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Food Chemistry 93 (2005) 521–530
Food Chemistry
www.elsevier.com/locate/foodchem

Changes of pectic composition of 'Annurca' apple fruit after storage

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Received 17 June 2003; received in revised form 5 October 2004; accepted 5 October 2004

Abstract

'Annurca' apple fruit, which is commonly cultivated in Southern Italy and undergoes, after harvest, a typical reddening treatment to turn the apples' skin red, is noticeable for its high firmness. An 'Annurca' variety, called 'Bella del Sud' is examined for its pectic composition after reddening and just after cold storage. Pectins, extracted with water and potassium oxalate, change their composition after different storage regimes with respect to unstored fruit. 'Annurca' pectin, as analyzed by gel-permeation chromatography, is composed of three types of polymers (high MW, >350 kDa, medium MW 350–110 kDa, low MW 40–9 kDa). Gel-permeation chromatograms revealed that the medium MW polymers decreased after normal atmosphere storage.

Eluates were subjected to enzymatic and chemical hydrolysis, then to HPLC analysis. The high and medium MW polysaccharides were rich in galacturonic acid. Low MW were rich in neutral sugars. The storage-induced differences in sugar composition revealed that the most important changes are loss of galacturonic acid in medium MW fragments, increase of glucose, rhamnose and arabinose and depletion of mannose–galactose units in low MW fragments.

Keywords: Annurca apple; Firmness; Water and oxalate-soluble pectin; Storage

1. Introduction

'Annurca' is a very old apple cultivar and it is one of the most important in Southern Italy. Today it is the most commonly grown cultivar in the Campania region. It accounts for 95% of southern Italy production and 3–4% of national apple production (Floris, 1997).

The 'Istituto Sperimentale di Frutticoltura' at Ciampino, Rome, in 1970 initiated a research programme aimed at resolving problems that can occur in Annurca apple. The research included studies of various aspects of its biology, breeding and storage (Fideghelli, Monastra, Della Strada, Quarta, & Donini, 1977; Lintas et al., 1993; Limongelli & Testoni, 1984).

This cultivar has also been proposed to the European Council for the "Protected Geographical Indication" (PGI), a European project related to the preservation of local and characteristic agriculture commodities (CEE rule nr. 2081, 1992).

Just after harvest, the fruits are treated with a special system to give redness. The apples are placed on a layer of straw on the soil and are daily sprayed with water. When the exposed fruit surface becomes red, they are manually turned to redden the opposite side. This treatment is prolonged for 20–30 days, depending on the weather conditions and the type of fruit. This cultivar shows good storability after reddening, maintaining a remarkable firmness. Therefore, if the fruits are kept at room temperature, after harvest, for a long time, they maintain high quality (Lintas et al., 1993).

This apple cv has already been examined for its characteristic aroma development in relation to ethylene biosynthesis (Lo Scalzo & Testoni, 1998) and for

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doi:10.1016/j.foodchem.2004.10.033

changes in pectic composition and aroma profile during the reddening process (Lo Scalzo, Testoni, & Genna, 2001).

Nowadays, great attention is paid to textural properties of fruits and vegetables, both for varietal characterization and for their quality characteristics (Gross, 1984; Selvedran, 1985; Van Buren, 1991).

Many studies relate to the change in pectin composition during apple softening. Knowledge of the molecular mechanism of fruit tissue softening, cell wall structure and cell wall breakdown is very important for improving texture and quality of apple fruit (Gheyas, Blankenship, Young, & Mc Feeters, 1997).

As reported by many authors, the pectic composition of the flesh is highly related to firmness changes during ripening; for example Gross (1984) reported the fundamental role of water-soluble pectin in the softening; other work also confirms that one of the major contributions to intercellular adhesion, and the consequent firmness, comes from chelator-soluble pectin (Knee & Bartley, 1981; Selvedran, 1985; Van Buren, 1991). Hence, changes in pectin composition could be valuable for the determination of apple fruit maturity in relation to softening (Knee, 1978; Knee & Bartley, 1981).

In more recent studies, it was found that cell wall materials decreased during apple development (Gheyas, Blankenship, Young, & Mc Feeters, 1998), while total pectin and total absolute polyuronide content of apple did not change during post-harvest apple softening (Jong-Pil-Chun, Yong-Soo-Hwang, Jae-Chang-Lee, & Huber, 1999; Nara, Kato, & Motomura, 2001; Yoshioka, Aoba, & Kashimura, 1992).

The main component of pectin is represented by galacturonic acid. It has been found that its relative proportion respect to other detected sugars shows an increase during apple softening (Gheyas et al., 1998 and during 'Annurca' apple reddening (Lo Scalzo et al., 2001). Some other authors confirm that the increase of galacturonic acid during apple ripening is limited to water-soluble pectin while, in chelator-soluble extracts, it shows a decrease (Yoshioka, Kashimura, & Kaneko, 1994).

The neutral sugar contents of ripening apples are subject to different changes in relation to the extract and the type of sugar: arabinose and galactose generally decrease, while rhamnose, glucose and xylose show little fluctuation (Gheyas et al., 1998; Jong-Pil-Chun et al., 1999; Nara et al., 2001; Yoshioka et al., 1994). Knee and Bartley have carried out detailed studies on the neutral sugar changes in ripening apples (Bartley, 1976; Bartley & Knee, 1982; Knee, 1973). They observed a decrease in arabinose and galactose during ripening, in the cell wall, and postulated that pectin solubilization was due to a de novo synthesis of pectin with a high degree of polymerization and less strongly bound to the middle lamella, leading to cell separation.

The neutral sugar evolution was also studied in 'Annurca' apple pectin during the reddening process. Glucose and rhamnose were the major compounds detected; they were mainly found in a fraction of low molecular weight (40–9 kDa) and they showed a clear decrease after reddening (Lo Scalzo et al., 2001). Arabinose was found mainly in medium molecular weight fractions (350–110 kDa) and also decreased with the ripening, as already confirmed by other authors (Gheyas et al., 1998; Jong-Pil-Chun et al., 1999; Nara et al., 2001; Yoshioka et al., 1994).

