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'Annurca' apple fruit, a southern Italy apple cultivar: textural properties and aroma composition

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Abstract

'Annurca' apple fruit, which is commonly cultivated in southern Italy and undergoes a typical reddening treatment and, is notable for its high firmness and its characteristic flavour, was examined for its pectic and aroma composition. Pectin, extracted with a water solution of potassium oxalate and dimethylsulphoxide, was found to be quantitatively and qualitatively important for its contribution to the high tissue firmness. Pectin changes its composition during the reddening process and there is even a difference in the pectic composition of the two 'Annurca' clones examined in this paper, 'Standard' and 'Rossa del Sud'. Volatile compounds were collected by headspace sampling of intact unreddened and reddened fruits. Thirty-one compounds of several chemical classes were identified, to establish their role in the characteristic flavor of this apple cv. Among these compounds, *n*-pentanol was unusually present in large amounts; also δ-octalactone, never found in apples, was present. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

'Annurca' has been known since ancient Roman times and it is one of the most important cultivars in Southern Italy. Today it is the most commonly grown cultivar in the Campania region. It accounts for 95% of the southern Italy and 3–4% of the national apple production (Floris, 1997).

'Annurca' is of excellent quality and commonly available in the Rome and Naples markets. However, it's sales have been markedly affected by a decline in acreage since the 1960s because of numerous drawbacks that have induced growers to switch to higher-yielding and more marketable cultivars. Given the cultivar's importance to Campania's economy, the 'Istituto Sperimentale di Frutticoltura' at Ciampino, Rome in 1970 initiated a research programme aimed at attenuating Annurca's defects. These efforts have included studies of various aspects of its biology, breeding and storage (Fideghelli, Monastra, Della Strada, Quarta & Donini 1977; Limongelli & Testoni, 1984; Lintas, Paoletti, Cappellani, Gambelli, Monastra & Ponziani 1993). 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 specially treated to obtain their redness. The apples are placed on a layer of straw on the soil and are daily sprayed with water. When the fruits' exposed surfaces becomes red, they are manually turned so as to redden the opposite side. This treatment is prolonged for 20–30 days, according to the weather conditions and types of fruit. This cultivar shows a good storability after reddening, maintaining a remarkable firmness and producing a typical, good aroma (Lintas et al., 1993).

Nowadays, great importance is given to studies of textural properties and aroma composition of fruits and vegetables, for varietal characterization and quality assessment. However, to date there are no available data on the contribution of pectin to the firmness and aroma composition of this apple cultivar during reddening. 'Annurca' apple flavour has already been briefly examined in relation to the production of ethylene in some clones (Lo Scalzo & Testoni, 1998). The changes in pectin composition and the evolution of aroma components could also be valuable for the determination of fruit maturity in relation to softening (Knee, 1978) and

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to the climacteric rise in respiration and production of ethylene (Lo Scalzo & Testoni, 1998; Song & Bangerth, 1996). The present study reports and discusses results of research on pectin composition and the production of volatile compounds, at harvest and after reddening. Two different clones of 'Annurca' were studied, one commonly cultivated called 'Standard', and another that shows a better percentage of red surface at harvest called 'Rossa del Sud' (Limongelli & Testoni, 1984).

2. Materials and methods

2.1. Preparation of fruit samples

'Annurca' apple fruit were harvested at commercial maturity from trees in a research orchard (Istituto Sperimentale per la Frutticoltura, Ciampino, Rome) on 10 October 1996 and were prepared for the reddening process. A fruit sample of each clone was transported to the laboratory and checked for soluble solids, colour, acidity and firmness, both at harvest (unreddened fruits) and after traditional reddening. Two aliquots of 10 fruits, one just at harvest and the second at the end of the reddening (30 days), were treated for the pectin extraction and the aroma headspace evaluation. The volatiles collection was performed on intact fruits (Mattheis, Buchanan & Fellman 1992).

2.2. Fruit colour, soluble solids, acidity, firmness measurements

'Annurca' background skin colour was measured using a colorimeter equipped with CIE, L^* a^* b^* system. Soluble solids were evaluated with a refractometer, while the acidity was evaluated with an automatic titroprocessor. 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 it was expressed in Newtons.

2.3. Pectin preparation

Fruits were 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. Four ethanol-insoluble residue (EIR) preparations were made from each clone of 'Annurca' apple. Each sample (400 mg) was twice treated overnight with 5 ml of EtOH 75% at room temperature and centrifuged at 6000 rpm for 30 min. After removing the supernatant, the EIR was suspended in 5 ml of acetone and re-centrifuged. The residue was dried overnight under vacuum and stored at −10°C until used. The EIR from 400 mg of each sample was treated with 2 ml of K oxalate (OX; 5 mM) and dimethylsulphoxide (DMSO) (0.06 mM), pH 6.1, shaken vigorously for 1 h and centrifuged on 10 μm cellulose acetate filters. The whole supernatant, containing the OX-DMSO soluble pectin, was directly applied to the column.

2.4. Pectin analysis

The molecular weight (MW) disributions of OX–DMSO soluble pectin from 'Annurca' fruit were analyzed by low pressure gel filtration chromatography using a 1.6×70 cm Sephacryl S-300 HR column (MW range for dextrans: 1000–400 000), with a void volume of 24.3 ml. The column elution profile was calibrated by dextran standards: Blue dextran, 464, 282, 181, 69, 42, 19.5 kDa, and 9.5 kDa at a flow rate of 0.8 ml/min. The eluant was the same used for pectin extraction. Seventeen fractions of 4 ml were collected and assayed for galacturonic acid, glucose, arabinose, mannose, xylose, galactose, rhamnose and methanol content. For the evaluation of pectin composition, the following fractions were pooled in order to give three clusters for each sample: 6–9, 10–13 and 14–17.

