Tokyo, Japan), which was kept at 30 °C. Deionized water was used as mobile phase at a flow rate of 1 mL/min. HPLC grade methanol was used for identification and quantification purposes. The DM was calculated according to Voragen et al. (1986).

Monosaccharide composition of pectin

Pectins were sequentially subjected to acid and enzymatic hydrolysis, according to Ramos-Aguilar et al. (2015). For acid hydrolysis, the pectin (100 mg) was mixed with 0.2 M trifluoroacetic acid (5 mL) and kept at 80 °C for 72 h. For enzymatic hydrolysis, the pH of the reaction was raised to 5 using 14 M NH₄OH and the volume adjusted to 25 mL with water. Then, 40 µL of Macerex PM (enzyme complex) were added to the reaction, which was kept at 50 °C for 24 h under reciprocal shaking. The enzymatic hydrolysis was stopped by heating (100 °C for 3 min). The hydrolyzed sample was filtered through a polyethylene membrane of 0.45 µm of pore size (Millipore Corp., Bedford, MA., USA) previous to be manually injected (20 µL) to the HPLC system described above. Each hydrolyzate was analyzed under two different chromatographic conditions. The glucose, arabinose, rhamnose and galactose (Glu, Ara, Rha and Gal) were separated at 58 °C in a Metacarb H + (7.8 × 300 mm; Varian Inc., Walnut Creek, CA, USA) ion-exchange column using 0.0085 N H₂SO₄ as mobile phase at a flow rate of 0.4 mL/min. The xylose and mannose (Xyl and Man) were separated at 70 °C in a Supelcogel Pb (7.8 x 300 mm; Sigma-Aldrich., St. Louis, MO., USA) ion exchange column using water as mobile phase at a flow rate of 0.5 mL/min. The identification and quantification of sugars were performed using standard compounds.

Mineral element content in pectin

The pectin (200 mg) was incinerated at 450 °C for 2 h. The ash was digested with a mixture of nitric and perchloric acids (5:1, v:v) for 30 min. The acid was removed from the mixture by heating. The obtained residues were dissolved in ultrapure water (10 mL), filtered, and analyzed by ICP-OES (VARIAN 730-ES; Varian Inc., Australia), including a blank digest as control reaction. High purity argon and nitrogen were used for plasma generation and auxiliary gas, respectively. The flow of plasma and auxiliary gases were of 15.0 and 1.5 L/min. The nebulizer flow, reading time, stabilization time, and pump velocity were 0.75 L/min, 3 s, 15 s, and 15 RPM, respectively. The instrument provided simultaneous measurements for Ca, B, Cu, K, Fe, P, Mg, Mn, Na, and Zn.

Thermogravimetric analysis

Pectin thermal stability was analyzed with a Q500 thermogravimetric analyzer (TA Instruments, Delaware, USA). Ten mg of pectin powder were weighed in aluminum pans (900793.901, TA Instruments, Delaware, USA) and heated from 30 to 600 °C at 10 °C/min under nitrogen flow

(15 mL/min). The thermogravimetric curves were analyzed with Universal Analysis 2000 ver. 4.5A software (TA Instruments-Waters LLC).

Statistical analysis

The statistical significance of the differences between treatments was determined using ANOVA followed by the Tukey–Kramer post hoc test; 0.05 was the significance limit. The data analysis was performed using JMP statistical software (SAS Institute Inc., Cary, NC, USA). Pearson's correlation coefficients were obtained using Excel 2003 (Microsoft; USA).

Results

Physicochemical changes in apples and their relation with firmness

Fruit harvested at 107 and 122 DAFB did not develop the characteristics of ripe fruit. The fruit harvested since 137 DAFB ripened normally at room temperature. The fruit firmness continuously decreased during the tested period, reaching a total firmness loss of 29.7% (Table 1). The values for the biometrical characteristics of fruit gradually increased during development and ripening (Table 1), although significant differences were not observed for weight and diameter of apples harvested at 152 and 167 DAFB. The diameter and height of fruit tended to be similar each to other as development advanced, with fruits harvested at 167 DAFB being almost spherical. The changes of weight, diameter, and height during fruit development and ripening were highly correlated with softening (r values ranged from -0.89 to -0.92).

