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Polyuronide content and correlation to optical properties measured by time-resolved reflectance spectroscopy in 'Jonagored' apples stored in normal and controlled atmosphere

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ABSTRACT

Time-resolved reflectance spectroscopy (TRS) studies on fruit have shown that the absorption coefficient (μ_a) at 670 nm is a good maturity index and that the scattering coefficient (μ'_s) at 780 nm allows an insight into the textural properties of apples. This work aimed at studying the relationship between the polyuronide pattern, firmness and TRS optical properties (μ_a and μ'_s) at 630, 670, 750 and 780 nm measured in 'Jonagored' apples at harvest, after 6 months' storage in normal (NA) and controlled atmosphere (CA), and after 7 days of post-storage shelf life at 20 °C. Results showed that fruit of different TRS maturity class had a different polyuronide content, even if their firmness was not different: 'less mature' (high μ_a630) fruit compared to 'more mature' (low μ_a630) ones showed at harvest higher total galacturonic acid (GA) content, residue insoluble pectin (RIP) and protopectin index (PI), and lower GA content in oxalate-soluble pectin (OSP) fraction, indicating a less advanced breakdown of insoluble protopectins to soluble pectins. The μ_a630 and μ_a670 were correlated to alcohol-insoluble substances (AIS) and GA content in RIP measured after storage: as maturity increased, GA decreased in RIP, showing pectin solubilisation. AIS, water-soluble pectin (WSP), RIP and PI measured after storage were highly correlated to scattering coefficients measured after storage. Generally, with increasing μ'_s, AIS and GA in WSP increased while firmness, RIP and PI decreased. CA apples were characterised by lower μ'_s values than NA ones, along with lower WSP and OSP and higher total GA, RIP and PI.

1. Introduction

Texture is one of the most important quality attributes of apples. A juicy and crispy texture is most appreciated by consumers, while the mealy one, which is characteristic of soft fruit causing a dry feeling in the mouth during mastication, could be a negative quality attribute for apple market (De Smedt, Pauwels, De Baerdemaeker, & Nicolaï, 1998; Jaeger, Andani, Wakeling, & MacFie, 1998; De Smedt et al., 2002). Texture is related to the structural, physiological and biochemical characteristics of the fruit tissues and to their changes over time (Szczesniak, 2002). Cell size and shape, and the amount of intercellular spaces are important properties of fruit tissues. Tissue from soft fruit has rounder cells, more cell separation and larger intercellular spaces than tissue from firm or freshly harvested fruit (De Smedt et al., 1998; Harker, Redgwell, Hallett, & Murray, 1997). In apple cortical tissue, cells are large (up to 300 μm in diameter), elongated along the direction of the fruit radius and organised in distinct columns (Khan & Vincent, 1993). Up to 30% of the volume of apple tissue may be gas-filled intercellular spaces, which indicates a low degree of cell packing and of cell-to-cell contact, which negatively correlate with tissue stiffness (Reeve, 1970; Vincent, 1989).

The metabolic events responsible for textural changes in fruit involve degradation and modifications of the cell wall and middle lamella structures, loss of turgor pressure, starch degradation and modifications in the symplast/apoplast relations (Goulao & Oliveira, 2008). Pectic substances are the main constituents of the middle lamella and they are the most abundant class of macromolecules within the cell wall matrix, playing a key role in fruit ripening and softening (De Smedt et al., 2002; Nara, Kato, & Motomura, 2001). Middle lamella is important for maintaining cell-to-cell adhesion and cell packing in fruit tissues, while primary cell wall determines cell shape and rigidity in many plant tissues (Harker et al., 1997). The changes in cohesion of the pectin matrix cause cells to be easily separated one from each other and, in turn, determines the final texture of the ripe fruit. When cell-to-cell adhesion is weaker than the individual cell walls, cell separation occurs and the intact cells are responsible for the mealy texture. On the other hand, when the individual cell walls are weaker than cell-to-cell adhesion, cell wall breakage occurs and the cellular content is released producing a juicy texture (De Smedt et al., 1998; Goulao &

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Oliveira, 2008; Harker, Maindonald, Murray, Gunson, & Walker, 2002).

Apple softening is usually associated with increased content of water-soluble pectin (WSP), decreased content of EDTA- and HCl-soluble polyuronides and reduced galactose and arabinose residues, with little depolymerisation occurring in each pectin fraction (Billy et al., 2008; Fisher & Amadò, 1994; Fisher, Arrigoni, & Amadò, 1994; Goulao & Oliveira, 2008; Lo Scalzo, Forni, Lupi, Giudetti, & Testoni, 2005; Nara et al., 2001; Siddiqui, Brackmann, Streif, & Bangerth, 1996; Vanoli et al., 2006; Yoshioka, Aoba, & Kashimura, 1992). Storage atmosphere affects pectin solubilisation and the final fruit texture. In 'Golden Delicious' and 'Cox' Orange Pippin' apples after 6 months of storage, it was found that both the decrease in total pectins and hemicellulose, and the increase in WSP were lowest in ultra-low oxygen conditions, higher in controlled atmosphere and highest in air (De Smedt et al., 2002; Siddiqui et al., 1996). Galacturonic acid is the main component of pectins and the most relevant biochemical marker of texture changes. Billy et al. (2008) found that galacturonic acid content in the WSP fraction was positively correlated to mealiness, and negatively related to juiciness, crunchiness and chewiness, and to instrumental firmness. Indeed, firmness was positively related to the protopectin index and inversely related to the content of galacturonic acid in the WSP fraction in apples (Van Buren, 1991; Vanoli et al., 2006).