For galacturonic acid and other monosaccharides, each fraction was dried under vacuum at room temperature, then treated for 1 h at 120°C with 0.5 ml of 2.5 N TFA, redried under vacuum, dissolved in 1 ml of 1mM H_2SO_4 and analysed by HPLC. The HPLC separation was carried out

Table 1
Soluble solids, fruit colour, titratable acidity and firmness of 'Annurca' apple at harvest and after reddening (averages of 20 individual fruits)[a]

	At harvest		After reddening	
	Standard	Rossa del Sud	Standard	Rossa del Sud
Soluble solids (%)	12.4±0.9a	13.2±1.0a	13.5±0.5a	13.4±1.0a
Colour (CIE)				
L^*	74.28±1.43a	69.35±2.42b	63.79±5.38b	56,54±7.05c
a^*	−10.29±2.66a	−4.33±2.36b	+ 5.05±4.56c	+ 16.67±9.78d
b^*	+ 36.46±3.18a	+ 34.24±3.19a	+ 30.58±4.11b	+ 23.82±6.22c
Titratable acidity (meq/100g)	13.9±1.14a	13.8±1.16a	9.43±0.79b	8.97±0.56b
Firmness (Newton)	113.66±7.18a	101.99±8.01b	72.37±5.52c	70.12±3.35c

[a] The data with the same letter are not statistically different ($P > 0.05$) in each row.

Table 2
Ethanol-insoluble residue (EIR) and potassium oxalate-dimethylsuphoxide-soluble pectin (OX-DMSO) of 'Annurca apple's clones (averages of four replications)[a]

	At harvest		After reddening	
	Standard	Rossa del Sud	Standard	Rossa del Sud
EIR (% f. w.)	4.83±0.25a	5.23±0.44a	2.62±0.12b	3.44±0.11b
OX-DMSO soluble pectin (% f. w.)	1.52±0.09a	1.41±0.10a	0.64±0.09b	0.79±0.14b

[a] The data with the same letter are not statistically different ($P > 0.05$) in each row.

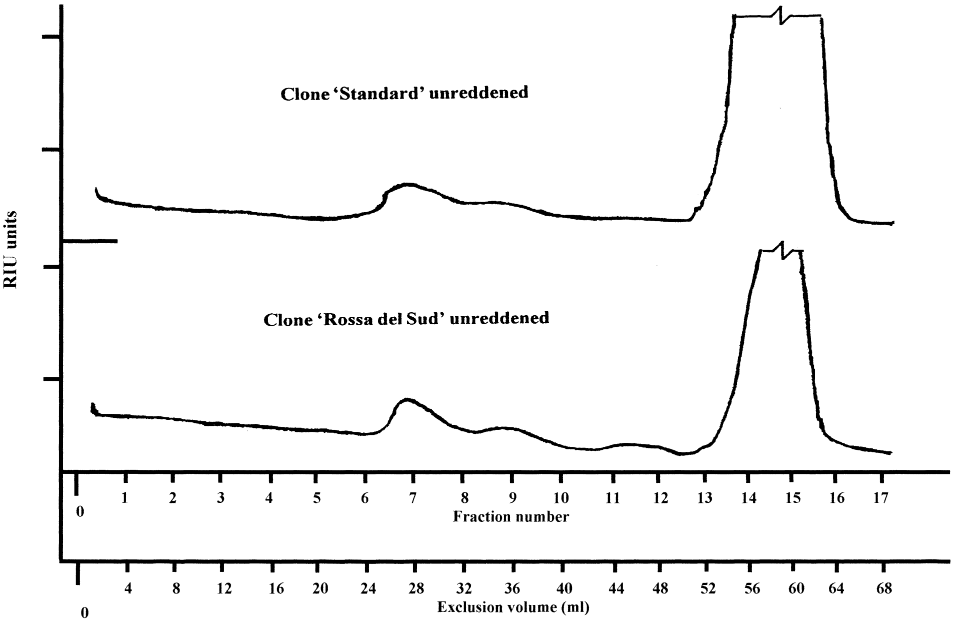

Fig. 1. Elution profiles of OX-DMSO-soluble pectin from unreddened fruits of 'Standard' and 'Rossa del Sud'.

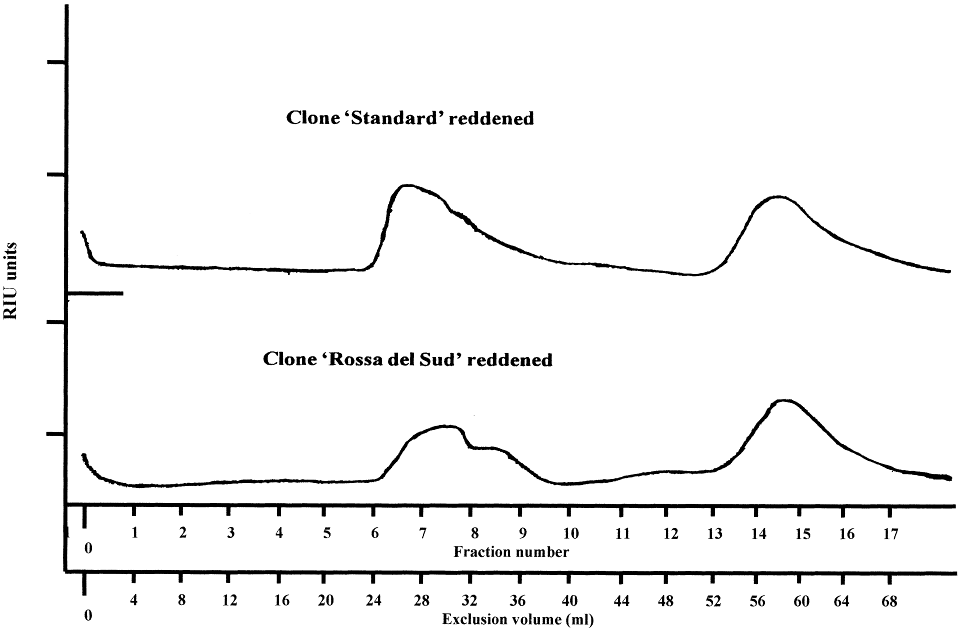

Fig. 2. Elution profiles of OX-DMSO-soluble pectin from reddened fruits of 'Standard' and 'Rossa del Sud'.

using a 6.5×300 mm Polispher OA HY column held at 40°C, eluted with 1 mM H_2SO_4 at a flow rate 0.3 ml min^{-1}, with refractive index (RI) as detector. Mannose, xylose and galactose were coeluted; hence the data are considered as one peak only. The sugars were quantified by calibrating with authentic standards, and the amounts were reported as percent w/w on the total content of a single cluster.