The moisture content in apples continuously increased during tested period. This variable was highly correlated

with fruit firmness ($r = -0.97$) (Table 1). The TSS increased as fruit firmness decreased (Table 1), showing a good correlation between these variables ($r = -0.80$).

The tristimulus color of the fruit changed during the tested period (Table 1). The L^* values continuously increased during tested period while the opposite was observed for a^* values. The C^* value increased from 107 to 137 DAFB and then decreased. The changes of L^* and a^* moderately correlated with fruit softening, showing r values of -0.76 and 0.70 , respectively.

Physicochemical changes in pectin and their relationship with apple firmness

The thermogravimetric analysis (TGA) of tested pectins showed two well-defined weight loss regions within the heating range (30 – 600 °C). The temperature for weight loss peaks (P_k) for these regions was determined by using the derivative of weight loss with respect to temperature (DTGA); however, no significant differences were observed for P_k values for tested pectins. The first weight loss region (30 – 150 °C) (Fig. 1) was associated to 10 – 12% of moisture release, with a P_k at 67.49 ± 2.60 °C. The second region (150 – 400 °C) (Fig. 1), with a loss of weight between 71 and 79% , corresponded to the pyrolytic decomposition of pectin. In all samples, the second region had two P_k at 229.13 ± 2.17 °C and 292.26 ± 0.26 °C, with the highest percentage of polysaccharide degradation occurring at the highest temperature. Considering the elevated concentrations of Glu in pectins (see below), this thermal stability of the last peak might be associated to some pectin-glucan complexes of high MW.

The pectin content remained almost constant in apples harvested between 107 and 152 DAFB, ranging from 12.5 to 13.5% in a dry weight basis (Table 2). Then, it continuously diminished until reach a value of 8.8% in fruit harvested at 167 DAFB. The firmness and pectin content

Table 1 Changes of physicochemical attributes of ‘Golden Delicious’ apples during on-tree development and ripening and their relationship with apple firmness

DAFB	Firmness (N)	Weight (g)	Diameter (cm)	Height (cm)	L^*	C^*	a^*	Moisture (%)	TSS (%)
107	89.6 ± 0.9^a	66.7 ± 1.5^d	5.3 ± 0.0^d	3.4 ± 0.1^d	65.7 ± 0.4^c	42.67 ± 0.3^c	118.3 ± 0.2^a	86.1 ± 0.2^{bc}	9.6 ± 0.1^c
122	82.4 ± 1.1^a	83.3 ± 1.4^c	5.7 ± 0.0^c	3.6 ± 0.1^d	66.6 ± 0.4^c	42.97 ± 0.2^{bc}	118.1 ± 0.2^a	86.0 ± 0.2^c	9.9 ± 0.1^c
137	70.2 ± 0.7^b	107.1 ± 2.9^b	6.2 ± 0.1^b	6.0 ± 0.1^c	69.3 ± 0.4^b	43.77 ± 0.2^a	116.8 ± 0.2^b	86.4 ± 0.2^{abc}	10.4 ± 0.1^b
152	66.1 ± 0.9^c	138.3 ± 3.3^a	6.7 ± 0.1^a	6.5 ± 0.1^b	69.5 ± 0.4^b	43.47 ± 0.1^{ab}	115.8 ± 0.2^{bc}	86.6 ± 0.1^{ab}	11.2 ± 0.1^a
167	63.0 ± 0.5^c	142.3 ± 3.7^a	6.8 ± 0.1^a	7.0 ± 0.1^a	71.5 ± 0.4^a	41.87 ± 0.1^d	115.2 ± 0.2^c	86.8 ± 0.1^a	11.6 ± 0.1^a
Correlation with firmness (r)		-0.89	-0.91	-0.92	-0.76	0.01	0.70	-0.97	-0.80

Values represent the mean of 20–80 individual measurements $\pm$ standard error. Values in the same column not connected by the same letter are significantly different ($p < 0.05$)

