

Full Length Research Paper

Isolation and determination of major anthocyanin pigments in the pericarp of *P. communis* L. cv. 'Red Du Comices' and their association with antioxidant activity

Wen-jiang Huang^{1,2}, Shao-ling Zhang¹, Gai-hua Qin¹, Le Wenquan³ and Jun Wu^{1*}

¹College of Horticultural Science, Nanjing Agricultural University, Nanjing 210095, China.

²College of life Science, Anhui Normal University, Wuhu 241000, China.

³Changli institute of Pomology, Changli Hebei 06600, China.

Accepted 29 February, 2012

The anthocyanins in the fruit skin of *Pyrus communis* cv. 'Red Du Comice' were isolated, identified and quantified using high-performance liquid chromatography/diode array detection (HPLC/DAD) and HPLC/electrospray ionization/mass spectrometry (HPLC/ESI/MS). The individual anthocyanins were identified by comparing their mass spectral data and retention times with those of standards and published data. Cyanidin-3-galactoside was the major compound, taking up to 93% of the total anthocyanin content. The content of Cyanidin-3-galactoside reached 23.7 ± 3.2 mg 100 g⁻¹ fresh weight, followed by the eighth peak (2.14%) and Cyanidin-3-glucoside (1.23%). Pelargonidin-3-rutinoside (pg-3-rutinoside) was identified for the first time in *P. communis* L. 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays showed that the extract from the fruit peel of 'Red Du Comice' contained anthocyanins and possessed high antioxidant capacity.

Key words: *Pyrus communis*, anthocyanin, high-performance liquid chromatography-electrospray ionization-mass spectrometry, antioxidant activity.

INTRODUCTION

Color is an important factor determining fruit outer quality which has been used successfully in the characterization of fruits (Gautier-Hion et al., 1985). This property has an important effect on overall acceptability to the consumer (Gamble et al., 2006). Anthocyanins are responsible for most of the red, blue and purple colors of fruits, vegetables, flowers and other plant tissues or products (Gould et al., 2002; Manetas, 2006; Steyn et al., 2002; Stintzing and Carle, 2004). The isolation and identification of anthocyanins are difficult as a result of their ability to undergo structural transformations and complex reaction. In addition, they are difficult to measure independently from other flavonoids as they have similar reaction characteristics. Paper and thin-layer chromatography have

traditionally been used for the identification of anthocyanins (Aneersen, 1985; Matysik and Benesz, 1991; Sherma, 2000; Timberlake and Bridle, 1976) but with the advance of analytical technology, analysis and identification of anthocyanins developed by using High performance liquid chromatography (HPLC) with mass spectrometry (MS) or with tandem MS (Bakker et al., 1986; Hong and Wrolstad, 1990). In recent years, HPLC coupled with MS has been becoming the standard method for identification and separation in most laboratories (Desai et al., 2010; Huang et al., 2009; Li et al., 2009; Zheng et al., 2010). HPLC can provide a faster and improved separation of complex compounds and allows their tentative identification based on their retentive characteristics. It is still difficult to obtain each reference compound for the same spectrum that represents the anthocyanins. Therefore, HPLC coupled with MS, ESI/MS are possibly the most powerful methods for identifying the structure of anthocyanins. These methods allow the

*Corresponding author. E-mail: wujun@njau.edu.cn. Tel: +86 25 84396485. Fax: +86 25 84396485.

sequential fragmentation of a given molecular ion and provide information on the identification of anthocyanins based on their ion fragment patterns (Wilson et al., 2005).

Several studies have been reported on the structural elucidation of anthocyanins by means of HPLC-DAD-MS. Using the HPLC-DAD-ESI-MS method, Lopes-da-Silva et al. (2002) discovered that strawberry extract contained three major anthocyanins, Pelargonidin-3-rutinoside (pg-3-rut), Pelargonidin-3-glucoside (pg-3-gluc), Cyanidin-3-glucoside (cy-3-gluc) and 12 minor anthocyanins, although identity could be only assigned to five of them as pg-3-acetylglucoside, cy-3-rutinoside, pg-3-malonylglucoside, pg-diglucoside, and cy-3-malonylglucosyl-1-5-glucoside, the latter three were a novel discovery in strawberry. So this technique has been shown to be appropriate for isolation and identification of complex mixtures. Many studies focused on the composition of anthocyanins in apple (DuPont et al., 2002; Shoji et al., 2006), strawberry (Aaby et al., 2007; Gil et al., 1997; Wu et al., 2006), blueberry (Gu et al., 2002; Prior et al., 2001), grape (Garca-Beneytez et al., 2003; Huang et al., 2009; Oliveira et al., 2010) and other fruits (Wu et al., 2006). Pear is one of the most important temperate fruits. Fruit skin color is a significant factor that affects the quality and customer acceptance. There is a few studies that have been conducted on pear phenolic compounds studies using liquid chromatography (LC) or liquid chromatography with mass spectrometric detection (LC-MS) have reported such polyphenols in pear materials (Francis, 1970; Muchuweti and Chikwambi, 2008; Oleszek et al., 1994).

This study aimed to isolate and identify the anthocyanin molecules responsible for fruit skin coloration in *P. communis* L. cv. 'Red Du Comices'.

MATERIALS AND METHODS

Plant samples

The fruit samples were picked at commercial maturity from Changli Institute of Pomology(Hebei, China), and transported to the laboratory immediately. Fruit free of defects and without evidence of mechanical damage were selected. Fruit skins were peeled and frozen in liquid nitrogen and kept at -70°C until being analysed.

Chemicals

HPLC-grade methanol and acetonitrile were obtained from Merck KGaA, Darmstadt, Germany. Formic acid, cyanidin-3-glucoside, cyanidin-3-galactoside, cyanidin-3-arabinoside, peonidin-3-glucoside, peonidin-3-galactoside and DPPH, 2,4,6-tripyridyl-s-triazine (TPTZ) were obtained from Sigma Chemical Co. All other chemicals used in this study were analytical grade.