Methanol was quantified by a capillary GC-headspace technique, using an automatic headspace sampler,

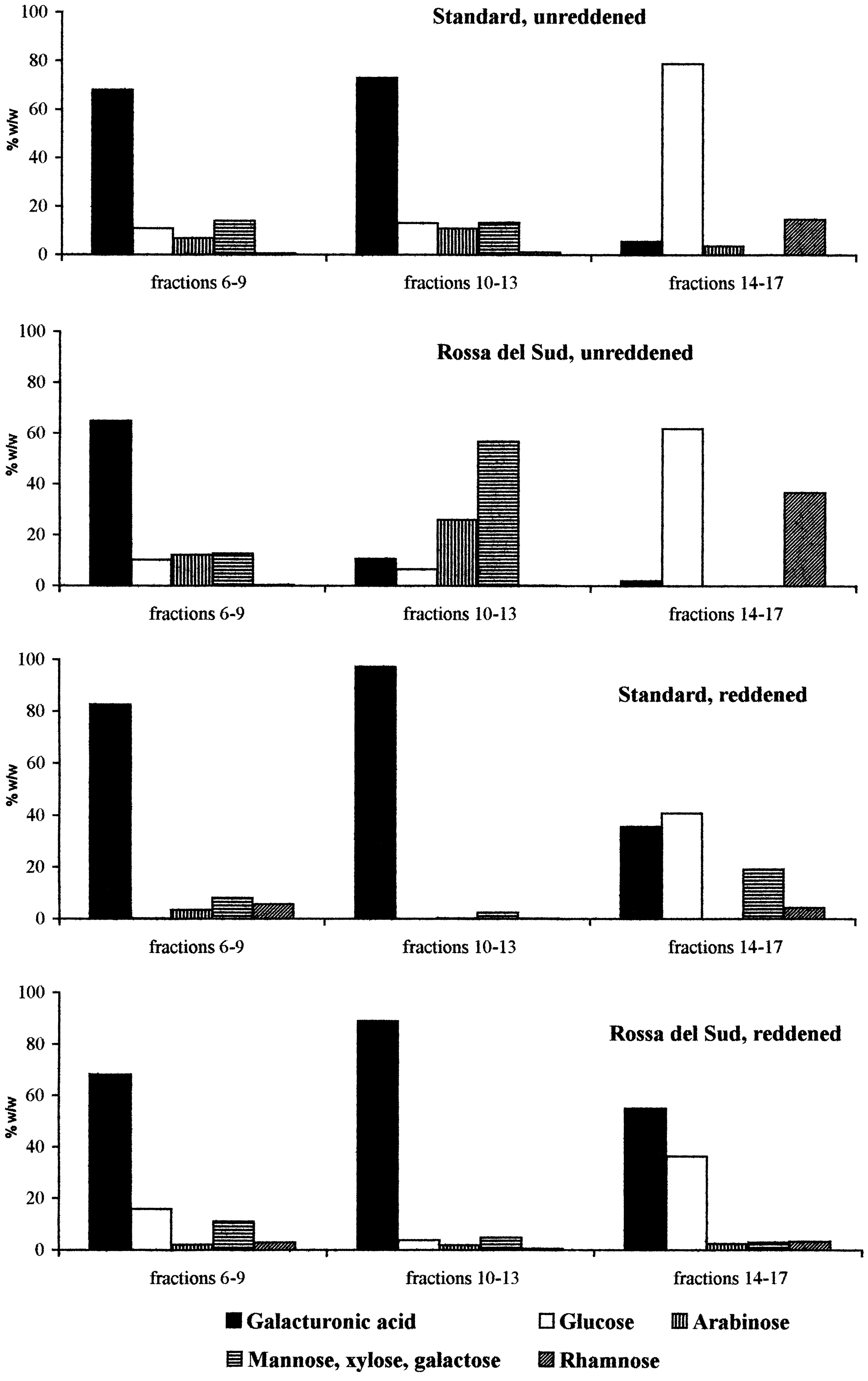

Fig. 3. Changes in galacturonic acid content and neutral sugars (% w/w on total content of each cluster) during the elution of OX-DMSO soluble pectin from ethanol-insoluble residue of 'Annurca' apple.