Fig. 1 Thermogravimetric and differential thermogravimetric curves of pectins extracted from 'Golden Delicious' apples during on-tree development and ripening

were slightly correlated ($r = 0.62$) each to other. Tested pectins contained two main fractions (Fig. 2). The MW of the first fraction (peak at 4411 kDa) was reduced 38% during fruit development and ripening and this reduction correlated well with fruit softening ($r = 0.89$). The second pectin fraction, which peaked at 1424 kDa, was clearly observed only in fruit harvested at 167 DAFB. The DM of pectin continually decreased during tested period (Table 2). It was strongly correlated with apple firmness ($r = 0.94$).

The most abundant sugars of tested pectins were GalA, Glu, Gal and Ara (Table 3), while Xyl and Man were detected at very low concentrations, representing only 2–10% of total sugars during ripening. The GalA content increased during development and ripening and was highly correlated with softening ($r = -0.95$). The Gal content tended to decrease from 107 to 152 DAFB, correlating well with fruit softening ($r = 0.88$). The Ara content in pectins increased during the first part of the tested period, reaching the highest concentration in pectin from apples harvested at 137 DAFB, and then decreased during the rest of the tested

Fig. 2 Molecular weight distribution of pectin from 'Golden Delicious' apples during on-tree development and ripening. [1] and [2] indicate the two characteristic pectin fractions/peaks of pectin

period. The changes of Glu in pectin also correlated well with apple softening ($r = 0.75$). The Rha, an important constituent of branched pectins, was detected at very low concentrations and showed a weak correlation with fruit softening. The Ara and Xyl content did not correlate well with fruit softening.

The content of the mineral elements in pectin that showed some correlation with apple softening is shown in Table 4. In all cases, the correlation coefficients were negative, indicating that the mineral element content in pectin increased as fruit firmness decreased. In our study, the changes of Ca content (821.0 ± 3 – 1087.9 ± 9.5 mg/Kg of pectin), the typical divalent ion with stabilizing activity of pectin, during development and ripening of tested apples did not correlate with apple firmness ($r = -0.28$). Similar results were found for B. The content of this element was very low and did not show significant changes during fruit development and ripening, ranging

Table 2 Changes of yield, tristimulus color, protein content and degree of methyl esterification of pectin from 'Golden Delicious' apples during on-tree development and ripening and their relationship with apple firmness

DAFB	Pectin content (%) ^a	L*	C*	°h	Protein in pectin (%)	Esterification (%)
107	13.5 ± 0.2^a	86.8 ± 0.6^b	10.4 ± 0.3^b	73.2 ± 0.2^c	0.2 ± 0.0^a	86.9 ± 1.1^a
122	12.3 ± 0.2^b	92.5 ± 0.2^a	8.8 ± 0.0^c	87.3 ± 0.1^a	0.2 ± 0.0^a	72.5 ± 1.7^b
137	13.2 ± 0.3^{ab}	93.0 ± 0.3^a	6.6 ± 0.1^d	82.8 ± 0.2^b	0.2 ± 0.0^a	58.6 ± 0.8^c
152	12.5 ± 0.2^{ab}	85.2 ± 0.0^c	11.7 ± 0.1^a	80.5 ± 0.1^c	0.2 ± 0.0^a	53.0 ± 0.8^c
167	8.8 ± 0.0^c	81.7 ± 0.1^d	10.8 ± 0.0^b	79.0 ± 0.1^d	0.3 ± 0.0^a	55.9 ± 1.5^c
Correlation with firmness (r)	0.62	0.51	-0.21	-0.06	-0.66	0.94

Values represent the mean of at least 3 individual measurements $\pm$ standard error. Values in the same column not connected by the same letter are significantly different ($p < 0.05$)

^ain a dry weight basis

Table 3 Changes in monosaccharide composition (g/Kg) of pectin from ‘Golden Delicious’ apples during on-tree development and ripening and their relationship with apple firmness