Extraction of anthocyanins

The extraction of anthocyanins was performed according to Wu (Wu et al., 2006), with some modifications. 20 ml methanol with 1% (v/v) formic acid was added into 100 ml Erlenmeyer flask containing

1 g peel powder. Anthocyanins were extracted at 4°C for 60 min in dark environment; this procedure was repeated three times to collect the extract solution. The extraction was concentrated under vacuum at 30°C using a rotary evaporator until dryness. The dry extraction was dissolved in 5 ml of 1% formic acid in distilled water. About 1 ml of extracted solution was passed through a 0.45 µm millipore filter for analysis.

HPLC-MS analysis

A Waters LC-MSD 1525 series, equipped with a UV detector and Agilent Zorbax SB-C18 column was used. The solvents were (A) aqueous 2% formic acid, and (B) acetonitrile: water (1:1 v/v) containing 2% formic acid. The gradient was from 6 to 10% B for 4 min, from 10 to 25% B for 8 min, isocratic 25% B for 1 min, from 25 to 40% for 7 min, from 40 to 60% for 15 min, from 60 to 100% for 5 min, from 100 to 6% for 5 min, at a flow rate of 1.0 ml/min. Injection volumes were 15 µl, and the detection wavelength was 516 nm. MS conditions were as follows: ESI interface, positive ion model, 35 psi nebulizer pressure, 10 L/min dry gas flow rate, 350°C dry gas temperature and scans at m/z 150 to 1000. All analyses were duplicated.

Antioxidant capacity determined by FRAP

This procedure involved the reduction of ferric ion (Fe³⁺) to the ferrous ion (Fe²⁺) to a blue colored complex Fe²⁺/TPTZ in the presence of bioactive compounds (antioxidants) which was revealed as the increase of absorption at 593 nm (Benzie and Strain, 1999). Extracts was added to the FRAP reagent. The FRAP reagent contained 10 mM TPTZ in 40 mM HCl, 20 mM FeCl₃ and 300 mM acetate buffer (pH3.6) that was freshly prepared in the ratio of 1:1:10 and warmed 30 min at 37.8°C before and after adding of sample extract. The FeSO₄·7H₂O compound was used to prepare the increasing concentrations of standard solutions. Results were expressed as ascorbic acid equivalent antioxidant capacity (mg ascorbic acid/100 g fresh weight).

Antioxidant capacity determined by DPPH-radical scavenging activity (DPPH assay)

The ability to scavenge DPPH free radical was determined based on the method of Brand Williams (Brand-Williams et al., 1995), with minor modification. Briefly, reaction mixtures containing 25 µl extracts and 2 ml 6.25 × 10⁻⁵ M DPPH solution were prepared, mixed, and then reacted in the dark for 30 min. A control sample containing the same volume of solvent in place of extract was used to measure the maximum 2,2-diphenyl-1-picrylhydrazyl (DPPH) absorption. The absorbance at 517 nm was recorded to determine the concentration of the remaining DPPH. Results were expressed as ascorbic acid equivalent antioxidant capacity (mg ascorbic acid/100 g fresh weight).

RESULTS AND DISCUSSION

Test for anthocyanins

The red, purple and blue colors found in many plants are due to two classes of water soluble pigments: anthocyanins and betacyanins. The anthocyanins are flavonoids, a class of phenolic molecules that are synthesized through the Shikimic acid pathway and are widespread in the plant kingdom. Betalains, a group of

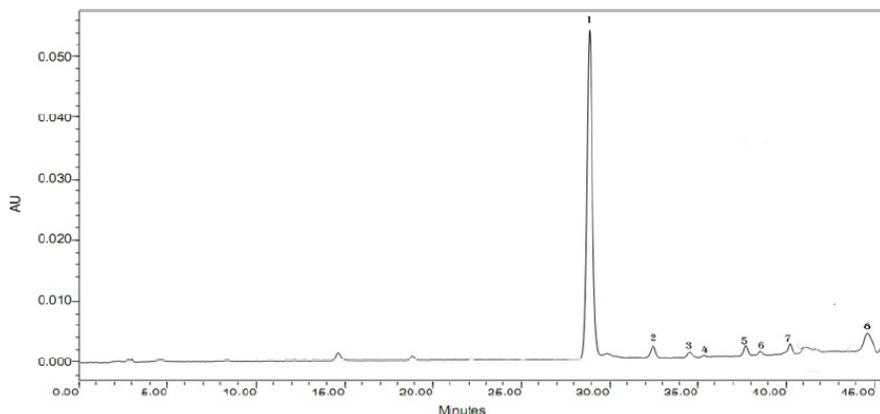


Figure 1. HPLC chromatograms of extract of 'Red Du Comice' pear peel of fruit (516 nm).

Table 1. HPLC/DAD and HPLC/ESI-MS of anthocyanins in fruit skin of 'Red Du Comice'.

Peak number	t_R (min)	$[M^+]$ m/z	MS^2 m/z	Compounds
1	28.82	449	287	Cyanidin-3-galactoside
2	32.42	449	287	Cyanidin-3-glucoside
3	34.50	419	287	Cyanidin-3-arabinoside
4	35.23	595	449/287	Cyanidin-3-rutinoside
5	37.64	579	433/271	Pelargonidin-3-rutinoside
6	38.51	463	301	Peonidin-3- galactoside
7	40.17	463	301	Peonidin-3- glucoside
8	44.52	549	287	Cyanidin-3-methylmalonylglucoside? Cyanidin-3-succinylglucoside? Cyanidin-malylrhamnoside?
Standard	28.76	449	287	Cyanidin-3-galactoside
Standard	32.33	449	287	Cyanidin-3-glucoside
Standard	34.30	419	287	Cyanidin-3-arabinoside
Standard	38.43	463	301	Peonidin-3- galactoside
Standard	40.1	463	301	Peonidin-3- glucoside

pigments that includes the betacyanins are indole-derived alkaloids and contain nitrogen. Pear peel extracts (100% methanol with 1% HCl) were tested for the presence of anthocyanins by observing pigment color under acidic or alkaline conditions by adding HCl or sodium hydroxide. 3 ml of extract and 3 ml HCl were mixed in a test-tube and then placed in boiling water bath for 5 min. The mixture was stable and did not lose color when boiled. A blue-green colour was observed when adding sodium hydroxide to the extract, which indicated the presence of anthocyanins in the extracts.