Few data were found about changes in pectin composition and apple storage regimes. A study, performed on 'Golden Delicious' apples, reported that different types of storage did not induce quantitative changes in pectin composition expressed as cell wall fraction/unit dry weight while, in stored apples, decreases of total pectin and hemicellulose contents were found; the greatest was in the apples stored in normal atmosphere (Siddiqui, Brackmann, Streif, & Bangerth, 1996). Changes in molecular sizes of polyuronides were not evident up to 6 months in any of the storage conditions.

However, to date, there are few available data on the quality parameters of the 'Annurca' apple cultivar during storage of reddened fruits and no data on pectin composition changes in relation to different storage regimes.

One early ripening variety of 'Annurca' apple, that shows a better percentage of red surface at harvest, called 'Bella del Sud' (Limongelli & Testoni, 1984), was studied. The present study describes the results of research on pectin composition, just after reddening and after cold storage, of reddened fruits in normal atmosphere (NA) and controlled atmosphere (CA).

2. Materials and methods

2.1. Preparation of fruit samples

'Annurca' apple fruits were harvested at commercial maturity from trees in a research orchard (Istituto Sperimentale per la Frutticoltura, Ciampino, Rome) on 13 October 1998 and were prepared for the reddening process. After 25 days of reddening, a sample of twenty fruits was transported to the laboratory and checked for firmness. The same fruits as analyzed for firmness were treated for the pectin extraction. Two aliquots of fruits, (50 kg) were, respectively, stored at low temperature, 0–1 °C, in NA for 5 months and CA for 6 months (1.0% O_2 and 1.2% CO_2).

Just after storage, twenty fruits from each treatment (NA and CA) were placed at room temperature for 24 h, analyzed for firmness and treated for the pectin extraction.

Firmness was measured by a dinamometer, probe 11 mm diameter, crosshead speed 200 mm min^{-1}; penetration of the probe was 8 mm. Firmness was measured on peeled flesh from opposite sides of each whole fruit and was expressed as kg/cm^2.

2.2. Pectin extraction

Fruits were divided into four samples of five each, cut, peeled and immediately frozen at −50 °C in an air-blast tunnel, then immediately lyophilized. The lyophilized tissue was homogenized at 0 °C, using a waring blender, and stored at −80 °C prior to use. Four ethanol-insoluble residue (EIR) preparations were made from each sample of 'Annurca' apple. Two grammes of each sample were treated for 2 h with 25 ml of 75% EtOH at 80 °C and then left overnight at room temperature (Gheyas et al., 1997). The following day the suspensions were centrifuged at 25,000*g* for 45 min. After removing the surnatant, the EIR was suspended in 25 ml of 75% EtOH and centrifuged as above. The residue was treated 6 h with 20 ml of acetone, centrifuged at 25000g for 45 min and, after decantation of the surnatant, the pellet was dried overnight in a vacuum and stored at −10 °C until used.

EIRs were extracted sequentially to obtain water-soluble and oxalate (OX)-soluble pectin. Fifty mg of EIRs were treated with 3 ml of H_2O, shaken vigorously for 12 h and filtered by centrifugation on 10 μm cellulose acetate filters. The insoluble residue was suspended in 3 ml of 0.05 M K oxalate and treated as above. Two ml of the filtrates, containing, respectively, the water and the OX-soluble pectin, were directly applied to the gel-permeation column. The residue coming from the OX extraction was dried at 100 °C to constant weight.

2.3. Pectin analysis

The water and the OX-soluble pectin from 'Annurca' fruit were analyzed for molecular weight (MW) distributions by low pressure gel filtration chromatography, using a 1.6 × 55 cm Sephacryl S-300 HR column (molecular weight range for dextrans: 1–400 kDa), with a void volume of 47.6 ml. Preliminary trials of elution were done using standard solutions of galacturonic acid and glucose, in order to evaluate possible interference or extracted pectin degradation during the elution of polymers. The analyzed chromatograms gave weak signals close to the total volume of the column, meaning no possible interference with the eluted pectic polymers. The column elution profile was calibrated by dextran standards: Blue dextran, 420, 280, 180, 69, 42, 20 and 10 kDa at a flow rate of 0.5 ml/min. The detection of signals was done by refractive index. The eluant was an aqueous solution of 0.06 mM dimetliylsulphoxide and 0.005 M K oxalate, pH 6.1, and the eluate was collected in aliquots of 5 ml each.

Three fractions of 20 ml were collected for each sample, corresponding to the following elution bands, as indicated in Figs. 1 and 2: fraction 1, from an elution volume of 40–60 ml (V_0 − 350 kDa, High MW); fraction 2, 60–80 ml (350–110 kDa, Medium MW); fraction 3, 100–120 ml (40–9 kDa, Low MW).

These fractions were considered for the evaluation of pectin composition in their contents of galacturonic acid, glucose, mannose, galactose, xylose, rhamnose and arabinose.

The fractions were also assayed for their methanol content, in order to evaluate the esterification index.

2.4. Galacturonic acid and neutral sugar analysis

Galacturonic acid content of eluates was determined by HPLC on enzymatically depolymerized eluted fractions, following a previous method (Forni, Polesello, & Braga, 1987), with some modifications: 2 ml of each fraction were treated for 4 h at room temp with 0.1 ml 1 N NaOH, buffered at pH 5.6 with 0.5 ml of 1 N acetate buffer and incubated with 0.1 ml of pectinase EC 3.2.1.15 Sigma, 365.8 U/ml diluted 10-fold with water. The samples were kept at 50 °C for 24 h, then dried under vacuum at room temperature with a centrifugal evaporator, dissolved in 1 ml of 1 mM H_2SO_4 analysed by HPLC.

The enzyme efficiency was assayed by treating (in the same way as above) a known amount (10 mg) of polygalacturonic acid purchased from Sigma dissolved in 2 ml of the column eluant and by subsequent quantification of the galacturonic acid by HPLC by the same method as used for the samples.