DAFB	GalA	Glu	Gal	Rha	Ara
107	262.4 ± 0.9 ^d	461.9 ± 2.2 ^{ab}	170.3 ± 5.0 ^a	11.2 ± 1.0 ^a	118.6 ± 7.4 ^a
122	286.7 ± 1.5 ^c	496.8 ± 11.1 ^a	167.3 ± 3.8 ^a	9.1 ± 1.0 ^a	135.1 ± 11.0 ^a
137	331.3 ± 2.8 ^b	483.4 ± 20.4 ^a	159.9 ± 8.0 ^a	10.7 ± 0.6 ^a	141.6 ± 11.6 ^a
152	324.8 ± 4.2 ^b	417.5 ± 7.9 ^{bc}	152.3 ± 5.1 ^a	9.0 ± 0.8 ^a	117.8 ± 5.1 ^a
167	369.0 ± 4.9 ^a	362.6 ± 17.9 ^c	159.7 ± 10.8 ^a	8.8 ± 0.3 ^a	111.2 ± 3.9 ^a
Correlation with firmness (r)	−0.95	0.75	0.88	0.50	0.33

Values represent the mean of at least 3 individual measurements ± standard error. Values in the same column not connected by the same letter are significantly different ($p < 0.05$)

Table 4 Changes in mineral composition (mg/Kg) of pectin from ‘Golden Delicious’ apples during on-tree development and ripening and their relationship with apple firmness

DAFB	Cu	Fe	K	Mg	Mn	Na	P
107	3.3 ± 0.2 ^c	82.8 ± 8.3 ^b	4067.5 ± 47.8 ^d	221.3 ± 4.8 ^c	3.0 ± 0.1 ^c	8.2 ± 0.8 ^b	31.6 ± 1.4 ^c
122	7.3 ± 0.0 ^d	45.3 ± 5.0 ^c	3766.1 ± 30.1 ^e	197.4 ± 0.4 ^d	8.6 ± 0.1 ^d	15.6 ± 3.4 ^b	32.8 ± 0.4 ^c
137	10.3 ± 0.3 ^c	62.5 ± 9.0 ^{bc}	5474.8 ± 28.7 ^a	321.8 ± 2.3 ^a	13.1 ± 0.1 ^c	36.1 ± 9.0 ^b	47.3 ± 1.0 ^b
152	26.6 ± 0.3 ^a	60.0 ± 4.0 ^{bc}	4246.7 ± 13.2 ^c	223.3 ± 1.1 ^c	21.5 ± 0.1 ^a	69.0 ± 11.0 ^a	53.3 ± 0.5 ^a
167	21.7 ± 0.1 ^b	115.4 ± 1.3 ^a	4413.6 ± 26.3 ^b	275.0 ± 1.5 ^b	15.6 ± 0.1 ^b	68.5 ± 1.4 ^a	53.3 ± 1.1 ^a
Correlation with firmness (r)	−0.89	−0.43	−0.47	−0.56	−0.89	−0.96	−0.99

Values represent the mean of at least 3 individual measurements ± standard error. Values in the same column not connected by the same letter are significantly different ($p < 0.05$)

from 0.4 to 0.5 mg/Kg of pectin. The content of Fe and Mg showed a weak correlation (r values of −0.43 and −0.56, respectively) with apple firmness.

The color of pectins consistently changed during tested period (Table 2); however, only L^* values showed some correlation with apple softening ($r = 0.51$). The relationship between the tristimulus color of pectin and apple softening had not been studied previously. The protein content of pectins showed small changes during tested period, ranging between 0.2 and 0.3%, and weakly correlated with fruit softening ($r = -0.66$) (Table 2).

Discussion

The relation between the firmness and several physicochemical characteristics of apples has been previously studied, but in a dispersed manner. In this work, the firmness of apples during development and ripening was correlated at the same time with several physicochemical attributes of the fruits and their pectin. It was observed that all the fruit harvested after 137 DAFB was able to ripen normally. Fruit harvested at 137 DAFB virtually showed the minimal recommended TSS value for apple harvesting (10.5%). Thus, the commercial harvesting time (167 DAFB) was delayed

30 days, compromising the firmness of fruit. ‘Golden Delicious’ apples from the same area have been harvested even after more DAFB (176) (Salas et al. 2011).