Identification of pear anthocyanins

Figure 1 showed the anthocyanin profile of the extract

from fruit skin of 'Red Du Comice' using the HPLC-DAD chromatograms at 516 nm. A total of eight anthocyanin compounds were identified by their elution order and by comparing the m/z of each anthocyanin molecule and its fragmentation to prepared standards and previous reports (Table 1). Five peaks (peaks 1, 2, 3, 4 and 8) showed fragment ions at m/z 287 in MS^2 analyses that could be tentatively identified as cyanidin derivatives. Peaks 1 and 2 showed identical molecular ions at m/z 499 and fragmentation patterns, but their retention time in the HPLC system were 28 and 32 min for peaks 1 and 2, respectively. Their elution order and MS characteristics suggest that they could be cyanidin-3-galactoside (Figure 2) and cyanidin-3-glucoside, respectively, which concurs with that found in previous studies (Cerezo et al., 2010) and coincides with the available standard used for

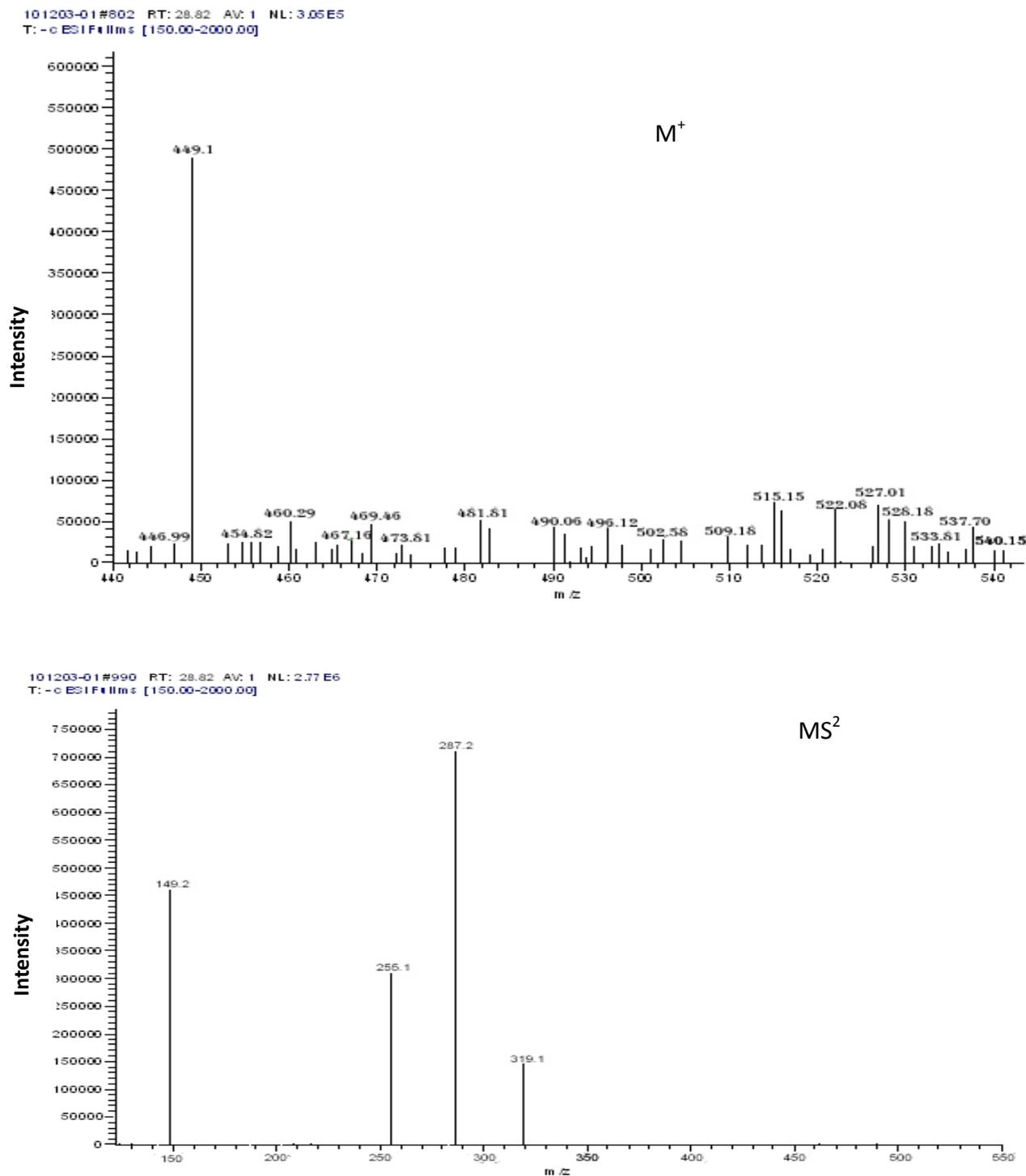


Figure 2. MS data of cyanidin -3-galactoside.