A common feature of most food and feed is their opacity to visible light. The majority of biological materials can in fact be modelled as a diffusive medium. In these media, due to the microscopic spatial changes in the refractive index, light undergoes multiple scattering events and its overall distribution (i.e. attenuation) is determined by the interplay between light scattering and light absorption. Scattering is very much dependent on the microstructure of the product. Classical VIS/NIR spectroscopy can only provide information on light attenuation, but does not allow the non-destructive assessment of both the absorption and scattering coefficients in diffusive media. As a result, continuous wave spectroscopy requests recalibration for each new batch of samples. On the contrary, time-resolved reflectance spectroscopy (TRS) and space-resolved reflectance spectroscopy (SRS) provide a complete characterisation with the simultaneous non-invasive measurement of the optical properties (absorption and scattering) of diffusive media (Bevilacqua, Piguet, Marquet, Gross, & Depeursinge, 1999; Cubeddu et al., 2001; Lu, Guyer, & Beaudry, 2000; Torricelli, Spinelli, Vanoli, Rizzolo, & Eccher Zerbini, 2008). In TRS, a short pulse of monochromatic light is injected into the fruit: whenever a photon strikes a scattering centre, it changes its trajectory and keeps on propagating in the tissue, until it is eventually re-emitted across the boundary, or it is captured by an absorbing centre. The temporal distribution of the re-emitted photons at a distance from the injection point will be delayed, broadened, and attenuated. Usually, the laser light is injected into and collected from the fruit pulp by using two optical fibres placed in contact with the surface at a distance of 1–2 cm. The laser light probes a banana-shaped volume of tissue to a depth of 1–2 cm. By an appropriate theoretical model of light penetration for the analysis of time distribution, it is possible to simultaneously measure the absorption (μ_a) and the transport scattering (μ_s') coefficients as a function of wavelength. The absorption coefficient is determined by pigments (chlorophyll, carotenoids) and chemical constituents of the pulp (water, sugar), while μ_s' is mainly due to cellular structure, as the photon path can be deviated by changes in the refractive index due to the presence in the tissue of membranes, cell walls, air, vacuoles, starch granules or organelles. The absorption coefficient measured at 630–690 nm, near the chlorophyll peak, is linked to fruit maturity, as found in apples and pears (Eccher Zerbini, Grassi, Cubeddu, Pifferi, & Torricelli, 2002; Vanoli et al., 2005, 2006) and in nectarines, where the measurement of μ_a670, expressed as biological shift factor and coupled to kinetic models, has been successfully used to predict their softening rate during shelf life and to select fruit for different market destinations (Eccher Zerbini et al., 2006; Tijskens et al., 2007). The scattering coefficient is related to fruit structure and texture. In apples, Vanoli et al. (2006, 2007) found that the relative internal space volume was positively related to μ_s'750 and μ_s'780 measured at harvest, while firmness, percent juice and sensory crispness were negatively correlated to μ_s'780 measured after storage. On the contrary, mealiness increased with increasing μ_s'780. Values of μ_s'780 lower than 11 cm^{-1} characterised crispy, not mealy fruit, with firmness higher than 50 N and percent juice higher than 30%. Valero et al. (2005) studied classification models based on TRS measurements at 672, 750, 818, 900 and 950 nm, in order to identify mealiness in apples. The performance of these models ranged from 47% to 100% of correctly identified mealy versus non-mealy apples. In pears, Nicolaï et al. (2008) by using TRS in the NIR region, from 875 to 1030 nm, found that firmness considerably increased with increasing μ_s' at 900 nm, following a non-linear relationship, and this fact prevented the construction of calibration models for firmness using μ_s' spectra. However, in kiwifruits, Valero et al. (2004), using μ_s' measured at 675 and 750 nm, obtained a model for firmness having an R^2 of 0.6, which rose to 0.8 when absorption spectra were added to the model. In a previous work on 'Jonagored' apples, Vanoli et al. (2006) found a positive correlation between μ_s' measured at 750 nm and WSP fraction and a negative correlation with residual insoluble pectin fraction (RIP).

The aim of this research was to investigate the polyuronide pattern of 'Jonagored' apples at harvest and after storage in normal and controlled atmosphere and its relationship with firmness and the TRS optical properties of absorption and scattering coefficients measured at four wavelengths.

2. Materials and methods

2.1. Fruit samples and experimental plan

Apples (*Malus domestica*, Borkh.) cultivar 'Jonagored' were harvested in 2002 on 30 August (H1) and 13 September (H2) in an experimental orchard in Laimburg (Bolzano, Italy), when starch index was 3.1 and 4.2, respectively (scale 1 = minimum, 5 = maximum starch hydrolysis). One-hundred and fifty fruits/harvest were individually weighed, measured by TRS at 630, 670, 750 and 780 nm, and ranked separately for each harvest by decreasing absorption coefficient at 630 nm (μ_a630), i.e. from less mature to more mature fruit. The range of μ_a630 at harvest was 0.05–0.20 cm^{-1} for H1 fruit and 0.05–0.16 cm^{-1} for H2. Ranked apples of each harvest were divided into 30 groups of five fruit (corresponding to 30 levels of μ_a630) and, therefore, into three classes of maturity (10 groups 'less mature', 10 'medium mature' and 10 'more mature'). Apples were analysed at harvest, at the end of 6 months' storage at +1 °C in normal atmosphere (NA) or in controlled atmosphere (CA, 1% O_2; 2% CO_2) (d1) and after 7 days (d7) of post-storage shelf life at 20 °C. In order to have the whole range of μ_a630 in all the five samplings, one fruit per group was randomly assigned to one of the five samplings. In this way a 30 fruit set/sampling was prepared.

At harvest, apples were analysed for flesh firmness, alcohol-insoluble substances (AIS) and polyuronide and total starch contents. The analyses "at harvest" were performed for all fruit after the second harvest; meanwhile, H1 fruit were kept at 1 °C. At the end of CA and NA storage, μ_a and μ_s' at 630, 670, 750 and 780 nm and fruit firmness were measured at d1 and d7 of shelf life, whereas AIS, total starch and polyuronide contents were investigated at d1 of shelf life. AIS and polyuronide content were deter-

mined on three individual fruits belonging to the 'less mature' class and three fruits of the 'more mature' class within each harvest and sampling; in each maturity class fruits were chosen according to their firmness: the highest, the lowest and the medium values. A total of 36 fruit were examined (3 firmness values × 2 maturity classes × 2 harvests × 3 samplings, i.e. at harvest, after CA and NA storage).