As expected, the firmness of the tested fruit continuously decreased during the development and ripening. Similar softening patterns have been previously described for ‘Golden Delicious’ and other apple cultivars (Felicetti and Mattheis 2010); however, the firmness of fruit at the commercial harvest date (63 N, 167 DAFB) was considerably lower than that reported for ‘Golden Delicious’ apples from the most important apple-producer regions (Felicetti and Mattheis 2010; Saftner 1999). Similar values of firmness have previously been reported for ‘Golden Delicious’ apples (~55–60 N) from the same geographical origin (Salas et al. 2011). Thus, Mexican ‘Golden Delicious’ apples seem to be softer than those from other geographical origins probably as a consequence of the delayed harvesting.

The size of tested apples (weight, diameter and height) increased during the development and ripening and correlated well with fruit softening. The diameter of apples at 167 DAFB was similar to that reported for ‘Golden Delicious’ apples from other countries; however, the weight of tested apples was 13.5–51.0% lower than that of fruit of the same apple cultivar but from other geographical origins

(De Salvador et al. 2006; Felicetti and Mattheis 2010). This suggests that tested fruits probably contained more intercellular spaces. Harker et al. (2010) observed that fruit firmness decreased as the intercellular spaces increased in fruit tissue. The diameter and height of fruit tended to be similar to each other as development and ripening advanced, with fruits at 167 DAFB being almost spherical. The changes of weight, diameter, and height during fruit development and ripening were highly correlated with softening. To date, the relationship between fruit firmness and size is unclear for apples. Volz et al. (2004) demonstrated that large ‘Royal Gala’ apples were softer than small fruits while Saei et al. (2011) found that large fruits of this cultivar were firmer than small fruits at harvest.

The moisture content and firmness were highly correlated. This correlation was also found for ‘Royal Gala’ and ‘Elstar’ apples (De Jager and De Putter 1999; Saei et al. 2011). It has been hypothesized that dry matter content is required for the formation of firm and dense apple tissue (De Jager and De Putter 1999; Johnson 1992). The water content of apples influences fruit texture by altering the cell turgor, ripening rate, and fruit firmness (Harker et al. 2010; Johnson 1992). The TSS and tristimulus color moderately correlated with firmness. The good correlation between fruit firmness and TSS or starch index of apples at harvest has been previously demonstrated (de Castro et al. 2007); however, in our study the TSS content did not show the expected high correlation with fruit firmness. In the case of the tristimulus color, our findings suggest that the carotenoid biosynthesis/chlorophyll degradation and the biochemical events causing softening do not occur exactly at the same time. Interestingly, both TSS and tristimulus color are widely used ripening indexes (Hoehn et al. 2003).

The softening of apples has been highly associated to the modification of cell wall materials. However, this association is still inconclusive and conflictive in some cases. The pectin content in tested apples was similar to that reported for unripe and ripe apples (Jin et al. 1999; Rascón-Chu et al. 2009). The pectin content decreased during the development and ripening, as occurred for firmness, but the correlation between these variables was slight. Similar changes in firmness and content of acid-soluble polyuronides have been reported for apples (Yoshioka et al. 1992). This slight correlation indicates the involvement of other factors on apple softening. The MW of pectin fractions was into the range reported for pectin from ‘Golden Delicious’ apples (100–10000 kDa) (Fischer et al. 1994). The reduction of the MW of the first pectin fraction correlated well with fruit firmness. Extensive depolymerization of pectin has been reported in several apple genotypes during ripening (Peña and Cárpita 2004). Recently, Gwanpua et al. (2016) demonstrated that pectin from ‘Golden Delicious’ apples experienced a high

depolymerization during the postharvest storage and that such depolymerization was involved in fruit softening. The DM of pectin also decreased during tested period, as reported for some apple varieties during ripening (Yoshioka et al. 1992). The DM of pectin from apples at 167 DAFB (DM = 55.9%) was similar to that reported for fruits (DM = 57.0%) from the same cultivar and geographical origin (Rascón-Chu et al. 2009). In general, the DM values found in this study were into the range previously reported for apple pectin (Rascón-Chu et al. 2009; Yoshioka et al. 1992). The DM was strongly correlated with apple firmness. It has been suggested that the deesterification of pectin is more responsible of the solubilization of pectins during apple softening than pectin depolymerization (Yoshioka et al. 1992), which agrees with our results.