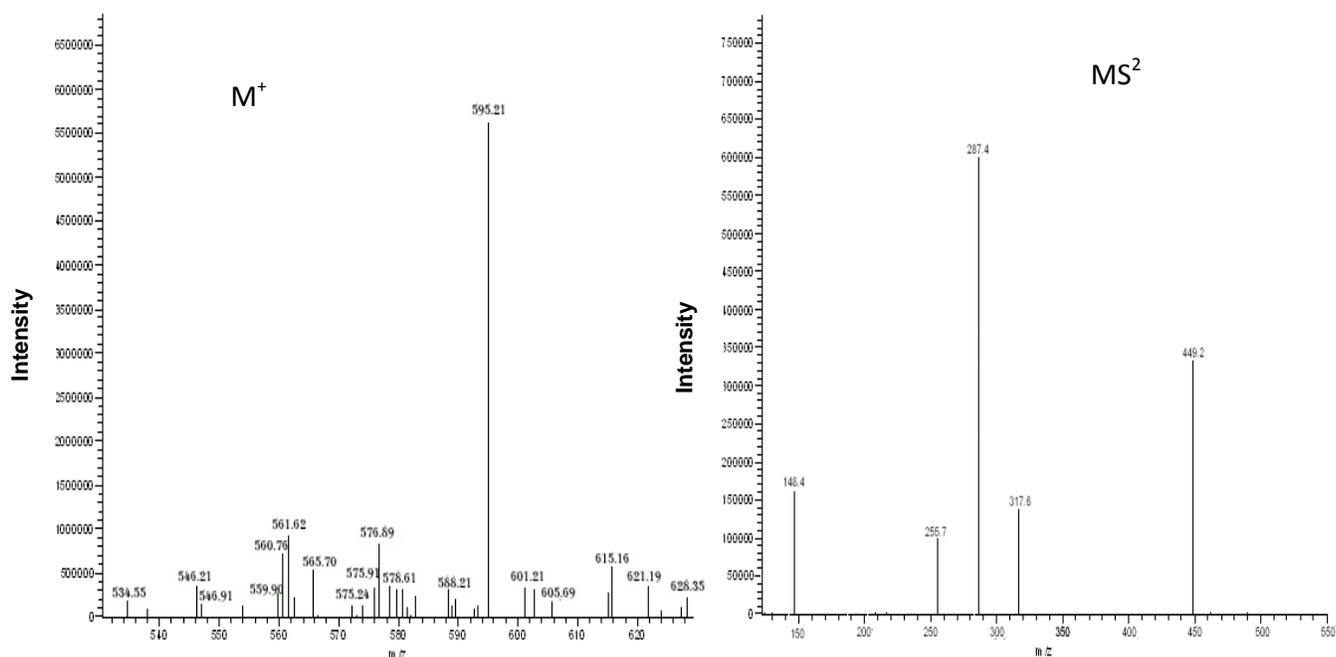
confirmation purposes (Table 2). Peaks 3 and 8 showed the same major cyanidin fragment ions at m/z 287, but different molecular ions at m/z 419 and 549, respectively. Peak 3 produced an MS pattern corresponding to cyanidin-3- arabinoside, which coincides with the confirmation marker. Peak 8 might correspond to the

methyl derivative of cyanidin-3-malonylglucoside. However, other structures that also match the molecular ion of peak 8 are cyanidin-malylrhamnoside and cyanidin-3-succinylglucoside (Da Silva et al., 2007). Unfortunately, no information could be obtained to confirm suggestions about its structure.

Table 2. Anthocyanins content and percentage in the extract of 'Red Du Comice' peel.

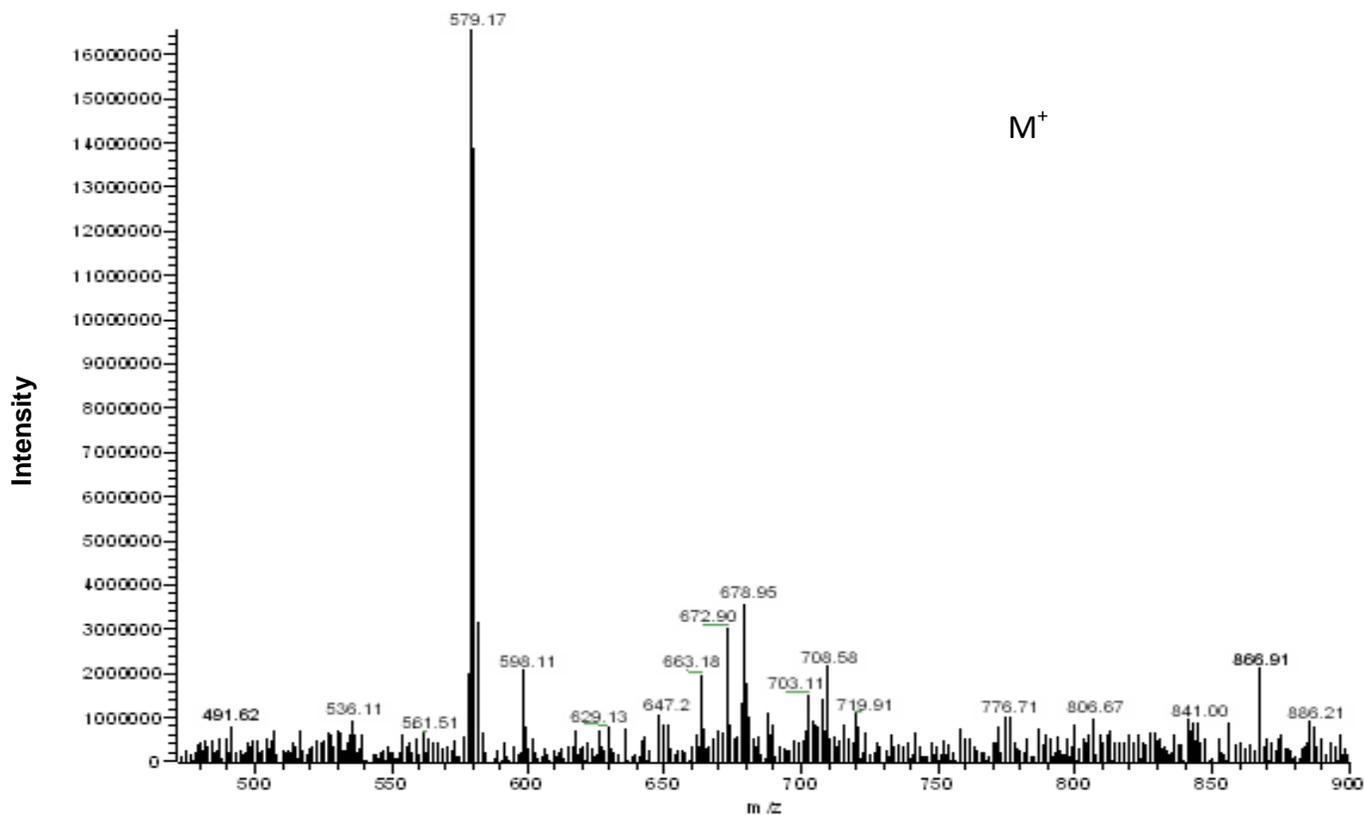
Compounds	Content ^a	% ^b
Cyanidin-3-galactoside	23.7±3.2	93.71
Cyanidin-3-glucoside	0.31±0.05	1.23
Cyanidin-3-arabinoside	0.03±0.00	0.12
Cyanidin-3-rutinoside	0.02±0.00	0.08
Pelargonidin-3-rutinoside	0.28±0.02	1.11
Peonidin-3- galactoside	0.19±0.03	0.75
Peonidin-3- glucoside	0.22±0.02	0.87
Peak 8	0.54 ±0.03	2.14