2.2. TRS measurements

TRS was measured on two opposite sides of each fruit and averaged per fruit, using a compact system based on a pulsed laser diode with 80 MHz repetition frequency, 100 ps duration, and 1 mW average power, with a compact photomultiplier and an integrated PC board for time-correlated single photon counting; typical acquisition time was 1 s/point. A couple of 1 mm plastic-glass fibres delivered light into the fruit and collected the emitted photones at a distance of 1.5 cm. The system was able to perform measurements at four wavelengths: 630, 670, 750 and 780 nm. Selection of wavelength was accomplished by a fibre optic switch. The reduced scattering coefficients and the absorption coefficients at 630, 670, 750 and 780 nm were obtained by fitting the experimental TRS data with a standard solution of the diffusion approximation to the transport equation for a semi-infinite homogenous medium. The extrapolated boundary condition was used (Contini, Martelli, & Zaccanti, 1997) to take into account the refractive index mismatch at the surface. With such a system, the reproducibility error (CV over repeated measurements in different days) was less than 4% for both μ_a and μ_s' at each wavelength.

2.3. Firmness

Each fruit was measured on two opposite peeled areas in the equatorial region of the apple using a 11 mm diameter plunger mounted on an Instron Universal Testing Machine (model 4301, Instron Ltd., Great Britain) with the crosshead speed at 200 mm/min. Then, firmness readings were averaged for each fruit.

2.4. Polyuronide analysis

Polyuronides were extracted from AIS and fractionated into three fractions: water-soluble pectin (WSP), oxalate-soluble pectin (OSP) and residual insoluble pectin (RIP). Each fraction was analysed for galacturonic acid (GA) content by HPLC after enzymic depolymerisation of pectin chains. Some data of pectin composition were missed at harvest (one fruit H1 less mature, one fruit H1 more mature) and after storage (one fruit H1 CA less mature, one fruit H2 CA less mature). So, all the data belonging to these four fruits were excluded.

2.4.1. Alcohol-insoluble substances determination

Alcohol-insoluble substances (AIS) were determined following the procedure by Forni, Torreggiani, Battiston, and Polesello (1986) with some modifications. The flesh from a single apple was homogenised in 96% boiling ethanol by means of an UltraTurrax homogeniser. The fruit tissue to ethanol ratio was 1:4. The mixture was allowed to stand overnight, then it was filtered through a G3 sintered funnel. The precipitate was washed successively with 200 mL × 5 times of 96% ethanol, then with 100 mL aliquots of acetone until total whitening. Afterwards, it was air-dried, weighed and ground to powder with a hammer mill, firstly through a 1 mm sieve and then through a 0.5 mm sieve. The obtained uncoloured powder was AIS, which was stored in a dry atmosphere till subsequent analysis. AIS yields were expressed as g/100 g of fresh tissue.

2.4.2. Polyuronide fractionation

The AIS fractionation was carried out in duplicate according to Forni et al. (1986) with some modifications. *WSP fraction*: 1 g of AIS was weighed into a 90 mL centrifuge tube and dispersed at room temperature in 40 mL HPLC-grade water by magnetic stirring for 30 min. The slurry was centrifuged at 6500 rpm (4670*g*) for 15 min. The supernatant was transferred to a 100 mL volumetric flask. The residue was re-dispersed in 40 mL water, stirred for 30 min, centrifuged at 6500 rpm (4670*g*) for 15 min, the supernatant transferred to the 100 mL volumetric flask and the volume was brought to the mark with HPLC-grade water. *OSP fraction*: the residue from WSP extraction was dispersed at room temperature in 35 mL 0.05 M kalium oxalate buffer, brought to pH 5.6 with 1 N H_2SO_4, by magnetic stirring for 30 min. The slurry was centrifuged at 6500 rpm (4670*g*) for 15 min. The supernatant was transferred to a 100 mL volumetric flask. The residue was re-dispersed in 35 mL kalium oxalate buffer, stirred for 30 min, centrifuged at 6500 rpm (4670*g*) for 15 min, the supernatant transferred to the 100 mL volumetric flask and the volume was brought to the mark with kalium oxalate buffer. *RIP fraction*: the residue from OSP extraction was suspended in 50 mL HPLC-grade water, transferred to a 100 mL volumetric flask and brought to volume with HPLC-grade water.

2.4.3. Galacturonic acid analysis

WSP, OSP and RIP fractions were analysed for GA content (two replicates/sample) by HPLC on enzymically depolymerised pectins (Forni, Penci, & Polesello, 1994). Two millilitres 1 M NaOH were added to 40 mL of fraction and, in order to de-esterify polyuronides, the mixture was held for 1 h at room temperature with magnetic stirring. De-esterified pectin solution was brought to pH 4.5 with 1 N H_2SO_4 and transferred to a 50 mL volumetric flask; 1 mL of an enzymic solution [50 mg cellulase (C9422 Sigma–Aldrich, Missouri, USA) plus 1 mL pectinase (P4716 Sigma–Aldrich, Missouri, USA) in 10 mL water] was added. Samples, brought to volume, were incubated at 50 °C for 20 h with magnetic stirring. Depolymerised pectins were filtered through a 0.45 μm Nylon 66 membrane unit with a 1 μm glass wool pre-filter prior to HPLC analysis. A Jasco (Tokio, Japan) HPLC system consisting of a PU-880 liquid chromatographic pump, a model AS-1555 autosampler and RI-930 detector was used. Chromatographic data were stored and processed with a Shimadzu CR3A Chromatopac data processor. Separations were performed on an Alltech IOA-1000 organic acid (7.8 mm i.d. × 300 mm length, 5 μm particle size) column at 50 °C which was maintained using a Jones Chromatography column thermostat. The sample injection volume was 20 μL. GA was determined using 0.001 N H_2SO_4 as mobile phase at a flow rate of 0.4 mL/min and RI detection (range 8×10^{-5}). Water solutions of monohydrate galacturonic acid at the concentrations of 0.05%, 0.025% and 0.0125% (w/v) were used as external standards. Quantitative data were obtained by relating the peak area of GA in the sample to that of standard solutions, and were expressed as mmol/100 g AIS. Total GA content was computed as the sum of GA content of WSP, OSP and RIP fractions. The percentage of each fraction to total GA content was also calculated.