Tested pectins showed similar sugar composition than that previously reported for apple pectin (Gross and Sams 1984; Jin et al. 1999). In our study, the content of GalA and Gal were highly correlated with fruit softening, as reported previously for apples (Gross and Sams 1984; Jin et al. 1999). However, it is still not known if the loss of Gal is causal, coincidental or a consequence of fruit softening (Redgwell et al. 1997). In this study, the Glu content also correlated well with apple softening, as reported for ‘Kinsei’ apples during ripening (Jin et al. 1999).

The content of several mineral elements in pectin correlated well with apple softening, especially that of Cu, Mn, Na, and P. Some of these mineral elements have been detected in the cell wall materials of ‘Golden Delicious’ apples (Chardonnet et al. 2003); however, their relationship with fruit firmness had not been studied. Except Na, these ions can exhibit divalent states and therefore they might interact with pectin chains. Interestingly, the highest correlation with firmness was observed for Na. Wang et al. (2014) hypothesized that Na, as other monovalent ions, is able to interact with the negatively charged carboxyl groups (shielding effect) of pectin, especially with those of depolymerized pectin, explaining the good negative correlation between the content of this element in pectin and fruit firmness. This phenomenon might also explain the moderate relationship observed between K content and fruit firmness. Surprisingly, the changes of Ca in pectin did not correlate well with apple softening. These findings were unexpected since it is well known that the Ca helps to maintain the cell-wall structure in fruits by interacting with pectin chains, forming cross-links between pairs of negatively charged homogalacturonans and tightening the cell wall (Lara et al. 2004). However, some studies have demonstrated that Ca infiltration in apples has a small positive effect on apple firmness retention and many other factors different to the Ca content in the cell wall have been suggested to be involved on apple firmness (Stow 1993).

Similarly, the B content in pectin did not correlate well with firmness of tested fruit. This was also unexpected since B is involved in pectin metabolism and the level of organization of cell growing in developing tissues (Hu and Brown 1994). Additionally, B is able to reduce transcription levels and activity of polygalacturonase, pectinesterase and β -galactosidase, slowing down the softening of some fruits (Hu et al. 1996). These findings might indicate a displacement of Ca and B in pectin by Cu, Mn, Na, and P, as demonstrated by the negative correlation coefficients.

Conclusion

The firmness of Mexican ‘Golden Delicious’ apples at the commercial harvest time was considerably low, as a consequence of the delayed harvesting based on ripening indicators that are not oriented to improve the postharvest firmness of apples. According to our results, the apples from tested geographical area should be harvested around 137 DAFB. The ripening indicators typically used in many apple producing areas showed good correlation with firmness but such correlation was lower than those obtained with other characteristics of fruits and their pectin. The biometrical characteristics and moisture content in apple fruit as well as the MW and DM of pectin and its content of GalA, Gal, Cu, Mn, Na and P may be used as ripening indicators to improve the firmness of apples in postharvest. The use of these ripening indicators is important because the quick and sudden change in the softening speed between 122 and 137 DAFB makes difficult to use the firmness measurement alone as a tool to determine harvest time. Other indicators with a slower speed of change during fruit development and ripening should be used along with firmness measurement. The results also suggested that climate change and global warming have modified the speed of change of several physiological processes of the fruit, dephasing much these processes and compromising the reliability of ripening indicators typically used to determine the harvesting date. Probably, cell division and expansion were two of the most affected physiological processes. Further studies are needed to determine the technological tools available to control the negative effects of climate change in the Mexican apple industry. The hormonal control of these changes might represent a solution.

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