For the evaluation of the neutral sugars, 4 ml of each fraction were dried under vacuum at room temperature with a centrifugal evaporator, then treated for 1 h at 120 °C with 0.5 ml of 2.5 N TFA, re-dried under vacuum, dissolved in 1 ml of 1 mM H_2SO_4 and analysed by HPLC.

The same HPLC separation was used both for galacturonic acid and neutral sugars. It was carried out using two columns in succession: a 6.5 × 300 mm Polispher OA HY connected with a Hyper-Rez 100 × 7.7 mm − 20 μm, both held at 40 °C, and eluted with 1 mM H_2SO_4 at a flow rate 0.3 ml min^{-1}, with a refractive index detector. The analyzed sugars showed the following retention times (minutes): galacturonic acid (17.6), glucose (20.7), mannose and galactose (22.9), xylose (23.2), rhamnose (23.7) and arabinose (24.6). Mannose, xylose and galactose did not show a good resolution with these separation conditions. Hence, a further HPLC separation was performed to separate the xylose: it was done by using a 7.8 × 300 mm Aminex RPX 87P column held at 55 °C, eluted with H_2O at a flow rate

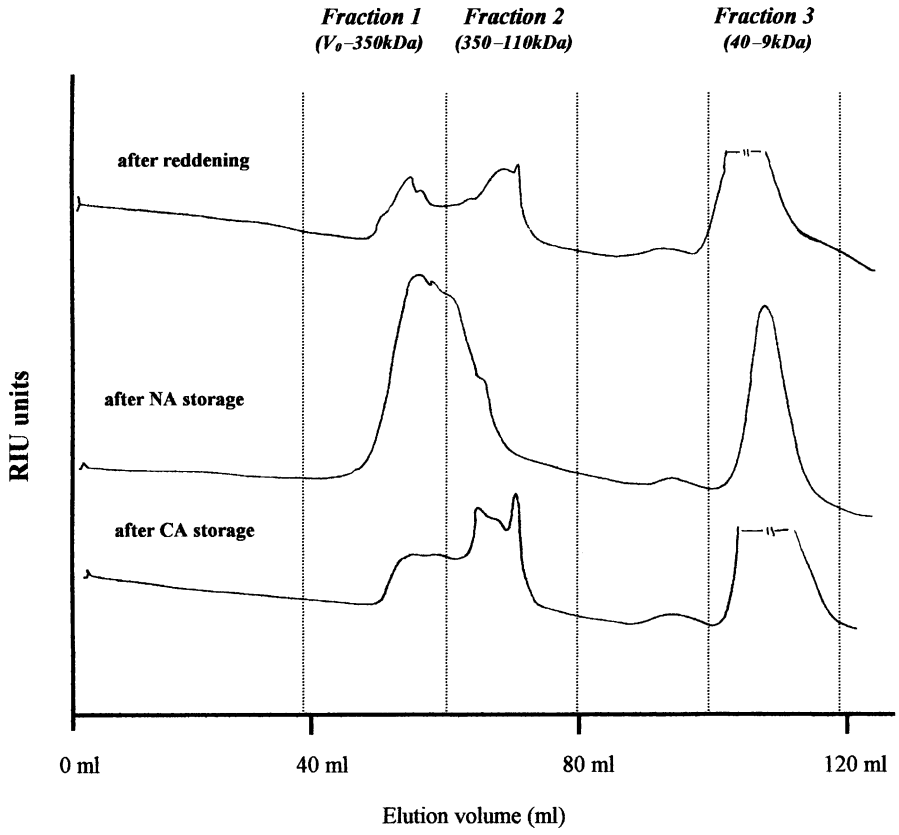

Fig. 1. Elution profiles of water-soluble pectin from 'Bella del Sud' variety of 'Annurca' apple after reddening, normal atmosphere (NA) storage and controlled atmosphere (CA) storage. The chromatograms are registered (RIU arbitrary units) with the same intensity. Fraction 1 is the elution volume collection from 40 to 60 ml and represents the High MW fractions (V_0 – 350 kDa), fraction 2 is from 60 to 80 ml (Medium MW, 350–110 kDa) and fraction 3 is from 100 to 120 ml (Low MW, 40–9 kDa).

0.4 ml min^{-1}, with refractive index as detector. The xylose showed a well resolved peak at a retention time of 19.5 min.

The sugars were quantified by calibrating with authentic standards and their amounts, for each fraction, were reported as percent millimolar of the total EIR content.

Mass balances, expressed as molar proportion of galacturonic acid and total neutral sugars vs total detected sugar content of EIR were also calculated and discussed.

2.5. Methanol analysis

Methanol was quantified by capillary GC-headspace technique, using a modified method already used for melon aroma (Senesi, Lo Scalzo, Prinzivalli, & Testoni, 2002) employing automatic headspace sampler, conditioning in a closed 3 ml vial, 2 ml of each fraction being treated with 0.1 ml of 1N NaOH at 80 °C for 1 h. A 30 m Carbowax 20M column was used with He as carrier at 0.8 ml/min, programmed at 40 °C for 5 min then 30 °C/min and 150° for 5 min; injector temperature was at 230 °C, FID was at 250 °C. The esterification index (EI) was determined by the equivalent ratio of the methanol to the galacturonic acid content.

3. Results and discussion

3.1. Firmness, EIR, residue and extraction yield

The firmness values of 'Annurca' apple are shown in Table 1. As expected, these apples show a value of 8.6 kg cm^{-2} after 25 days of the reddening process in the sun; the NA storage lowered this value to 6.5 kg cm^{-2}, and the fruits stored in CA showed a better firmness retention than the NA stored apples with a value of 7.6 kg cm^{-2}.

Table 1 also shows the EIR content for all reddened fruits. The EIR content of apples after reddening did not show a significant diminution after NA and CA storage, confirming the data of other authors (Gheyas et al., 1998: Jong-Pil-Chun et al., 1999; Siddiqui et al., 1996). However, the EIR content of 'Annurca' apple was subject to a drastic decrease during the reddening process (Lo Scalzo et al., 2001).