2.4.4. Protopectin index

The protopectin index (PI) is the ratio insoluble pectin (RIP) to soluble pectins (WSP and OSP) (Forni, Senesi, Viganò, Bertolo, & Maestrelli, 1989) and it was computed according to: PI = RIP/(WSP + OSP), where RIP, WSP and OSP were the relative amounts of GA as mmol/100 g AIS.

2.5. Total starch in AIS

Starch content was determined on 100 mg AIS following the total starch assay procedure based on the use of thermostable

Table 1
Average ($n = 10$) ± standard error of firmness (N) of 'Jonagored' apples in function of harvest date and TRS maturity class at harvest, and after controlled atmosphere (CA) and normal atmosphere (NA) storage at day 1 (d1) and day 7 (d7) of post-storage ripening at 20 °C.

	Firmness (N)		
	Less mature	Medium mature	More mature
H1			
At harvest	65.1 ± 0.90	66.6 ± 1.40	65.4 ± 2.69
CA d1	51.9 ± 1.17	53.3 ± 2.46	50.9 ± 2.47
CA d7	51.1 ± 2.45	51.1 ± 2.81	54.9 ± 2.28
NA d1	34.6 ± 1.02	35.1 ± 0.92	40.3 ± 1.79
NA d7	33.3 ± 0.90	32.8 ± 1.64	34.8 ± 1.76
H2			
At harvest	61.6 ± 1.48	65.8 ± 1.75	65.2 ± 1.46
CA d1	63.6 ± 1.29	63.5 ± 2.55	65.6 ± 2.65
CA d7	62.2 ± 1.20	62.3 ± 1.42	61.2 ± 2.45
NA d1	33.2 ± 0.77	34.8 ± 1.16	36.3 ± 1.52
NA d7	29.0 ± 0.98	30.3 ± 0.63	32.9 ± 0.83
Main effects[a]			
A: Harvest date		***	
B(A): Maturity class (harvest)			ns
C: Sampling[b]		***	
Interactions			
A × C		***	
B(A) × C			ns

[a] *P*-value of *F* ratio: ns = not significantly different $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.
[b] *Sampling*: at harvest, after CA storage at day 1 (CA d1) and day 7 (CA d7) and after NA storage at day 1 (NA d1) and day 7 (NA d7).

α-amylase and amyloglucosidase (McCleary, Gibson, & Mugford, 1997) using the Total Starch Assay kit (Megazyme International Ireland Ltd.).

2.6. Statistical analyses

Data were submitted to multifactorial analysis of variance considering harvest date, maturity class and sampling (at harvest, after CA and NA storage) as sources of variation (PROC GLM, SAS/STAT, SAS Institute Inc., Cary, NC, 1999). As the classification based on $\mu_a 630$ was made within each harvest date, the maturity class factor was nested within the harvest date. Means were compared by Tukey's test at $P \leqslant 0.05\%$. Correlations between firmness, chemical and optical data at harvest and after storage were studied using PROC CORR procedure (SAS/STAT, SAS Institute Inc., Cary, NC, 1999).

3. Results

3.1. Firmness

Firmness was affected by harvest date, sampling and their interaction, while maturity class had no significant effect (Table 1). With storage, firmness decreased in NA in both harvests and in CA in H1 fruit, while it did not change in CA stored H2 fruit. Post-storage shelf life did not influence firmness of fruit from both harvests stored in CA, while H2 apples stored in NA were less firm at d7 than at d1 of shelf life at 20 °C.

3.2. AIS and GA content

AIS was about 10% at harvest and its value markedly decreased to about 1.5% after storage, with no differences between storage atmospheres. AIS was not influenced by harvest date and maturity class. Total GA content depended on harvest date, maturity class and storage, with no interactions (Table 2). On average total GA increased from 95.7 mmol/100 g at harvest to the end of storage, more in CA (123.5 mmol/100 g) than in NA (116.8 mmol/100 g); it was higher in H2 apples and in 'less mature' fruit class.

Considering the three polyuronide fractions (Table 2), WSP was low at harvest and markedly increased after storage, especially in NA. At harvest, GA content in WSP fraction was higher in H1 'more mature' fruit and in H2 'less mature' ones. After storage, WSP was the highest in NA fruit without differences between maturity clas-

Table 2
Average ($n = 3$) and standard error (between brackets) of galacturonic acid content (mmol/100 g AIS) in water-soluble (WSP), oxalate-soluble (OSP) and residual insoluble (RIP) pectic fractions and protopectin Index (PI) in function of harvest date and TRS maturity class at harvest and after controlled atmosphere (CA) and normal atmosphere (NA) storage.