^a Individual anthocyanins were analyzed using HPLC-ESI-MS. Each anthocyanin was quantified in cyanidin equivalents (mg/100 g fresh weight). ^b Percentage of the total content.

**Figure 3.** MS data of cyanidin-3-rutinoside.

Peak 4 produced a fragment pattern matching cyanidin-3-rutinoside (Figure 3). Peaks 6 and 7 were assigned as Peonidin-3-galactoside (Figure 5) and Peonidin-3-glucoside, both with the mass spectrometric characteristics and elution orders matching the reference compounds and those that had previously been reported in grape (Huang et al., 2009). Peak 5 showed an $[M]^+$ at 579, which gave MS^2 fragments that were at m/z 271 and 433 and was identified as Pelargonidin-3-rutinoside (Figure 4). Due to the lack of corresponding reference compounds for Cyanidin-3-rutinoside, Pelargonidin-3-rutinoside and the compound representing peak 8, the identified anthocyanins were quantitated through a

calibration curve determined using cyanidin-3-glucoside. Other identified anthocyanins were quantitated through the calibration curves of corresponding reference compounds. Table 2 shows the individual concentration of the anthocyanins in the extracts of 'Red Du Comice' peel. Cyanidin-3-galactoside, the major compound, accounts for 93.7% of total anthocyanin which confirms in previous studies (Sanchez et al., 2003). Percentages of 94.2% for Cyanidin-3-galactoside and 4.5% for Cyanidin-3-rutinoside were found by Sanchez et al. (2003) in RedD'Anjou. It is followed by the compounds representing peak 8 (2.14%), Cyanidin-3-glucoside (1.23%), Pelargonidin-3-rutinoside (1.11%), Peonidin-3-glucoside (0.87%) and Peonidin-3-



101203-01#990 RT: 37.72 AV: 1 NL: 2.77 E6
T: -o ESIFilms [150.00-2000.00]

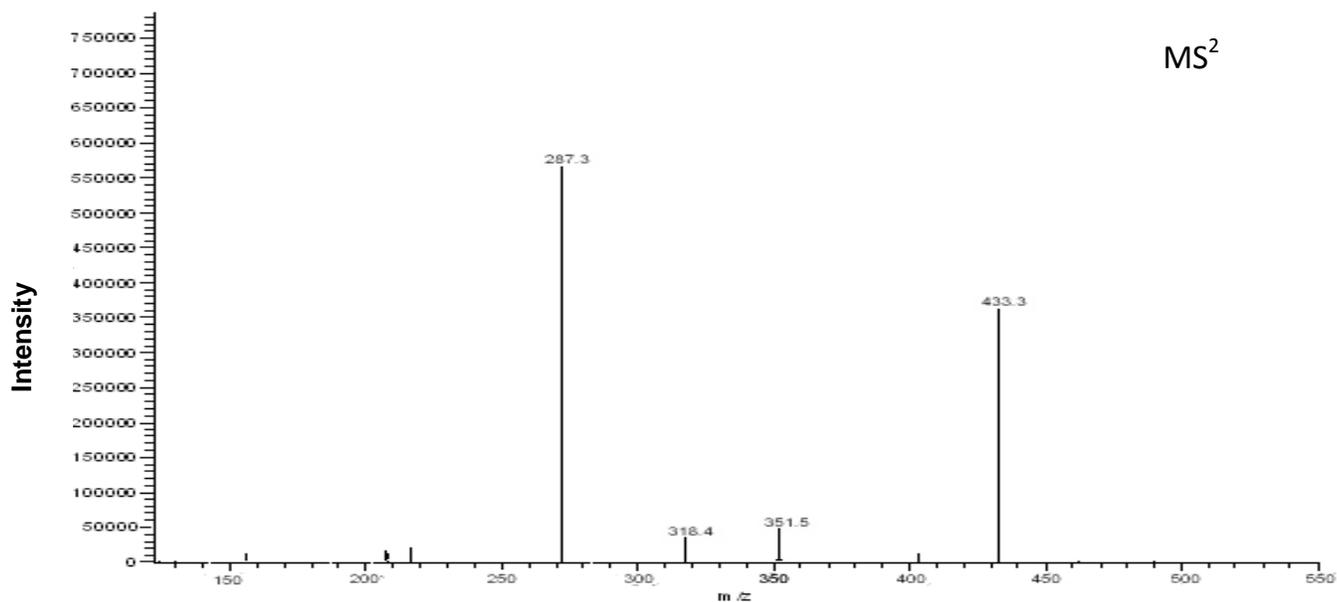


Figure 4. MS data of pelargonidin-3-rutinoside.