The EIR solubility, related to the extraction efficiency, could be an important marker of ripening stage. The yield in water-soluble pectic material was increased after both storage regimes, while OX-soluble material showed the highest content in fruits after reddening (Table 1). This result was also found by Yoshioka et al.

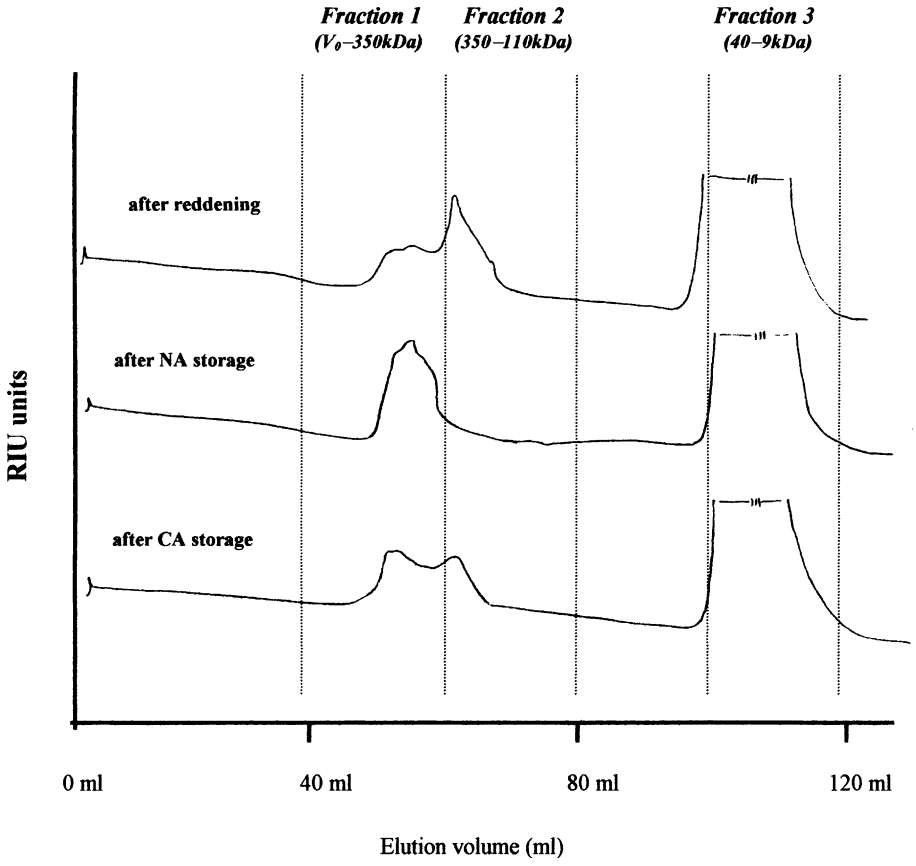

Fig. 2. Elution profiles of OX-soluble pectin from 'Bella del Sud' variety of 'Annurca' apple after reddening, normal atmosphere (NA) storage and controlled atmosphere (CA) storage. The chromatograms are registered (RIU arbitrary units) with the same intensity. Elution volumes and fractions collection are the same as in Fig. 1.

Table 1
Firmness, ethanol insoluble residue (EIR), residue from extractions, water- and OX-soluble pectin contents of 'Annurca' apple, cv 'Bella del Sud'

	Firmness[a] (kg cm^{-2})	EIR (g kg^{-1} f.w.)	Water-soluble (%EIR w/w)	OX-soluble (%EIR w/w)	Residue (%EIR w/w)
After reddening	8.6a	25.4a	11.4b	9.2a	69.8a
After NA storage	6.5c	24.3a	14.3a	6.2b	66.0b
After CA storage	7.6b	23.4a	16.1a	6.9b	67.6b

EIR and residue were calculated by direct weights. Water-and OX-soluble pectin data were obtained from HPLC data. The data (averages of 4 replications) with the same letter are not statistically different ($p > 0.05$) in each column.

[a] Average of 40 replications.

(1992) during softening of apples and pears. Generally, the amount of water-soluble material is higher than in OX-soluble samples.

The residue after both extractions (Table 1) was high after reddening (69.8%), indicating a low EIR solubility. On the other hand, it showed the lowest value in NA fruits (66.0%), indicating higher extraction of the sum water- and OX-soluble cell wall material.

3.2. Gel-permeation fractionation

The first extraction of the EIR from 'Annurca' apple was done with H_2O, so the pectin examined was defined as water-soluble pectin. The second extraction of the EIR was done a solution of 0.05 M, K oxalate so the pectin examined was defined as OX-soluble pectin.

Both extracts were chromatographed to detect some possible change in molecular weight distribution of pectin from non-stored or stored apple fruit.

The elution of water- and OX-soluble pectin from 'Annurca' apple was difficult: so a very small aliquot of dimethylsulphoxide was added to the eluant solution of the gel-filtration in order to maintain the solubilisation during the chromatographic separation.

First of all, three groups of polymers (Figs. 1 and 2) are observed in all the gel-permeation profiles: one close to the exclusion volume, collected in fraction 1, and another at 350–110 kDa, corresponding to fraction 2.

Fraction 1 and 2 polymers are also defined was 'high and medium MW' chains, respectively. The last polymer was eluted at an interval between 40 and 9 kDa, at low MW, represented by fraction 3.

In Fig. 1, the elution profiles coming from samples after reddening and CA storage, show a large band in fraction 2 if compared with the data from fruit stored in NA. The chromatograms from extracts of fruits stored in CA showed an important contribution to fraction 2 with a narrow peak eluted at 210 kDa. In NA-stored fruit, the very large peak in fraction 1 was partially collected in fraction 2. There was no narrow peak of lower MW as is seen in CA fruit or fruit after reddening.