	Galacturonic acid content (mmol/100 g AIS)									
	WSP		OSP		RIP		Total GA		PI	
	Less	More	Less	More	Less	More	Less	More	Less	More
H1										
At harvest	3.31 (−0.382)	6.7 (−0.43)	4.39 (−0.467)	6.1 (−0.825)	87.27 (−8.714)	71.14 (−1.633)	95.39 (−9.23)	83.93 (−2.353)	11.16 (−1.006)	5.9 (−0.524)
After CA storage	17.25 (−0.832)	20.99 (−1.093)	9.91 (−1.606)	8.17 (−1.545)	106.41 (−6.177)	86.43 (−3.467)	133.57 (−8.308)	115.59 (−5.51)	4.02 (−0.213)	3.15 (−0.239)
After NA storage	31.15 (−0.924)	30.59 (−0.469)	11.53 (−0.454)	7.55 (−0.99)	81.83 (−1.643)	65.17 (−2.297)	124.62 (−2.631)	103.42 (−2.567)	1.96 (−0.045)	1.71 (−0.06)
H2										
At harvest	6.79 (−0.999)	3.49 (−0.181)	5.51 (−0.911)	6.71 (−1.019)	93.52 (−7.067)	82.99 (−3.188)	105.83 (−6.331)	93.29 (−3.74)	8.83 (−1.391)	8.5 (−0.734)
After CA storage	13.69 (−1.015)	16.88 (−1.001)	8.78 (−0.834)	7.74 (−0.767)	114.23 (−4.463)	91.36 (−4.989)	136.71 (−5.706)	115.99 (−4.89)	5.19 (−0.255)	3.81 (−0.272)
After NA storage	33.82 (−2.78)	31.09 (−2.362)	18.21 (−1.557)	10.73 (−1.804)	77.86 (−0.987)	70.84 (−2.522)	130.86 (−4.929)	112.12 (−5.939)	1.59 (−0.14)	1.83 (−0.109)
Main effects[a]										
A: Harvest date	ns		*		*		*		ns	
B(A): Maturity class (harvest)	ns		*		***		***		***	
C: Sampling[b]	***		***		***		***		***	
Interactions										
A × C	*		**		ns		ns		ns	
B(A) × C	ns (0.052)		***		ns		ns		***	

[a] *P*-value of *F* ratio: ns = not significantly different $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.
[b] *Sampling*: at harvest, after CA storage and after NA storage.

ses or harvests, while in CA it was higher in 'more mature' fruit whatever the harvest date. In H2 fruit, CA stored apples had half GA content with respect to the NA ones. As for OSP fraction, GA increased from harvest to the end of storage, especially in H2 apples stored in NA, while in H1 fruit there was no difference between storage atmospheres. At H1 harvest, OSP was higher in 'more mature' apples, while at H2 harvest there was no difference between the maturity classes. After storage, OSP in CA stored apples was not affected by maturity class whatever the harvest date, while in NA OSP was higher in 'less mature' apples. GA content in RIP was higher in H2 fruit and in the 'less mature' class. RIP increased with storage in CA and decreased in NA.

Table 3
Correlation coefficients (r)[§] of firmness with AIS, galacturonic acid content in water-soluble (WSP), oxalate-soluble (OSP) and residual insoluble (RIP) pectic fractions, total galacturonic acid (total GA) and protopectin Index (PI) at harvest (n = 10) and after storage (n = 22).

	Firmness	
	At harvest	After storage
AIS	0.071	0.271***
WSP	−0.592	−0.854***
OSP	−0.529	−0.526
RIP	−0.006	0.596
Total GA	−0.161	0.007
PI	0.634*	0.928***

§ Significance of r *$P \leqslant 0.05$; **$P \leqslant 0.01$; and ***$P \leqslant 0.001$.

On average protopectin index (PI) was high at harvest (8.5) and markedly decreased with storage, more in NA (1.8) than in CA (3.9). At harvest, even if on average PI values of H1 and H2 were not different, H1 'less mature' fruit had twice as much PI than H1 'more mature' ones, whereas there was no difference between maturity classes in H2 apples. After storage, 'less mature' class had higher PI value, except for NA H2 apples. Furthermore, CA stored apples had twice as much PI than NA stored ones, independently of maturity class and harvest date.

At each sampling, the proportion of GA of each fraction to total GA varied: the percent GA of RIP fraction decreased from 88% at harvest to 79% in CA and 63% in NA; simultaneously, GA of WSP fraction rose from 6% at harvest to 15% after CA and 27% after NA; minor changes were found for OSP fraction, which was 6% at harvest and after CA storage, and increased to 10% after NA storage.

3.3. Starch content in AIS

At harvest total starch content in AIS was (average ± s.e.) 6.36 ± 1.43 g/100 g in H1 and 2.40 ± 0.36 g/100 g in H2 apples. After CA and NA storage, no starch was detected for either of the harvests.

3.4. Correlation analyses

As AIS and polyuronide pattern greatly changed from harvest to the end of storage, correlation analyses were carried out separately for data at harvest and after storage.

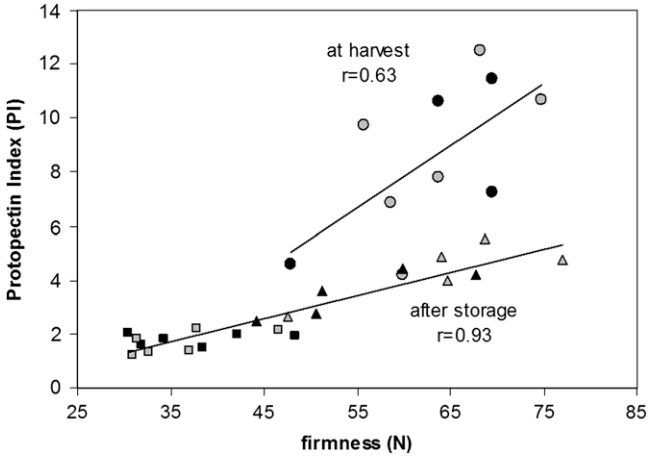

Fig. 1. Correlation of firmness vs. protopectin index of H1 (black) and H2 (grey) 'Jonagored' apples at harvest (circle) and after 6 months' storage in normal (square) and controlled (triangle) atmospheres.