galactoside (0.75%). Muchuweti et al. (2008) considered that only one anthocyanidin present the pear peel according to paper chromatography and Uv-vis and

infrared-spectra. Cyanidin-3-o-galactoside, Quercetin-3-o-glucoside and Cyanidin-3-o-arabinoside were identified only in the 'Max Red Bartlett' fruit skin using HPLC-MS by

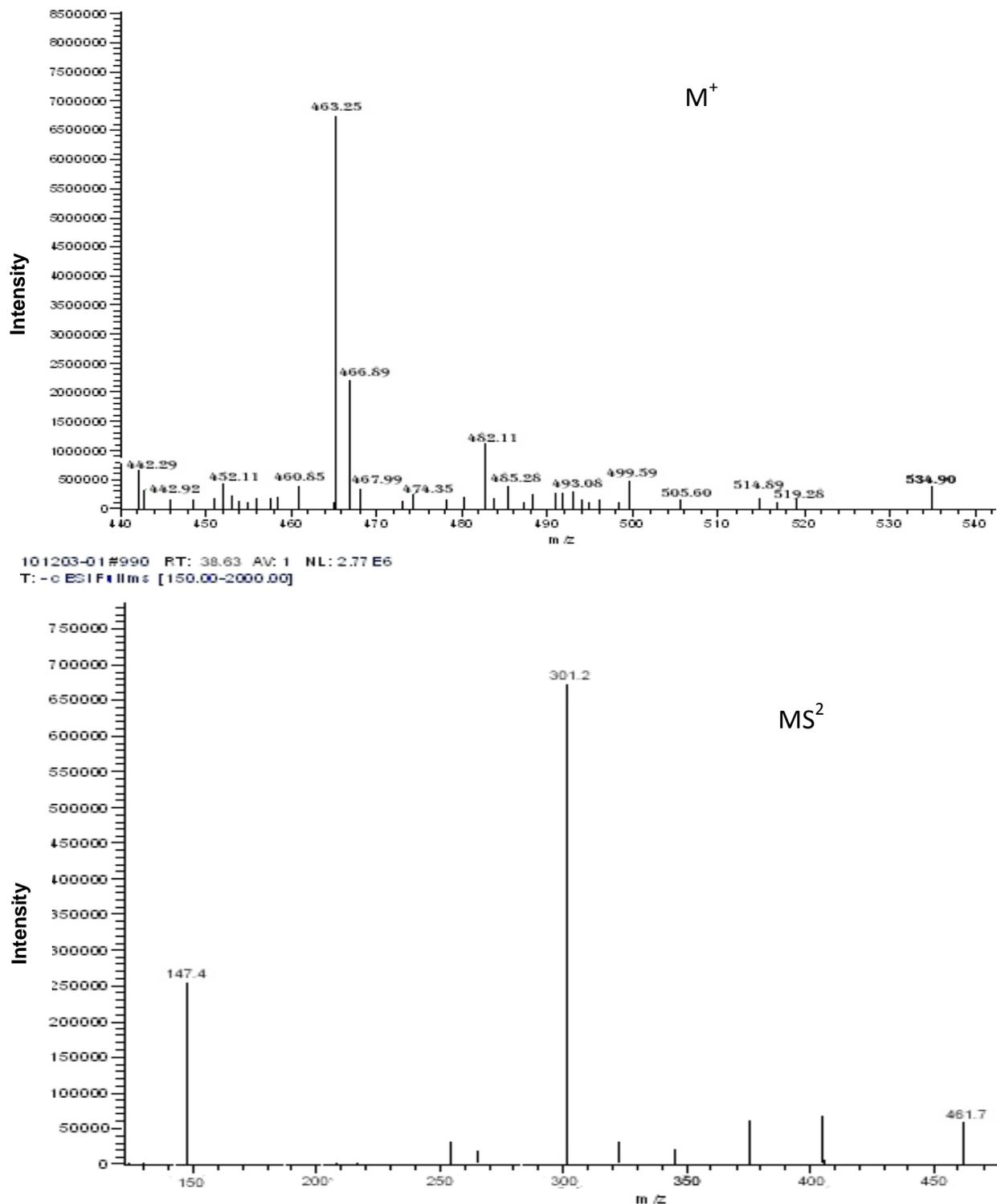


Figure 5. MS data of peonidin-3-galactoside.

Luca et al. (2010). According to Tamura's works (1995), most of anthocyanin contain Pelargonidin (Pg) as aglycone and Pg-3-rutinoside is commonly found in

strawberry (Ana et al., 2010). Pelargonidin-3-rutinoside has also been previously reported in Concord grape (Baublis et al., 1994), cranberry (Heinonen, 2007),

strawberry (Da Silva et al., 2007b), blackberry (Wrolstad, 1993), sweet cherry (Gao and Mazza, 1995) and black plum (Wu et al., 2006); but not in pear (Lin and Harnly, 2008; Sanchez et al., 2003). This study is tentatively proposing, for the first time, that Pelargonidin-3-rutinoside is present in pear.

Antioxidant capacity

The antioxidant capacity of the total anthocyanin obtained from fruit peel of 'Red Du Comice' was evaluated with the DPPH and FRAP test. The free radical scavenging activity determined by DPPH was $165.23 \pm 11.6 \text{ mg}/100 \text{ g}^{-1} \text{ FW}$ and the value of total antioxidant capacity determined by FRAP was $87.88 \pm 9.8 \text{ mg}/100 \text{ g}^{-1} \text{ FW}$ (ascorbic acid equivalent antioxidant capacity). The extract possessed higher antioxidant capacity than that found in previous studies. This difference might be due to the differing maturation rate of individual fruit and different methods of extraction and analysis.