Fig. 2 shows the gel-filtration chromatograms from oxalate extracts of EIR, with the problem of the signal interference at low MW given by the oxalate, present in higher concentration in the sample than in the eluant. The signals between 40 and 80 ml of elution volume are lower than in the water extracts, but they replicate the situation previously explained for water extracts, showing the NA samples with a poor band in fraction 2.

It seems that the elution profiles from CA-stored apple are more similar to those 'after reddening' than are the chromatograms from NA-stored fruit.

3.3. Galacturonic acid and neutral sugar changes

The composition of galacturonic acid in each fraction from gel-permeation is shown in Table 2. The amount of galacturonic acid was highest in fraction 1 (high MW) of NA samples of water-soluble pectin with a value of 27.5 mmol%EIR. In fraction 2 (medium MW), it showed a drastic decrease after NA with respect to 'after reddening' and CA, while it had low values in fraction 3 (low MW) for all samples.

The changes in galacturonic acid of the fractions eluted by the gel-permeation of OX-soluble pectin generally resemble the situation in water-soluble pectin, except for the low amount found in fraction 1 after NA storage with respect to the highest value found in fruit after reddening. The fraction 2, after NA, stood out for the low value, reaching 1.0 mmol % with respect to 'after reddening' and CA. Little presence of galacturonic acid was confirmed in low MW fragments, like water-soluble pectin.

Galacturonic acid (absolute amount) in fraction 1 of NA samples showed an increase in water-soluble and a decrease in OX-soluble pectin with respect to 'after reddening' samples, thus confirming the data of Yoshioka et al. (1994). Total content of galacturonic acid was clearly depleted in NA samples of both extracts with respect to after reddening and CA apple fruit.

In water-soluble pectin, galacturonic acid from fraction 1 showed a mass balance of 75% with respect to total detected sugars in after-reddening fruit, while

Table 2
Galacturonic acid and neutral sugar contents (mmol/100 g EIR of single fractions) in water- and OX-soluble pectin of eluted gel-permeation fraction

Fraction	After reddening		NA		CA	
Water-soluble						
Galacturonic acid						
1	18.9b	*75.2*	27.5a	*78.8*	18.5b	*73.2*
2	15.4a	*74.7*	3.8b	*29.6*	19.9a	*76.4*
3	4.9a	*30.4*	2.4a	*7.8*	3.2a	*8.6*
Total	39.2a	*63.4*	33.7b	*42.8*	41.6a	*47.0*
Glucose						
1	1.2b	*4.9*	2.3a	*6.7*	3.7a	*14.8*
2	1.1b	*5.1*	2.9a	*22.8*	2.2a	*8.6*
3	6.4b	*39.7*	20.6a	*66.2*	24.6a	*66.4*
Total	8.7b	*14.0*	25.8a	*32.8*	30.5a	*34.6*
Mannose–galactose						
1	0.0b	*0.0*	1.4a	*4.0*	0.1b	*0.2*
2	1.6a	*7.8*	1.1a	*8.2*	0.3b	*1.1*
3	2.6a	*16.2*	0.3c	*0.9*	1.1b	*2.9*
Total	4.2a	*6.8*	2.8b	*3.4*	1.5c	*1.6*
Xylose						
1	0.1a	*0.5*	0.4a	*1.1*	0.1a	*0.3*
2	0.1a	*0.6*	0.1a	*0.5*	0.1a	*0.5*
3	0.9b	*5.4*	2.0a	*6.4*	1.5a	*4.0*
Total	1.1b	*1.8*	2.5a	*3.1*	1.7a	*1.9*
Rhamnose						
1	1.2a	*4.8*	1.8a	*5.2*	1.5a	*6.0*
2	1.2b	*5.9*	2.7a	*21.3*	1.9b	*7.3*
3	1.3b	*7.9*	4.0a	*12.9*	5.0a	*13.5*
Total	3.7b	*6.0*	8.5a	*10.9*	8.4a	*9.5*
Arabinose						
1	3.7a	*14.6*	1.5b	*4.2*	1.4b	*5.6*
2	1.2b	*5.8*	2.3a	*17.6*	1.6b	*6.2*
3	0.1b	*0.4*	1.8a	*5.8*	1.7a	*4.7*
Total	5.0a	*8.0*	5.6a	*7.0*	4.7a	*5.4*
OX-Soluble						
Galacturonic acid						
1	18.3a	*90.1*	12.1b	*59.8*	11.4b	*71.1*
2	13.3a	*76.4*	1.0c	*14.1*	10.0b	*67.2*
3	2.9a	*25.5*	1.1a	*15.2*	2.5a	*39.2*
Total	34.5a	*70.3*	14.2c	*41.0*	23.9b	*64.1*
Glucose						
1	0.9b	*4.7*	2.2a	*11.0*	1.4b	*9.0*
2	1.9a	*10.9*	0.8b	*11.2*	2.2a	*15.0*
3	4.2a	*36.8*	2.7b	*35.8*	2.2b	*35.2*
Total	7.0a	*14.3*	5.7b	*16.4*	5.8b	*15.8*
Mannose–galactose						
1	0.6b	*3.0*	2.3a	*11.3*	0.9b	*5.9*
2	1.3b	*7.4*	2.4a	*35.2*	1.7ab	*11.3*
3	1.3a	*11.8*	0.1c	*0.7*	0.6b	*9.7*
Total	3.2b	*6.6*	4.8a	*13.8*	3.2b	*8.7*
Xylose						
1	0.2a	*1.0*	0.2a	*1.0*	0.1a	*0.8*
2	0.1b	*0.8*	1.1a	*15.4*	0.4b	*2.7*

Table 2 (*continued*)

Fraction	After reddening		NA		CA	
3	0.3a	*2.4*	0.2a	*2.7*	0.2a	*3.2*
Total	0.6b	*1.2*	1.5a	*4.2*	0.7b	*2.0*
Rhamnose						
1	0.1c	*0.6*	2.0a	*9.7*	0.9b	*5.3*
2	0.1b	*0.4*	0.6a	*8.8*	0.4a	*2.5*
3	0.0b	*0.0*	0.1b	*0.8*	0.6a	*9.7*
Total	0.2b	*0.4*	2.7a	*7.6*	1.9a	*4.9*
Arabinose						
1	0.1b	*0.7*	1.5a	*7.3*	1.3a	*7.9*
2	0.7a	*4.2*	1.1a	*15.4*	0.2b	*1.4*
3	2.7a	*23.6*	3.3a	*44.7*	0.2b	*3.2*
Total	3.5b	*7.2*	5.9a	*17.0*	1.7c	*4.5*

Each result is the average of four replicates and the data with the same letters are not statistically different in each row for each extract ($p > 0.05$). The numbers written in italics beside each value represent the percent proportion respect to the total content of each fraction. Fraction/identity and collection is explained in Fig. 1.

fruits after NA and CA storage had 79% and 73%, respectively.