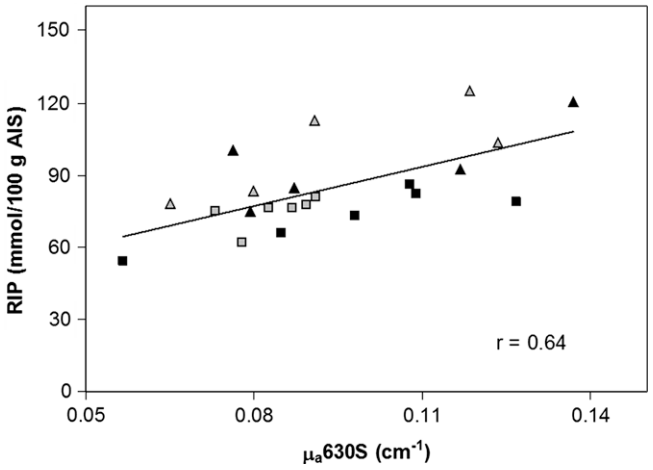

Fig. 2. Galacturonic acid content in residual insoluble pectin (RIP) fraction of H1 (black) and H2 (grey) 'Jonagored' apples stored for 6 months in normal (square) and controlled (triangle) atmosphere in function of absorption coefficient at 630 nm measured after storage (μ_a630S).

Table 4
Correlation coefficients (r)[§] of the absorption coefficients (μ_a) measured at harvest (H) and after storage (S) with firmness, alcohol-insoluble substances (AIS), galacturonic acid content in water-soluble (WSP), oxalate-soluble (OSP) and residual insoluble (RIP) pectic fractions, total galacturonic acid (total GA) content and protopectin index (PI) measured after storage (n = 22).

	μ_a630H	μ_a670H	μ_a750H	μ_a780H	μ_a630S	μ_a670S	μ_a750S	μ_a780S
Firmness	−0.211	−0.270	0.009	−0.160	0.213	−0.033	0.290	0.280
AIS	−0.650**	−0.625**	−0.436*	−0.265	−0.497*	−0.457*	−0.181	−0.203
WSP	0.107	0.202	−0.026	0.195	−0.242	−0.052	−0.121	−0.151
OSP	0.332	0.394	0.370	0.387	0.104	0.165	0.242	0.199
RIP	0.334	0.297	0.333	0.086	0.636**	0.444*	0.405	0.451*
Total GA	0.466*	0.496*	0.399	0.259	0.526*	0.453*	0.406	0.432*
PI	0.026	−0.051	0.114	−0.111	0.421	0.164	0.371	0.383

§ Significance of r *$P \leqslant 0.05$; **$P \leqslant 0.01$; and ***$P \leqslant 0.001$.

Table 5
Correlation coefficients (r)[§] of scattering coefficients (μ'_s) measured at harvest (H) and after storage (S) with firmness, alcohol-insoluble substances (AIS), galacturonic acid content in water-soluble (WSP), oxalate-soluble (OSP) and residual insoluble (RIP) pectic fractions, total galacturonic acid (total GA) content and protopectin index (PI) measured after storage (n = 22).

	μ'_s630H	μ'_s670H	μ'_s750H	μ'_s780H	μ'_s630S	$\mu\mu'_s$670S	μ'_s750S	μ'_s780S
Firmness	−0.364	−0.432*	−0.318	−0.289	−0.563**	−0.592**	−0.806***	−0.782***
AIS	0.116	0.150*	0.433*	0.496*	0.062	0.474*	0.075	0.054
WSP	0.484*	0.601**	0.503*	0.457*	0.610**	0.634**	0.767***	0.747***
OSP	−0.020	0.120	−0.134	−0.194	0.224	0.083	0.271	0.270
RIP	−0.418	−0.437*	−0.623**	−0.622**	−0.366	−0.711***	−0.663***	−0.621**
Total GA	−0.161	−0.083	−0.385	−0.422	0.021	−0.343	−0.176	−0.145
PI	−0.397	−0.512*	−0.473*	−0.456*	−0.590**	−0.755***	−0.843***	−0.815***

[§] Significance of r $^{*}P \leqslant 0.05$; $^{**}P \leqslant 0.01$; and $^{***}P \leqslant 0.001$.

3.4.1. Polyuronides and firmness

The correlation analysis of firmness vs. AIS content, pectin fractions, PI and total GA showed that firmness was positively correlated at harvest only to PI (Table 3) as well as after storage, but with a higher correlation coefficient, and negatively related after storage to GA content in the WSP fraction. Fig. 1 shows the observed data of firmness vs. PI at harvest and after storage; at harvest, there was a greater range of PI data (from 4 to 13) in a narrow range of firmness (from 48 to 75 N), while after storage there was a larger range of firmness (from 30 to 77 N) with a narrower range of PI (from 1 to 5.5).

3.4.2. Polyuronide content and TRS measurements

At harvest, polyuronide content, AIS and firmness did not show any correlation to μ_a coefficients (data not shown), while among scattering coefficients only μ'_s measured at 750 nm was negatively correlated to GA in OSP fraction (r = −0.626). The correlation analysis of firmness and polyuronide content measured after storage vs. optical properties was carried out considering either the μ_a and μ'_s values measured at harvest (H), or those measured after storage (S). The absorption coefficients μ_a630H, μ_a670H, μ_a630S and μ_a670S were negatively related to AIS and positively related to total GA. In addition, μ_a750H was negatively related to AIS, and μ_a630S, μ_a670S and μ_a780S were positively related to GA content in RIP fraction, and μ_a780S to total GA (Table 4). The highest correlations were found between μ_a630H and AIS (r = −0.65) and between μ_a630S and RIP (r = 0.64). As shown in Fig. 2, GA content in RIP fraction decreased with decreasing μ_a630S (i.e. increasing maturity), irrespective of the harvest date and the storage atmosphere. No correlations were found between any of μ_as and firmness, WSP, OSP or PI.