Conclusion

HPLC coupled with mass spectrometry has become the standard and most powerful method used for anthocyanins analysis. However, mass spectra alone are not 100% effective because MS cannot provide complete structural information for different anthocyanins with the same mass spectra. Therefore, it is necessary to combine it with sources of other useful information that can be obtained in order to identify peaks. Retention time is very important for the determination of anthocyanins even with MS data. A simple elution order for some common anthocyanidin glycosides using reverse-phase HPLC that seems to fit most experimental conditions is delphinidin, cyanidin, petunidin, pelargonidin, peonidin and malvidin. For different glycoside and/or acylated groups with the same anthocyanidin (cyanidin), it is cyanidin-3,5-diglucoside, cyanidin-3-diglucoside, cyanidin-3-galactoside, cyanidin-3-sambubioside, cyanidin-3-glucoside, cyanidin-3-arabinoside, cyanidin-3-rutinoside and cyanidin-3-(malonyl)glucoside (Wu and Ronald, 2005). By comparing their elution orders and mass spectrometric characteristics with reported data in the literature and the values of a standard substance, seven anthocyanidins were tentatively identified in the 'Red Du Comice' pear samples. Most anthocyanins showed Cyanidin aglycon, although Peonidin and Pelargonidin derivatives were also present.

Cyanidin-3-galactoside was the major anthocyanin pigment in red skin of pear fruit, accounting for 93.7% of total anthocyanin. This agreed with the results of Muchuweti and Chikwambi (2008). Muchuweti and Chikwambi found out the compound responsible for the red pigmentation in pear fruit. Anthocyanin compositions vary greatly with genotype. For example, pelargonidin-3-

glucoside was the major anthocyanin in strawberry (*Fragaria x ananassa*) (Cerezo et al., 2010). Delphinidin, petunidin and malvidin were the major contributors to total anthocyanin content but the proportions of each compound were cultivar-dependent. High bush has more polar anthocyanins than rabbit eye cultivars (Lohachoompol et al., 2008). The anthocyanin differences in fruit peels constitute a biochemical marker, so the gathered information in this study helps towards characterizing pear cultivars and their mutants. This will assist in breeding good sensory quality attributes and defense against ultraviolet light or aggression by pathogens (Hamauzu et al., 2005).

ACKNOWLEDGMENT

This work was supported by the Natural Science Fund of China (300900974). The authors would like to thank Dr. Huping Zhang for helpful discussions on antioxidant measurements and Mr. Xu (State key laboratory of Crop Genetics and Germplasm Enhancement) for technical assistance with HPLC-MS.

REFERENCES

- Aaby K, Ekeberg D, Skrede G (2007). Characterization of phenolic compounds in strawberry (*Fragaria x ananassa*) fruits by different HPLC detectors and contribution of individual compounds to total antioxidant capacity. *J. Agric. Food Chem.*, 55: 4395-4406.
- Ana BC, Elyana C, Winterhalter P, Garcia-Parrilla MC, Troncoso AM (2010). Isolation, identification, and antioxidant activity of anthocyanin compounds in *Camarosa* strawberry. *Food Chem.*, 123: 574-582.
- Aneersen M (1985). Chromatographic Separation of Anthocyanins in Cowberry (*Lingonberry*) *Vaccinium vitis-idaea* L. *J. Food Sci.*, 50: 1230-1232.
- Bakker J, Preston NW, Timberlake CF (1986). The determination of anthocyanins in aging red wines: comparison of HPLC and spectral methods. *Am. J. Enol. Viti.*, 37: 121-126.
- Baublis A, Spomer A, Berer-Jimenez M (1994). Anthocyanin pigments: comparison of extract stability. *J Food Sci.*, 59: 1219-1221.
- Benzie IFF, Strain JJ (1999). Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods Enzymol.*, 299: 15-27.
- Brand-Williams W, Cuvelier ME, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. *Lwt-Food Sci. Technol.*, 28: 25-30.
- Cerezo AB, Cuevas EP, Winterhalter MC, Garcia-Parrilla, Troncoso AM (2010). Isolation, identification, and antioxidant activity of anthocyanin compounds in *Camarosa* strawberry. *Food Chem.*, 123: 574-582.
- Da Silva FL, Escribano-Bailn MT, Perez Alonso JJ, Rivas-Gonzalo JC, Santos-Buelga C (2007). Anthocyanin pigments in strawberry. *Lwt-Food Sci. Technol.*, 40: 374-382.
- Desai KGH, Olsen KF, Mallery SR, Stoner GD, Schwendeman SP (2010). Formulation and In Vitro-In Vivo Evaluation of Black Raspberry Extract-Loaded PLGA/PLA Injectable Millicylindrical Implants for Sustained Delivery of Chemopreventive Anthocyanins. *Pharmaceut. Res.*, 27: 628-643.
- DuPont MS, Bennett RN, Mellon FA, Williamson G (2002). Polyphenols from alcoholic apple cider are absorbed, metabolized and excreted by humans. *J. Nutr.*, 132: 172.
- Francis FJ (1970). Anthocyanins in pears. *Hort. Sci.*, 5: 42.
- Gamble J, Jaeger SR, Harker FR (2006). Preferences in pear