The NA storage showed proportions of galacturonic acid in fraction 2 in water- and OX-soluble pectin of 30% and 14%, respectively, if compared with the amounts in fruit after reddening and CA, averaging about 70%, thus enforcing the hypothesis of the loss in galacturonic acid in medium MW fragments for apple stored in NA.

Glucose is the major neutral sugar found in 'Annurca' apple pectin; it showed high amounts in fraction 3 of all water-soluble samples (Table 2). It showed a general increase in both NA- and CA-stored fruit, reaching the highest amounts of all detected neutral sugars in fraction 3 and a proportion of 66%. In OX-soluble pectin, its amount was generally lower than in water-soluble polymers. In fraction 3 of apple after reddening it was 4.2 mmol%, higher than the amount found in fruit after both types of storage, but with the same mass balance (about 35%).

The amount of glucose in fraction 3 becomes important, confirming previous data (Lo Scalzo et al., 2001). Its increase after storage and an assay with Lugol solution, directly on fruit with negative response, gives the opportunity to confirm that it does not orginate from starch molecules. Besides, a similar situation, with polysaccharides rich in glucose, was found in water-soluble cell wall material from apple and peach (Massiot, Baron, & Drilleau, 1996; Zhou et al., 2000).

In water-soluble pectin, the total content of mannose–galactose showed a high value after reddening. In fraction 1, the NA pectin showed a mannose–galactose amount of 1.4 mmol%, while it was very low in other samples; instead in fraction 3 the mannose–galactose content was low in NA and CA fruit if compared with apple after reddening, meaning a decrease in these sugars for the low MW fraction after storage. For OX-soluble pectin, mannose–galactose showed the highest amounts of high and medium MW fragments after NA storage and a loss in low MW fragments, as was already found for the water-soluble pectin.

The loss of galactose from pectic polymers during apple softening is well described by other authors (Gheyas et al., 1998; Jong-Pil-Chun et al., 1999; Yoshioka et al., 1994) but comparison with this study is difficult, owing to the coelution of mannose and galactose in HPLC separation of pectin hydrolysates. The xylose amount is higher in fraction 3 than in other fractions and it is very low in other fractions, except for fraction 2 of NA fruit of OX-soluble pectin. Xylose is known to be associated with pectic polymers (Schols, Vierhuis, Bakx, & Voragen, 1995; Renard, Weightmann, & Thibault, 1997). The similar xylose evolution to that of glucose is confirmed in the present results of water-soluble pectins, but its pectic association is not clear, owing to the low amounts of galacturonic acid found in low MW fractions; a possible presence of water-soluble fragments of xyloglucans is hypothesized.

A remarkable presence of rhamnose was found in the low-MW fraction of water-soluble pectin from fruit after both types of storage. As for OX-soluble pectin, rhamnose remained at very low values in all fractions after reddening, with high presence in fraction 1 of samples after NA and CA storage.

It seems that the presence of glucose, xylose and rhamnose is enhanced in low MW fragments of water-soluble pectin after both kinds of storage. This result was confirmed by other authors: Gheyas et al. (1998) found an increase of glucose in softening apples, while Yoshioka et al. (1994) confirmed the increase of rhamnose in water-soluble pectins. On the other hand, this result was in contrast with the 'Annurca' apple softening during reddening (Lo Scalzo et al., 2001); the fruit showed decreases of both glucose and rhamnose during the stringent reddening conditions.

The arabinose of water-soluble samples showed a decrease in both types of stored fruit in fraction 1, confirming other authors' data (Yoshioka et al., 1994; Gheyas et al., 1998; Jong-Pil-Chun et al., 1999), while in fraction 3 it showed an increase. Total content did not show changes.

Arabinose of OX-soluble chains showed an increase in stored apples with respect to those after reddening in fraction 1, an inverse trend with respect to water-soluble pectin. It has to be noted that, in fractions 2 and 3, the values after CA storage were very low.

The increase of a pectic component during fruit postharvest ripening could be due to the higher solubility of pectic polymers in soft than in firm fruit, apart from changes in molecular weight or in degree of polymerization.

It is also reported that polyuronides poor in neutral sugars, are preferentially released during apple softening (Yoshioka et al., 1994); this means an increase in the ratio of galacturonic acid to neutral sugars, during apple softening. On the other hand, a decrease in the ratio of galacturonic acid to neutral sugars indicates loss of galacturonic acid.

Specifically, the ratio of galacturonic acid to rhamnose indicates the amount of 'hairy' regions present in the sample. Published data (de Vries, den Uijl, Voragen, Rombouts, & Pilnik, 1983; Legentil, Gunchard, Piffaut, & Haluk, 1995; Renard, Thibault, Voragen, van der Broeck, & Pilnik, 1993; Van Buren, 1991) quantify this parameter, establishing that a ratio lower than about 50 could mean a potentially hairy polymer with a proportion of rhamnose with respect to galacturonic acid, of 2%.

In the present study, the amount of rhamnose did not reach this value, except for fractions 1, 2 and 3 of OX-soluble pectin after reddening, indicating the presence of smooth rhamnogalacturonan polymers. All other values are higher (Table 2), with a maximum of 21.3% with respect to total detected sugars in fraction 2 of NA samples of water-soluble pectin, so demonstrating the clear loss of water-soluble galacturonic acid polymers in medium MW chains of NA-stored fruit.