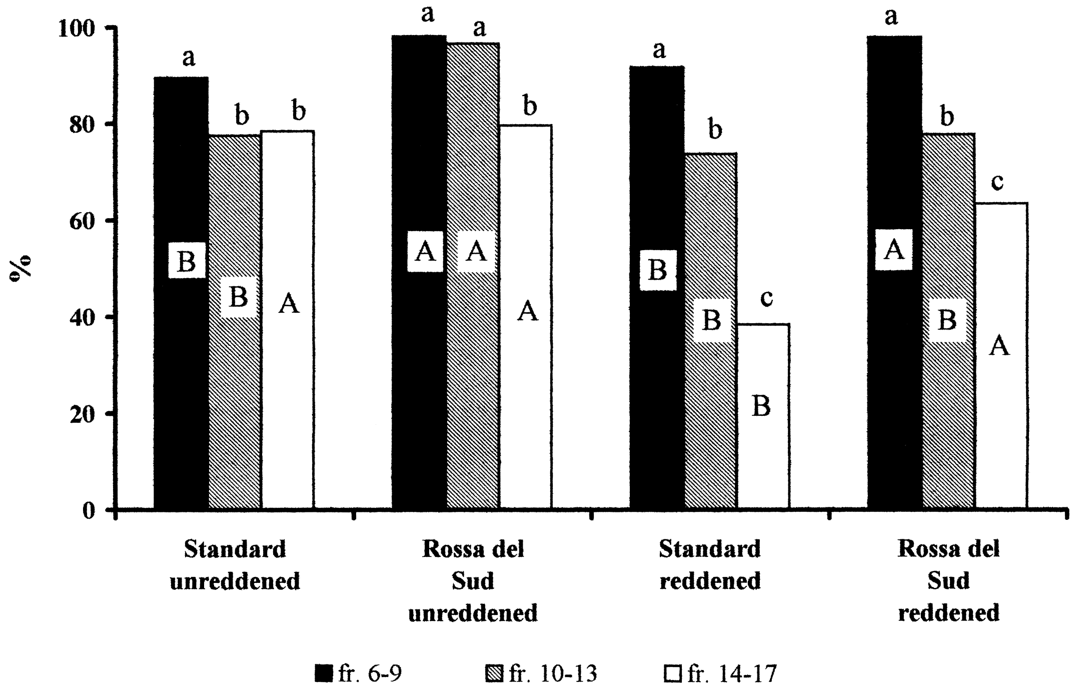

Fig. 4. Changes in esterification index (EI) in each cluster of unreddened and reddened samples of ‘Standard’ and ‘Rossa del Sud’ clones. The data with different letters are statistically different ($P > 0.05$), capital letters among same cluster for different samples, small letters among the different clusters in each sample.

conditioning in a closed 3 ml vial; 2 ml of each fraction were treated with 0.1 ml of 1 N NaOH at 80°C for 1 h. A 30 m Carbowax 20M column was used, programmed at 40°C for 5 min; then 30°C/min and 150°C for 5 min, injector was at 230°C, flame ionization detector (FID) was at 250°C. The esterification index (EI) was determined by the equivalent ratio of the methanol to the galacturonic acid content. The evaluation of the EI was made by considering the 3 clusters.

2.5. Headspace extraction

Glass jars (3 l) were used for sample collection (about 1 kg each of intact fruits); the jars were fitted with teflon lids having two gas ports, and were equilibrated for 15 min prior to sample collection with a stream of purified compressed air flowing at 200 ml min^{-1}. Blank analyses conducted with empty jars ensured there were no trace contaminants. Each dynamic headspace sampling was conducted for 2 h in a flow of purified air of 600 ml min^{-1} . Volatile compounds of each sample were collected onto 150 mg of 20–40 mesh activated coconut charcoal cartridges packed in a glass tube (0.6 cm id×7 cm) attached at the outlet port on the jar lid. Headspace components were subsequently desorbed from the trap using 500 µl of CH_2Cl_2 in 3-ml teflon-fitted vials, shaken for 40 min at room temperature, and then decanted at 0° C. The extract was concentrated 10-fold in a Micro-Kuderna-Danish apparatus at room temp and press, and directly introduced into the gas chromatography–mass spectrometry (GC–MS) and GC–FID systems.

2.6. GC–MS and GC–FID

Volatile components were separated on a GC–MS apparatus using a 60 m×0.25 mm DB-1 column (0.25 µm film thickness) through a split-splittless injector and temperature programmed as follows: 50°C for 5 min, increased to 200°C at 2°C min^{-1}, and then at 200°C for 5 min; carrier was He at 1 ml min^{-1}. The injector temperature was 200°C, that of the transfer-line was 240°C. Mass spectra were obtained at 70 eV with a quadrupole mass spectrometer. The injection volume was 0.5 µl at a split ratio 1:20. Identification was based on comparison of spectra by library match (National Bureau of Standards, Washington, DC). Authentic standards and relative retention times were used to confirm these identities.

Headspace components were also separated by GC–FID using a 30 m×0.53 mm Carbowax 20 M (1.00 µm film thickness) wide-bore column and temperature programmed as follows: 50°C for 5 min, increased to 200°C at 3°C min^{-1} and then at 200°C for 5 min; the injection port was equipped with a PTV apparatus, programmed at 50°C for 0.5 min, increased at 240°C at 270°C min^{-1} and then kept at 240°C for 15 min; the FID was at 250°C; carrier gas was He at 3 ml min^{-1}. The injection volume was 4 µl at a split ratio 1:15. The identification of compounds was based on the comparison of relative retention times with those of the same standards used for GC–MS. A different type of column was used for MS and FID determinations in order to have two different responses, avoiding eventual problems of identification derived from coelution phenomena. The estimates of concentrations of the head-space compounds were made by GC-FID, using response factors determined from the standards combined with fruit weight and air flow rate through the sampling jars. Concentrations are, therefore, expressed as amount per unit weight per unit time, i.e. $\text{ng kg}^{-1}\ \text{h}^{-1}$.

3. Results and discussion

3.1. Pectin composition

Apples were harvested at an acceptable maturity for commercial use (Table 1). The two clones show differences at harvest and after reddening; as we expected, ‘Standard’ presents, at harvest, higher firmness, and greener background, demonstrating a late ripening stage. The same differences are maintained after reddening: clone ‘Rossa del Sud’ shows a better red colour (a^* value), accompanied by an higher increase during reddening with respect to ‘Standard’. The firmness value decreases during the reddening: about 40 Newtons for ‘Standard’, 30 for ‘Rossa del Sud’.

The literature data affirm that one of the major contributors to intercellular adhesion and the consequent firmness is the chelator-soluble pectin that is derived from the middle-lamella (Knee & Bartley, 1981; Selvedran, 1985, Van Buren, 1991). However, the water-soluble pectin plays a fundamental role in the softening of the fruits during ripening (Gross, 1984). For this reason, both types of pectin were examined together. Their solubilisation was difficult, so a small aliquot of DMSO was added to the water solution of potassium oxalate in order to facilitate the extraction of the pectin from the EIR, and to maintain the solubilisation during chromatographic separation. Thus, the pectin examined was defined as OX-DMSO-soluble pectin.