Considering scattering coefficients (Table 5), no correlations were found between any of the scattering coefficients with total GA content and GA in OSP fraction. On the contrary, most of the μ'_s were correlated to WSP (positively), PI and RIP (negatively). Firmness was significantly correlated to all μ'_s measured after storage. Among the significant correlations, the highest r values were found for μ'_s750S and μ'_s780S vs. PI, firmness, and GA content in WSP fraction. Protopectin index and firmness decreased with increasing μ'_s750S, while WSP increased with increasing μ'_s750S (Fig. 3). A μ'_s750S value of 12 cm^{-1} allowed the separation of CA stored apples from NA stored ones: CA apples had lower scattering values than NA ones, signifying higher firmness and PI and lower WSP.

4. Discussion

It is well known that AIS contains cellulose, hemicellulose, pectic substances, proteins, nucleic acids, starch and polyphenols (Fisher & Amadò, 1994). The majority of authors extracted AIS from starch-free apples (Massiot, Baron, & Drilleau, 1994; Renard, 2005)

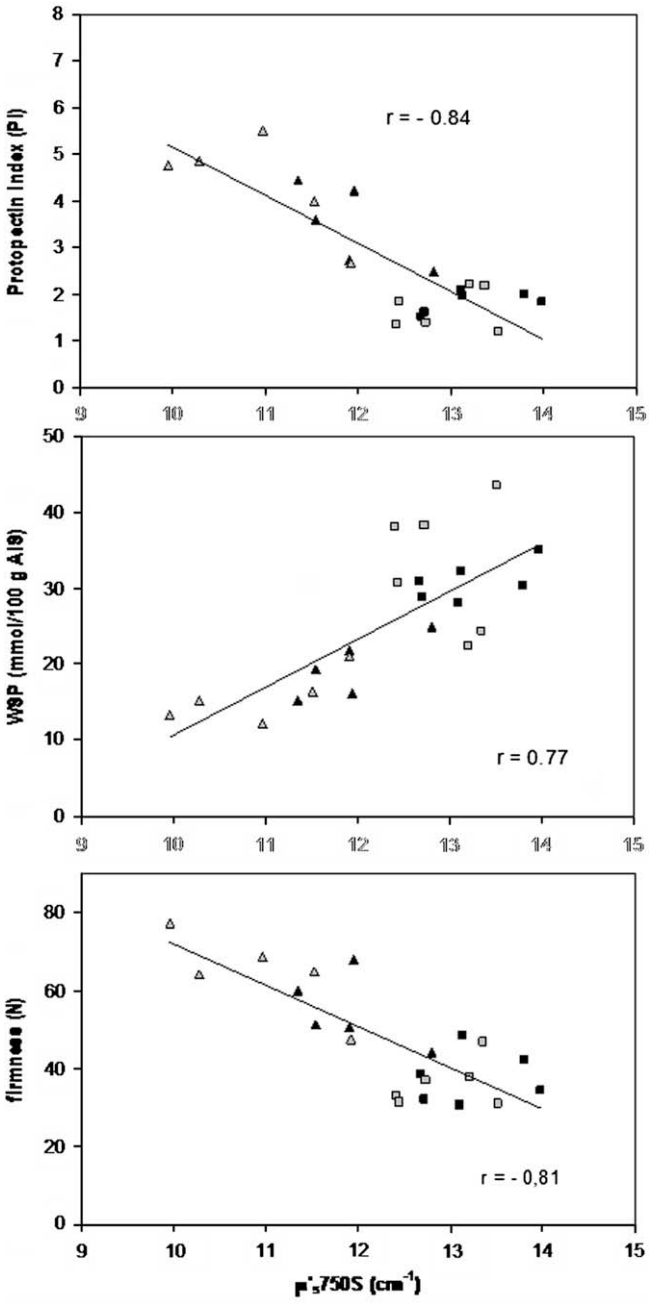

Fig. 3. Protopectin index, galacturonic acid content (GA) in water-soluble pectin (WSP) fraction and firmness of H1 (black) and H2 (grey) 'Jonagored' apples stored for 6 months in normal (square) and controlled (triangle) atmosphere in function of reduced scattering coefficient at 750 nm measured after storage (μ'_s750S).

or removed starch during cell wall extraction (Billy et al., 2008; Siddiqui et al., 1996), so obtaining AIS values at harvest of about 2–3%. In unripe 'Golden Delicious' apples, Fisher and Amadò (1994) found that starch accounted for about 2% of the fruit flesh (up to 40% of the AIS), it decreased to 0.7% (24% in the AIS) at harvest, and, after 3 weeks of cold storage, even if no starch could be detected in fruit pulp by iodine staining, 9% of AIS was starch. In addition, Brookfield, Murphy, Harker, and MacRae (1997), comparing starch analyses and starch pattern index, found that iodine staining clearing occurred when starch was still present in the pulp, even if at low concentration (2–3 mg/g fresh weight). In this experiment, the AIS percentage at harvest was about 10%, about 4–5 times higher than the starch-free AIS values reported in literature, and twice the data reported by Lo Scalzo, Testoni, and Genna (2001), who found a value of about 5% in 'Annurca' apples. At harvest the starch content of AIS was close to the amount reported in literature for a starch pattern index correspondent to iodine staining clearing, but not so high to explain the difference in AIS percentage found between harvest and storage. So it was hypothesised that the high value of AIS found at harvest in our experiment could be due to unintentional presence of some tissue from the carpel region, containing high amounts of cell wall polysaccharides, especially cellulose and hemicellulose (Massiot et al., 1994). Therefore, the carpel region was accurately removed prior to AIS preparation in all the samples after storage. After 6 months' storage starch is completely hydrolised, and the amounts of AIS found after storage in our experiment were similar to those reported in literature (Fisher & Amadò, 1994; Lo Scalzo et al., 2005; Siddiqui et al., 1996). AIS at the end of storage did not show any difference regarding storage atmospheres, as also found by Lo Scalzo et al. (2005) and Siddiqui et al. (1996).