Another parameter could be the size of side chains which could be evaluated by the presence of arabinose. High concentrations of this sugar could represent long side chains.

In water-soluble pectins, a considerable amount of arabinose was found in fraction 1 after reddening and in fraction 2 after NA storage. This could mean that fruit before storage have pectic polymers with long side chains of high MW while, in NA-storage, these polymers decrease their MW mainly by the loss in galacturonic acid previously reported.

In OX-soluble pectins, high concentrations of arabinose are found in low MW polymers, indicating long side chain regions, also accompanied by polymers rich in glucose (Table 2).

The esterification index (Table 3), evaluated for all the fractions, was generally very low with respect to those found in unreddened apples, probably due to the stringent conditions of the reddening process. It must be noted that these 'Annurca' apples were subjected to a very severe post-harvest treatment by their deposition on the soil, only protected by straw in the groundspot, and hence complete exposure to weather conditions, that in October could show high temperature shifts between night and day. To this stress was added the stress represented by cold storage conditions of several months.

Table 3
Esterification index (molar%) in fractions from gel-permeation of pectin from Annurca cv 'Bella del Sud' (averages of four replications)

	After reddening	After NA storage	After CA storage
Water-soluble			
Fraction 1	22.6b	21.2b	26.9a
Fraction 2	24.8a	22.0a	17.8b
Fraction 3	7.4a	1.7b	3.2b
OX-soluble			
Fraction 1	20.1b	23.9b	43.4a
Fraction 2	22.3b	40.1a	17.2b
Fraction 3	3.6a	5.9b	1.4c

The data with the same letter are not statistically different ($p > 0.05$) in each row. Fraction identity and collection is explained in Fig. 1.

In fractions 1 and 2 the value was generally higher than in fraction 3. These last result were already found in previous data from 'Annurca' apple (Lo Scalzo et al., 2001).

All values for fractions 1 and 2 were at about 20%, except for two samples in OX-soluble pectin at about 40%: fraction 2 of NA and fraction 1 for CA fruit, confirming the finding of Yoshioka et al. (1992) that polyuronides with a high degree of methoxylation were preferentially extracted from chelator-soluble fragments. The only clear decrease of esterification index with storage occured in fraction 3 of water-soluble pectin; this was already reported by Klein, Hanzon, Irwin, Ben-Shalom, & Lurie (1995) on Golden Delicious apples, with very low values in apples treated at 38 °C before storage.

4. Conclusions

The firmness after-NA storage was lower than in samples after reddening. After CA storage, the firmness value was retained.

Annurca apple pectin was composed of three types of polymers; those of higher MW were mainly composed of galacturonic acid, while the polymers of lower MW were mainly composed of neutral sugars, especially glucose.

It seems that the major changes in composition related to storage of reddened fruits of 'Annurca' apple occured in polymers of 350–110 kDa (medium MW) and of 40–9 kDa (low MW).

Both storages induced an increase in total water-soluble pectin and a decrease in OX-soluble polysaccharides. The galacturonic acid was depleted, both by its absolute amount and by its relative presence, in medium MW chains of NA-stored fruit both in water and OX-soluble pectin, while it showed a maximum of its relative proportion in high MW chains of OX-soluble pectin from fruits after reddening.

Glucose, rhamnose and arabinose increased in fraction 3 of water-soluble pectin stored fruit, while mannose and galactose showed loss. In OX-soluble pectins, neutral sugars were generally reduced with respect to water-soluble ones, and a stable trend is not so evident.

Finally, the main compositional changes of 'Annurca' apple pectin structure during the reddening process

agreed with those of other authors (Yoshioka et al., 1994): the reddening process induced a release of smooth polymers mainly composed of galacturonic acid with low rhamnose (Lo Scalzo et al., 2001).

A further storage-induced softening of reddened apples, discussed in the present paper induced a loss of smooth fragments, demonstrated by the decrease in galacturonic acid and by the increase of rhamnose.

The changes in biochemical parameters during reddening evaluated in a previous paper and the storage-induced modifications often contrast; for example, glucose was decreased during reddening, but increased after storage, meaning that the earlier softening of 'Annurca' apples at high temperature and in the sun, related to the reddening, was surely different from the softening of already reddened and successively stored apples.

It is clear that this study is not exhaustive: other studies should be carried out to better understand the particular features of this apple cv.

References

Bartley, I. M. (1976). Changes in the glucans of ripening apples. *Phytochemistry, 15*, 625–626.

Bartley, I. M., & Knee, M. (1982). The chemistry and textural changes in fruit during storage. *Food Chemistry, 9*, 47–58.

CEE rule nr. 2081/92, Europe Council of 14/07/1992 – related to the protected geographical indication (PGI) and protected designation of origin (PDO) of agricultural products (*Gazzetta Ufficiale Italiana* L208 24/07/1992).

de Vries, J. A., den Uijl, C. H., Voragen, A. G. J., Rombouts, F. M., & Pilnik, W. (1983). Structural features of the neutral sugar side chains of apple pectic substances. *Carbohydrate Polymers, 3*, 193–205.

Fideghelli, C., Monastra, F., Della Strada, G., Quarta, R., & Donini, B. (1977). Mutazioni indotte nelle varietà di melo Annurca. *Rivista Ortoflorofrutticoltura Italiana, 61*(6), 360–367.

Floris, M. (1997). Melo e Pero nella Frutticoltura Italiana ed Europea. Federchimica Agrofarma eds., Milano, Italy, p. 1.

Forni, E., Polesello, A., & Braga, F. (1987). Research on a standardization of a combined enzymatic and HPLC method for the evaluation of pectins from their galacturonic acid content. *Food Hydrocolloids, 1*(5–6), 531–536.

Gheyas, F., Blankenship, S. M., Young, E., & Mc Feeters, R. (1997). Dietary fiber content of thirteen apple cultivars. *Journal of the Science of Food and Agriculture, 75*, 333–340.