The percentage of EIR in the fruit pulp, calculated on fresh weight (Table 2), was higher at harvest, and it became lower after reddening in both clones. After harvest, the yield in EIR was about 5% while, after reddening, this percentage became lower, about 3% in both clones. Consequently, the percentage of OX-DMSO-soluble pectin shows the same evolution as the EIR, while the ratio OX-DMSO pectin to EIR is about 30% at harvest and 25% after reddening. It seems clear that the reddening causes a loss of structural polysaccharides in both EIR and OX-DMSO-soluble pectin.

The elution profiles of OX-DMSO-soluble pectin (Figs. 1, 2) show great changes between unreddened fruits and reddened ones; small differences are noted between the clones. At harvest (unreddened fruits), there is a greater presence of polymers of low MW (15 000, fractions 14 and 15), compared to those of reddened fruits. The pulp of reddened fruits shows an increase of the polymers of large size (fractions 7 and 8), eluting close to the exclusion volume of the column. The elution profiles show some differences among the clones. In unreddened fruits of ‘Rossa del Sud’, an early ripening clone with respect to ‘Standard’, the presence of units of low MW (fractions 14 and 15) is reduced (Fig. 1). In these fruits, the clone ‘Rossa del Sud’ shows two small peaks at intermediate MW (fractions 9 and 12). In ‘Standard’ these two peaks are less evident. After reddening, a great difference is noted: the clone ‘Rossa del Sud’ shows a fragment in fraction 8 that is absent in

Table 3
Volatile substances emanated by “Annurca” fruits, identified by GC–MS and GC–FID

Peak No.	R_t (GC–MS)	R_t (GC–FID)	Aroma compounds[a]
Alcohols			
9	nd[b]	12.16	Butanol
29	7.33	nd	3-Penten-2-ol
30	9.03	nd	2-Methylbutanol
13	10.09	15.30	Pentanol
20	15.77	22.27	Hexanol
26	32.03	31.60	Octanol
Esters			
1	nd	6.30	Ethyl propanoate
2	nd	7.03	Propyl acetate
3	11.71	7.58	Ethyl butanoate
4	12.34	8.89	Butyl acetate
6	14.66	9.76	Ethyl 2-methylbutanoate
5	15.34	9.23	Isobutyl propanoate
7	17.58	10.90	Propyl butanoate
8	18.27	11.52	Butyl propanoate
10	21.91	13.25	Isobutyl butanoate
12	24.83	15.03	Butyl butanoate
14	25.19	16.04	Ethyl hexanoate
15	26.19	17.99	Hexyl acetate
17	32.51	20.10	Pentyl butanoate
18	33.77	21.35	Hexyl propanoate
19	37.16	22.45	Isobutil hexanoate
22	40.35	24.92	Hexyl butanoate
23	40.68	25.75	Ethyl octanoate
24	43.78	27.02	Hexyl isovalerate
25	47.25	28.49	Pentyl hexanoate
27	54.46	33.58	Hexyl hexanoate
28	54.97	35.16	Octyl butanoate
Other compounds			
11	23.69	13.55	6-Methyl-5-hepten-2-one
16	31.66	19.33	Octanal
21	34.49	23.97	Nonanal
31	44.69	40.97	δ-Octalactone

[a] Compounds identified by comparison of mass spectra and GC retention data from fruit components with those from authentic standards.

[b] nd, not detected

'Standard' (Fig 2). It seems that the presence of this fragment might be associated with the more advanced ripening stage of 'Rossa del Sud'.

The differences in pectin composition were analyzed considering three clusters of fractions, that reflected three zones of the elution profile of different MW: fractions 6–9 for high MW (near the exclusion volume), fractions 10–13 for medium MW (about 100 000) and fractions 14–17 for low MW (about 15 000). Fig. 3 shows the changes in galacturonic acid and neutral sugar contents in the three clusters of fractions of unreddened and reddened samples of both clones. The percentage of galacturonic acid in unreddened clones is about 60% in fractions 6–9 and becames very low (below 10%) in fractions 14–17; this value becomes high in reddened fruits, with a peak in fractions 10–13 (about 80%) and a noticeable amount in fractions 14–17 (40–50%). The fractions 14–17 are characterized by a high content of glucose (60–80%) and rhamnose (20–40%) in unreddened apples while, in reddened fruits, these amounts

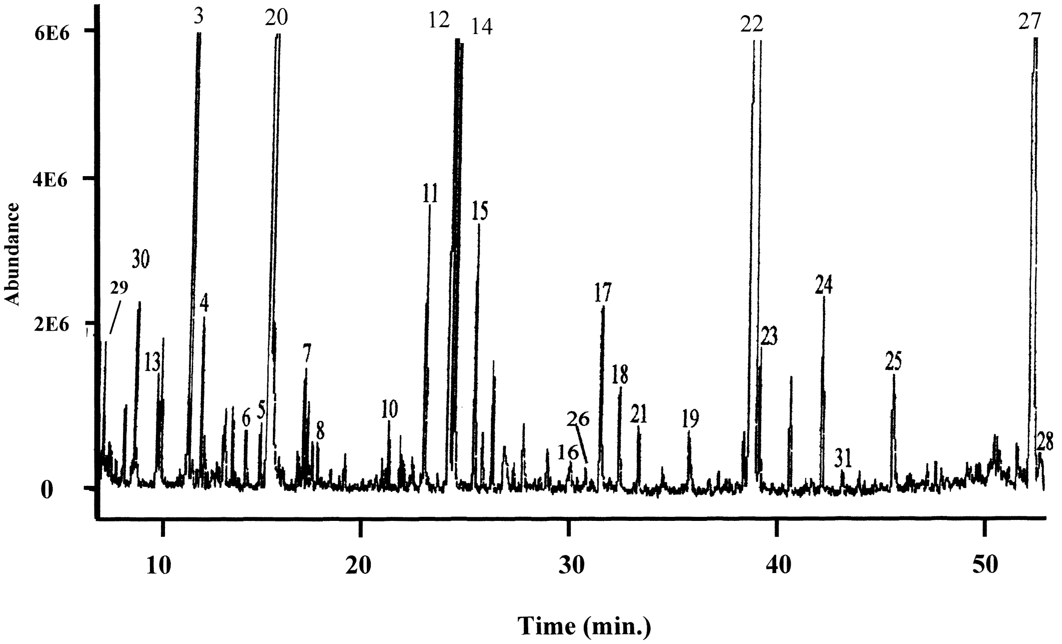

Fig. 5. Characteristic GC–MS chromatogram from 'Annurca' fruits' headspace (substances identified by GC–MS are listed in Table 3).