Despite the high AIS value found at harvest, total content of galacturonic acid in AIS was close to that found by Billy et al. (2008), Fisher et al. (1994), Massiot et al., 1994, and Renard (2005), both at harvest and after storage. Considering polyuronide pattern in relation to fruit softening, at harvest 'Jonagored' apples were characterised by high GA content in RIP and low GA content in WSP and OSP fractions. With storage, there was an increase of GA in WSP and OSP fractions coupled to a decrease of firmness in NA apples. These results confirm the general finding that, during apple storage, cell walls and middle lamellae undergo gradual modifications, causing flesh softening especially in NA: an increase of WS polyuronides and a decrease of chelator- and HCl-soluble polyuronides suggest that insoluble protopectins may be converted to water-soluble polyuronides during storage (Billy et al., 2008; Fisher et al., 1994; Nara et al., 2001; Yoshioka et al., 1992). Pectin solubilisation might result from the enzymatic cleavage of linkages between pectin and other cell wall components (Siddiqui et al., 1996).

With CA storage, on average firmness either slightly decreased (H1) or did not change (H2), while WSP and OSP fractions significantly increased, but at a lower extent than in NA apples, so that CA apples were firmer than NA ones and were characterised by lower WSP and OSP and higher total GA, RIP and PI than NA ones. Similar results have been obtained by De Smedt et al. (2002), who found a higher rate of degradation of middle lamellae in NA than in CA storage, and by Lo Scalzo et al. (2005) and Siddiqui et al. (1996), who found the least increase of WSP content and the highest firmness in apples at the end of the storage in CA as compared to NA.

Considering the single fruit data, the uronic acid content of the WSP fraction showed a high negative correlation with firmness, whatever the storage conditions confirming the findings by Billy et al. (2008) and Siddiqui et al. (1996). Our results also showed a positive correlation between protopectin index and firmness, even if it was not univocal, as at the higher PI values observed at harvest corresponded firmness values which were also recorded in CA stored apples, having much lower PI values. Even so, for fruit belonging to the same batch, the higher the firmness, the higher the PI, that is, the higher the RIP and the lower the WSP and OSP. This polyuronide pattern is related to less advanced maturity of fruit, as shown by our results on pectin composition in relation to TRS measurement. In fact fruit of different TRS maturity class had a different polyuronide content, even if their firmness was not different. Fruit selected at harvest with a high μ_a630, i.e. less mature, if compared to more mature (low μ_a630) apples, had higher total GA content, RIP and PI (except for H2 at harvest and after NA storage), and lower GA content in OSP fraction at harvest, indicating a less advanced breakdown of insoluble protopectins to soluble pectins. The absorption coefficient measured near the chlorophyll peak is related to fruit maturity (Tijskens et al., 2007). The absorption coefficients measured at 630 and 670 nm were correlated to AIS and GA content in RIP measured after storage: with increasing maturity, that is decreasing μ_a, GA in RIP decreased, showing pectin solubilisation.

The absorption coefficients did show no significant correlations with polyuronide content, AIS and firmness measured at harvest. Also considering scattering coefficient we did not found any correlations between scattering and firmness or between scattering and pectin composition (except for OSP) at harvest. However, many significant and high correlations were found with measurements made after storage. In a parallel study on 'Jonagored' apples, concerning the relationship between scattering properties and apple quality, Vanoli et al. (2007) found that the scattering coefficients were correlated at harvest only to acidity and to intercellular space volume, while after storage μ_s' were correlated to texture parameters (firmness, stiffness, percent juice, sensory mealiness and crispness) and to acidity. The lack of correlation between scattering coefficient and apple structure at harvest could be due in part to the presence of starch granules at harvest, which could have influenced TRS measurement (Contini et al., 1997). Starch is hydrolysed during storage, so the optical measurements after storage can be assumed to be not affected by starch, which is no more present. Polyuronide content measured after storage (AIS, WSP, RIP and PI) was correlated to scattering coefficients measured both at harvest and after storage, but with higher correlation coefficients value for μ_s' measured after storage. Furthermore, firmness showed significant and high correlations only with μ_s' measured after storage. Generally, CA apples were characterised by lower μ_s' values than NA ones, and as μ_s' increased, AIS and GA in WSP increased, and firmness, RIP and PI decreased. Similarly, Vanoli et al. (2007), on the same cultivar, found that sensory firmness and crispness decreased and mealiness increased with increasing scattering values and that μ_s' values were lower in CA stored apples, which were firmer, crisper and without mealiness when compared to NA ones. These findings suggested that GA in the WSP was positively related to mealiness and negatively related to crispness, as found by Billy et al. (2008).

The hydrolysis of polysaccharides could induce a decrease in the size and an increase in the number of scattering centres, so increasing scattering values in NA stored apples, compared to CA ones. In fact, according to Mie theory, μ_s' depends on wavelength and it is related to the size and density of scattering centres. Under the hypothesis that the scattering centres are homogeneous spheres behaving individually, the relationship between μ_s' and wavelength (λ) can be empirically described as follows: $\mu_s'(\lambda) = a\lambda^{-b}$, where a and b are free parameters. In particular, a depends on the density of the scattering centres and b on their size (Mourant, Fuselier, Boyer, Johnson, & Bigio, 1997; Nilsson, Sturesson, Liu, & Andersson-Engels, 1998). Actually, the situation in tissues is much more complex than just described, as the scattering centres are not expected to be homogeneous spheres.

In conclusion our results highlighted that pectin hydrolysis occurred in a different way according to the storage atmosphere, hence modifying not only the textural characteristics of apple tissue, but also its optical properties. So, the TRS technique is useful to get an insight into the quality and textural properties of apples. Absorption coefficient was more linked to fruit maturity, while scattering coefficient was related to fruit texture not only at macroscopic level (firmness, stiffness, mealiness, crispness) but also, from the biochemical point of view, to cell wall and middle lamella pectic compositions. Further studies are needed to look into the relationships among TRS optical properties and texture analysing other cultivars. The final aim would be to elucidate the relationship between scattering coefficient and microscopical features of apple tissues, such as the distribution of starch and intercellular space, the cell size and shape in different apple cultivars.

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