Gheyas, F., Blankenship, S. M., Young, E., & Mc Feeters, R. (1998). Dietary fibre composition in developing apple fruits. *Journal of Horticultural Science and Biotechnology, 73*(5), 631–639.

Gross, K. C. (1984). Fractionation and partial characterization of cell walls from normal and non ripening mutant tomato fruit. *Physiologia Plantarum, 62*, 25–32.

Jong-Pil-Chun Yong-Soo-Hwang Jae-Chang-Lee & Huber, D. J. (1999). Cell wall component changes during maturation and storage in Tsugaru and Fuji apple fruits. *Journal of Korean Society for Horticultural Science, 40*(6), 705–710.

Klein, J. D., Hanzon, J., Irwin, P. L., Ben-Shalom, N., & Lurie, S. (1995). Pectin esterase activity and pectin methyl esterification in heated Golden Delicious apples. *Phytochemistry, 39*(3), 491–494.

Knee, M. (1973). Polysaccharides changes in cell walls of ripening apples. *Phytochemistry, 12*, 1543–1549.

Knee, M. (1978). Properties of polygalacturonate and cell cohesion in apple fruit cortical tissue. *Phytochemistry, 17*, 1257–1260.

Knee, M., & Bartley, I. M. (1981). Composition and metabolism of cell wall polysaccharides in ripening apples. In J. Friend & M. J. C. Rhodes (Eds.), *Recent advances in it the biochemistry of fruit and vegetables* (pp. 133–148). New York: Academic Press.

Legentil, A., Gunchard, I., Piffaut, B., & Haluk, J. P. (1995). Characterization of strawberry pectin extracted by chemical means. *Lebensmittel-Wissenschaft & Technologie, 28*, 569–576.

Limongelli, F., & Testoni, A. (1984). Annurca Bella del Sud e Annurca Rossa del Sud. *Atti IVTPA, 7*, 139–149.

Lintas, C., Paoletti, F., Cappelloni, M., Gambelli, L., Monastra, F., & Ponziani, G. (1993). Agronomic, nutritional and texture evaluation of 'Annurca' apple clones. *Advanced Horticultural Science, 7*, 165–168.

Lo Scalzo, R., & Testoni, A. (1998). Ethylene development in different clones of 'Annurca' apple and its influence on the biosynthesis of aroma esters and alcohols. In A. K. Kanellis, C. Chang, H. Klee, A. B. Bleecker, J. C. Pech, & D. Grierson (Eds.), *Biology and biotechnology of the plant hormone ethylene II* (pp. 419–425). Dordrecht, The Netherlands: Kluwer Academic.

Lo Scalzo, R., Testoni, A., & Genna, A. (2001). 'Annurca' apple fruit, a Southern Italy apple cultivar: textural properties and aroma composition. *Food Chemistry, 73*, 333–343.

Massiot, P., Baron, A., & Drilleau, J. F. (1996). Effect of storage of apple on the enzymatic hydrolysis of cell wall polysaccharides. *Carbohydrate Polymers, 29*, 301–307.

Nara, K., Kato, Y., & Motomura, Y. (2001). Involvement of terminal-arabinose and -galactose pectic compounds in mealiness of apple fruit during storage. *Postharvest Biology and Technology, 22*(2), 141–150.

Renard, C. M. G. C., Thibault, J. F., Voragen, A. G. J., van der Broeck, L. A. M., & Pilnik, W. (1993). Studies on apple protopectin. VI. Extraction of pectin from apple cells walls with rhamnogalacturonase. *Carbohydrate Polymers, 22*, 203–210.

Renard, C. M. G. C., Weightmann, R. M., & Thibault, J. F. (1997). The xylose-rich pectins from pea hulls. *International Journal of Biological Macromolecules, 21*(1–2), 155–162.

Schols, H. A., Vierhuis, E., Bakx, E. J., & Voragen, A. G. J. (1995). Different populations of pectic hairy regions occur in apple cell walls. *Carbohydrate Research, 275*(2), 343–360.

Selvedran, R. R. (1985). Development in the chemistry and biochemistry of pectic and hemicellulosic polymers. In K. Roberts, A. W. B. Johnston, C. W. Lloyd, P. Shaw, & H. W. Woolhouse (Eds.), *The cell surface in plant growth and development* (pp. 51–88). Cambridge: Company of Biologists.

Senesi, E., Lo Scalzo, R., Prinzivalli, C., & Testoni, A. (2002). Relationships between volatile composition and sensory evaluation in eight varieties of netted muskmelon (*Cucumis melo*L var reticulates Naud.). *Journal of the Science of Food and Agriculture, 82*(6), 655–662.

Siddiqui, S., Brackmann, A., Streif, J., & Bangerth, F. (1996). Controlled atmosphere storage of apples: cell wall composition and fruit softening. *Journal of Horticultural Science, 71*(4), 613–620.

Van Buren, J. P. (1991). Function of pectin in plant tissue structure and firmness. In R. H. Walter (Ed.), *The chemistry and technology of pectin* (pp. 1–22). New York: Academic Press.

Yoshioka, H., Aoba, K., & Kashimura, Y. (1992). Molecular weight and degree of methoxylation in cell wall polyuronide during softening in pear and apple fruit. *Journal of the American Society for Horticultural Science, 117*(4), 600–606.

Yoshioka, H., Kashimura, Y., & Kaneko, K. (1994). Solubilization and distribution of neutral sugars residues derived from

polyuronides during the softening in apple fruit. *Journal of the Japanese Society for Horticultural Science, 63*(1), 173–182.

Zhou, H-W., Sonego, L., Khalchitsk, A., Ben-Arie, R., Lers, A., & Lurie, S. (2000). Cell wall enzymes and cell wall changes in 'Flavortop' nectarines: mRNA abundance, enzyme activity, and changes in pectic and neutral polymers during ripening and in woolly fruit. *Journal of the American Society for Horticultural Science, 125*(5), 630–637.