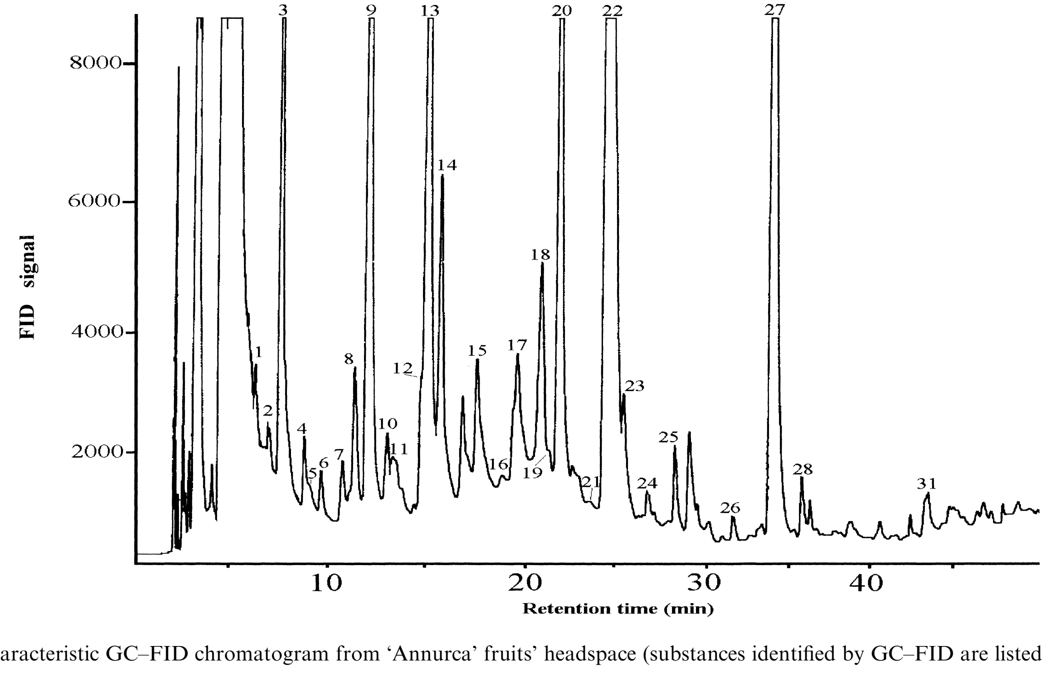

Fig. 6. Characteristic GC–FID chromatogram from 'Annurca' fruits' headspace (substances identified by GC–FID are listed in Table 3).

are lower, especially for rhamnose. The role of rhamnose residues, that are interspersed in the chains of polygalacturonic acid, is to form side chains with different amounts of neutral sugar residues. It may be possible that the high firmness in unreddened fruits could be related to the high number of potential branch sites. The difference between the unreddened clones, is noted especially in fractions 10–13: the contents of arabinose and mannose, xylose and galactose are higher in 'Rossa del Sud'. As regards the reddened clones, the glucose is present in fractions 6–9 of 'Rossa del Sud' and practically absent in 'Standard'. The mannose, xylose and galactose are well represented in fractions 14–17 of 'Standard' clone with respect to 'Rossa del Sud'. Examining the elution profiles, it seems possible that the low MW fractions represented starch, but: (1) other sugars than glucose were detected; (2) the single fractions didn't react with the Lugol solution; and (3) the EIR treated with a solution of amyloglucosidase (3.2.1.3), washed with ethanol 75% and then extracted with OX-DMSO did not show the diminution of the peak at 15 000 MW.

The OX-DMSO-soluble pectin of 'Annurca' apple are in a highly esterified form, especially in the high MW fragments (6–9, Fig. 4). This index decreases strongly in fractions 14–17: this is noted in all the samples analyzed except for 'Standard' unreddened. The reddening process make the EI lower in the low MW fractions (14–17)

Table 4
Amounts of volatiles emanated by Annurca, 'Standard' clone (mean of two determination each using about 1000 g fruit)

Peak No.	R_t	Aroma compounds[a]	At harvest		After reddening	
			M±S.E. (ng kg^{-1} h^{-1})[b]	Percent of total aroma	M±S.E. (ng kg^{-1} h^{-1})[b]	Percent of total aroma
Alcohols						
9	12.16	Butanol	15.3±0.9	9.7	1.17±1.6	5.1
13	15.30	Pentanol	12.02±2.6	7.6	95.92±2.8	4.2
20	22.27	Hexanol	6.29±1.1	4.0	111±9.2	4.9
26	31.60	Octanol	nd[c]	3.66±0.4	0.2	
Total alcohols			33.6±3.1	21.3	327± 4.2	14.3
Ester						
1	6.29	Ethyl propanoate	0.02±0.0	0.01	22.50±0.1	1.0
2	7.03	Propyl acetate	nd		nd	
3	7.58	Ethyl butanoate	nd		nd	
4	8.89	Butyl acetate	nd		2.64±0.5	0.1
5	8.92	Isobutyl propanoate	nd		nd	
6	9.76	Ethyl 2-methylbutanoate	0.44±0.2	0.3	3.56±0.8	0.2
7	10.90	Propyl butanoate	1.29±0.2	0.8	6.15±1.2	0.3
8	11.52	Butyl propanoate	nd		nd	
10	13.25	Isobutyl butanoate	nd		53.91±10.11	2.4
12	14.99	Butyl butanoate	nd		21.86±3.7	1.0
14	16.04	Ethyl hexanoate	0.12±0.1	0.1	325±22.3	14.2
15	17.99	Hexyl acetate	0.15±0.1	0.1	16.02±3.6	0.7
17	20.10	Pentyl butanoate	8.98±2.7	5.7	23.6±6.6	1.0
18	21.35	Hexyl propanoate	2.96±0.1	1.9	43.76±0.1	1.9
19	21.74	Isobutil hexanoate	nd		nd	
22	24.92	Hexyl butanoate	84.6±10.3	53.6	115±37.8	50.1
23	25.75	Ethyl octanoate	0.01±0.0	0.01	2.76±0.1	0.1
24	27.03	Hexyl isovalerate	0.02±0.0	0.01	2.96±0.7	0.1
25	28.49	Pentyl hexanoate	nd		9.39±0.1	0.4
27	33.58	Hexyl hexanoate	25.64±4.8	16.2	242±14.9	10.6
28	35.16	Octyl butanoate	0.01±0.0	0.01	1.00±0.3	0.04
Total Esters			124±5.4	78.7	1922±8.2	84.2
Other compounds						
11	13.55	6-Methyl-5-hepten-2-one	nd		20.82±2.6	0.9
16	19.33	Octanal	nd		6.03±1.0	0.3
21	23.97	Nonanal	nd		3.80±0.7	0.2
31	40.87	δ-Octalactone	0.03±0.0	0.02	3.76±0.8	0.2
Total			158±15.2	100.0	2284±114.9	100.0

[a] Compounds identified by GC retention data from fruit components

[b] Values are normalized per unit weight per unit time due to collection via dynamic headspace sampling using a flow system.

[c] nd = not detectable

in the ‘Standard’ clone and in fractions 10–13 for ‘Rossa del Sud’. ‘Rossa del Sud’, unreddened, shows a higher EI in fractions 6–9 and 10–13 with respect to ‘Standard’ unreddened. According to the literature data (Knee, 1978; Van Buren, 1991) the different firmness values between the unreddened clones (Table 1) may be ascribed to pectins with smaller EI, allowing the free carboxyl groups to increase their interactions.

3.2. Total aroma analysis

The volatiles identified from the headspace of intact fruits represent those responsible for the ‘initial fragrance’ of fruit smell, which is a characteristic of this fruit. Using the dynamic headspace, only the compounds with high volatility can be detected; additional compounds with insufficient volatility to evaporate from intact fruit may be extracted using destructive sampling techniques (i.e. steam distillation, vacuum extraction; Mattheis et al., 1992).

Thirty-one aroma compounds produced by ‘Annurca’ fruits were identified by GC–MS and GC–FID (Table 3). Characteristic chromatograms obtained from 10-fold concentrated reddened samples are shown in Fig. 5 (GC–MS) and Fig. 6 (GC–FID). In both clones (at harvest and after reddening), the majority of volatiles were

Table 5
Amounts of volatiles emanated by Annurca, ‘Rossa del Sud’ clone. (mean of two determination each using about 1000 g fruit)

Peak No.	R_t	Aroma compounds[a]	At harvest		After reddening	
			M±S.E. (ng kg^{-1} h^{-1})[b]	Percent of total aroma	M±S.E. (ng kg^{-1} hr^{-1})[b]	Percent of total aroma
Alcohols						
9	12.16	Butanol	43.66±1.2	12.4	186±15.6	8.3
13	15.30	Pentanol	35.33±3.4	10.0	136±22.8	6.1
20	22.27	Hexanol	16.15±2.8	4.6	146±5.0	6.6
26	31.60	Octanol	nd[c]		9.54±0.1	0.4
Total alcohols			95.2±6.3	27.0	477±2.1	21.4
Esters						
1	6.29	Ethyl propanoate	0.06±0.0	0.02	18.8±0.6	0.8
2	7.03	Propyl acetate	nd		3.61±0.1	0.2
3	7.58	Ethyl butanoate	nd		4.16±0.1	0.2
4	8.89	Butyl acetate	nd		2.89±0.3	0.1
5	8.92	Isobutyl propanoate	nd		3.49±0.2	0.2
6	9.76	Ethyl 2-methylbutanoate	0.12±0.1	0.03	1.56±0.1	0.1
7	10.90	Propyl butanoate	6.97±0.2	2.0	103±0.3	0.5
8	11.52	Butyl propanoate	nd		nd	
10	13.25	Isobutyl butanoate	nd		6.35±2.0	0.3
12	14.99	Butyl butanoate	nd		18.9±4.9	0.8
14	16.04	Ethyl hexanoate	0.27±0.1	0.1	202±1.5	9.0
15	17.99	Hexyl acetate	0.36±0.1	0.1	10.30±8.5	0.5
17	20.10	Pentyl butanoate	53.04±3.5	15.1	27.6±3.7	1.2
18	21.35	Hexyl propanoate	4.01±0.7	1.1	34.4±4.4	1.5
19	21.74	Isobutil hexanoate	nd		nd	
22	24.92	Hexyl butanoate	134.11±18.8	38.1	1113±12.3	49.9
23	25.75	Ethyl octanoate	0.04±0.0	0.01	1.62±0.1	0.1
24	27.03	Hexyl isovalerate	21.92±0.0	6.2	7.99±0.1	0.4
25	28.49	Pentyl hexanoate	nd		4.39±0.3	0.2
27	33.58	Hexyl hexanoate	33.63±5.7	9.6	2445±17.4	11.0
28	35.16	Octyl butanoate	0.30±0.0	0.1	0.35±5.8	0.02
Total esters			255±9.8	72.5	1716±22.0	76.9
Other compounds						
11	13.55	6-Methyl-5-hepten-2-one	nd		26.12±0.7	1.2
16	19.33	Octanal	nd		5.80±0.4	0.3
21	23.97	Nonanal	nd		3.09±0.1	0.1
31	40.87	δ-Octalactone	1.95±0.3	0.6	3.84±0.4	0.2
Total			352±25.6	100.0	2232±35.6	100.0

[a] Compounds identified by GC retention data from fruit components.

[b] Values are normalized per unit weight per unit time due to collection via dynamic headspace sampling using a flow system.

[c] nd, not detectable.

esters and alcohols, with the addition of two aldehydes (octanal and nonanal), one ketone (6-methyl-5-hepten-2-one) and one lactone (δ-octalactone). The differences among the samples were mainly quantitative.

Tables 4 and 5 report the quantitative evaluation of aroma compounds made by GC–FID in unreddened and reddened samples. Generally, there is a great increase of total aroma, caused by the reddening in both clones, with a higher increase in 'Standard', probably due to the lower ripening stage.

Esters were the most abundant substances found and show percentages, on the total volatiles, of 79% at harvest and 84% after reddening in 'Standard'. The same volatiles were present in 'Rossa del Sud' at lower percentages (72 and 77% by mass). Reddened fruits show an increase in the percentage of esters in the total aroma, compared to unreddened ones, probably improving the organoleptic aspect of aroma. Butanoates are characteristic of red-skinned apples (Paillard, 1979), and they were the dominant compounds in both clones, including a homologous series with alcohol moieties at carbon atoms 2, 3, 4, 5, 6 and 8. Hexyl butanoate, the predominant compound, is augmented about 10-fold during the reddening process. Low quantities of acetates (butyl and hexyl acetates) were also observed. They are the characteristic compounds of yellow-skinned apples (i.e. Golden Delicious) together with the propanoates (ethyl, isobuthyl, *n*-butyl, hexyl; Paillard, 1979).

Branched chain esters, especially isobutyl esters, derived from branched amino-acid biosynthesis pathway (Sanz, Olias & Perez 1997) are detectable only in reddened fruits, comprising about 2.4 and 0.5% of the volatile mass, for 'Standard' and 'Rossa del Sud', respectively. Ethyl-2-methyl-butanoate, known for its organoleptic threshold value of 100 ng kg^{-1} (Flath, Black, Guadagni, MacFadden & Schulz, 1967) was found in reddened samples (3.56 for 'Standard' and 1.56 ng kg^{-1} h^{-1} for 'Rossa del Sud') like other apple cultivars (Dimick & Hoskin, 1983; Van Straten, 1983); at harvest, these values were lower (0.44 and 0.12 ng kg^{-1} h^{-1}, respectively). Considerable quantities of hexanoates and octanoates, partial products of β-oxidation of fatty acids (Sanz et al., 1997) were found in the aroma composition. The amount of ethyl hexanoate ranges from 0.12 to 325 and 0.27 to 202 ng kg^{-1} h^{-1} in 'Standard' and 'Rossa del Sud', respectively.

The absolute amounts of the alcohols increase in reddened clones (for 'Standard' about 10 fold). However, the percentage contributions of the alcohols to the overall volatiles, during reddening, decrease (21–14% in 'Standard; 27–21% in 'Rossa del Sud'), they include *n*-butanol, *n*-pentanol, *n*-hexanol and *n*-octanol; whereas 3-penten-2-ol and 2-methylbutanol were detected only by GC–MS. The dominant alcohol was *n*-butanol; *n*-octanol was detected only in reddened apples. The amounts of *n*-pentanol are surprisingsly high, if compared with other cultivars of apples (Dimick & Hoskin, 1983; Van Straten, 1983): the high level of this compound could be considered as a marker of 'Annurca' apple aroma.

The compounds identified in this work have previously been found in other cultivars of apples (Dimick & Hoskin, 1983; Van Straten, 1983) with the exception of δ-octalactone, so it may be possible to distinguish the cv Annurca from others by the presence of this compound. At harvest, this compound is present at 0.03 ng kg^{-1} h^{-1} in 'Standard' and 1.95 in 'Rossa del Sud'. After reddening, δ-octalactone reaches about 3.8 ng kg^{-1} h^{-1} in both clones.

4. Conclusions

4.1. Texture properties

There is a decrease of firmness in the reddened fruits for both clones; at harvest this difference is also due to the different ripening stage of the clones. The decrease of firmness after reddening may be related to the increase of large-size polymers in OX-DMSO pectins and to the decrease of low MW fragments. At harvest, the two clones differ in low MW fragments, reflecting the different firmness values: the higher the firmness, the higher the amount of low MW fragments. Polymers of intermediate MW are present in both unreddened and reddened samples of 'Rossa del Sud'.

The large-size polymers are mainly composed of galacturonic acid and, the low MW fragments are composed mainly of glucose and rhamnose, this is evident in unreddened samples.

The reddening process shows an increase of galacturonic acid in all the clusters, while the amounts of rhamnose, responsible for the branch sites, and of glucose, decrease in low MW clusters. If low MW clusters are considered as 'hairy' regions of pectic molecules and responsible for the intercellular adhesion, their diminution during the reddening can be associated with the lack of potential linkages between the cell walls: this fact could be related to the fruit softening.

4.2. Aroma composition

In both clones, the reddening process causes an increase in the quantity of total aroma; the main components of aroma, namely esters and alcohols, show an increase in absolute content. The quality of aroma could also be enhanced by evaluations of the contributions of esters and alcohols to the overall aroma: esters increase, alcohols decrease.

The flavour of 'Annurca' apples presents the following features:

1. the presence, as a marker, of high amounts of *n*-pentanol, and the consequent presence of pentyl

esters (butanoate and hexanoate);
2. the presence of high amounts of butanoates;
3. the presence of branched-chain esters (isobutyl esters), propanoates and hexanoates; and
4. the presence, as a marker, of a lactone, unusual for apple aroma (δ-octalactone).

These features could signify that *n*-pentanol and δ-octalactone have a unique role in the characteristic aroma composition of this apple cultivar. Further studies based on GC olfactometry should be carried out to clarify this concept.

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