- appearance and response to novelty among Australian and New Zealand consumers. *Postharvest Biol Technol.*, 41: 38-47.
- Gao L, Mazza G (1995). Characterization, quantitation, and distribution of anthocyanins and colorless phenolics in sweet cherries. *J. Agric. Food Chem.*, 43: 343-346.
- Garca-Beneytez E, Cabello F, Revilla E (2003). Analysis of grape and wine anthocyanins by HPLC-MS. *J. Agric. Food Chem.*, 51: 5622-5629.
- Gautier-Hion A, Duplantier JM, Quris R, Feer F, Sourd C, Decoux JP, Dubost G, Emmons L, Erard C, Hecketsweiler P (1985). Fruit characters as a basis of fruit choice and seed dispersal in a tropical forest vertebrate community. *Oecologia*, 65: 324-337.
- Gil MI, Holcroft DM, Kader AA (1997). Changes in strawberry anthocyanins and other polyphenols in response to carbon dioxide treatments. *J. Agric. Food Chem.*, 45: 1662-1667.
- Gould KS, McKelvie J, Markham KR (2002). Do anthocyanins function as antioxidants in leaves? Imaging of H₂O₂ in red and green leaves after mechanical injury. *Plant Cell Environ.*, 25: 1261-1269.
- Gu L, Kelm M, Hammerstone JF, Beecher G, Cunningham D, Vannozzi S, Prior RL (2002). Fractionation of Polymeric Procyanidins from Lowbush Blueberry and Quantification of Procyanidins in Selected Foods with an Optimized Normal-Phase HPLC- MS Fluorescent Detection Method. *J. Agric. Food Chem.*, 50: 4852-4860.
- Hamauzu Y, Yasui H, Inno T, Kume C, Omanyuda M (2005). Phenolic profile, antioxidant property, and anti-influenza viral activity of Chinese quince (*Pseudocarya sinensis* Schneid.), quince (*Cydonia oblonga* Mill.), and apple (*Malus domestica* Mill.) fruits. *J. Agric. Food Chem.*, 53: 928-934.
- Heinonen M (2007). Antioxidant activity and antimicrobial effect of berry phenolics—a Finnish perspective. *Mol. Nutr. Food Res.*, 51: 684-691.
- Hong V, Wrolstad RE (1990). Use of HPLC separation/photodiode array detection for characterization of anthocyanins. *J. Agric. Food Chem.*, 38: 708-715.
- Huang Z, Wang B, Williams P, Pace RD (2009). Identification of anthocyanins in muscadine grapes with HPLC-ESI-MS. *Lwt-Food Sci Technol.*, 42: 819-824.
- Li PCH, Wong MCK, Adomat H, Guns EST (2009). Blueberry anthocyanins analyzed by absorption spectroscopy and HPLC-UV-MS. *Can. J. Food Appl. Sci.*, pp. 765-772.
- Lin Long-Ze, James Harnly M (2008). phenolic compounds and chromatographic profiles of pear skin (*Pyrus* spp.). *J. Agric. Food Chem.*, 56: 9094-9101.
- Lohachompol V, Mulholland M, Szrednicki G, Craske J (2008). Determination of anthocyanins in various cultivars of highbush and rabbiteye blueberries. *Food Chem.*, 111: 249-254.
- Lopes-da-Silva F, De Pascual-Teresa S, Rivas-Gonzalo J, Santos-Buelga C (2002). Identification of anthocyanin pigments in strawberry (cv Camarosa) by LC using DAD and ESI-MS detection. *Eur. Food Res. Technol.*, 214: 248-253.
- Luca P, Luca D, Paolo DF, Stefano M, Brenda SJ, Silviro S (2010). Mapping of an anthocyanin-regulating MYB transcription factor and its expression in red and green pear, *Pyrus communis*. *Plant Physiol. Biochem.*, 48: 1020-1026.
- Manetas Y (2006). Why some leaves are anthocyanic and why most anthocyanic leaves are red? *Flora*, 201: 163-177.
- Matysik G, Benesz M (1991). Thin-layer chromatography and densitometry of anthocyanins in the petals of red poppy during development of the flowers. *Chromatographia*, 32: 19-22.
- Muchuweti M, Chikwambi Z (2008). Isolation and identification of anthocyanins in the fruit peels of starkrimson and marx red bartlett common pear cultivars and their bud mutants. *Am. J. Food Technol.*, 3: 1-12.
- Oleszek W, Amiot MJ, Aubert SY (1994). Identification of some phenolics in pear fruit. *J. Agric. Food Chem.*, 42: 1261-1265
- Oliveira J, Azevedo J, Silva AMS, Teixeira N, Cruz L, Mateus N, De Freitas V (2010). Pyranoanthocyanin Dimers: A New Family of Turquoise Blue Anthocyanin-Derived Pigments Found in Port Wine. *J. Agric. Food Chem.*, 58: 5154-5159.
- Prior RL, Lazarus SA, Cao G, Muccitelli H, Hammerstone JF (2001). Identification of procyanidins and anthocyanins in blueberries and cranberries (*Vaccinium* spp.) using high-performance liquid chromatography/mass spectrometry. *J. Agric. Food Chem.*, 49: 1270-1276.
- Sanchez AC, Galvis A, Gil-Lzquierdo, M, Gil I (2003). comparative study of six pear cultivars in term of their phenolic and vitamin c contents and antioxidant capacity. *J. Sci. Food Agri.*, 83: 995-1003.
- Sherma J, (2000). Thin-layer chromatography in food and agricultural analysis. *J Chromatogr. A.*, 880: 129-147.
- Shoji T, Masumoto S, Moriichi N, Kanda T, Ohtake Y (2006). Apple (*Malus pumila*) procyanidins fractionated according to the degree of polymerization using normal-phase chromatography and characterized by HPLC-ESI/MS and MALDI-TOF/MS. *J. Chromatogr. A.*, 1102: 206-213.
- Steyn WJ, Wand SJE, Holcroft DM, Jacobs G (2002). Anthocyanins in vegetative tissues: a proposed unified function in photoprotection. *New Phytol.*, 155: 349-361.
- Stintzing FC, Carle R (2004). Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. *Trends Food Sci. Tech.*, 15: 19-38.
- Tamura H, Takada M, Yoshida Y (1995). Pelargonidin 3-O-(6-O-malonyl-β-D-glucopyranoside) in *Fragaria xananassa* Duch cv Nyoho. *Biosci Biotech Bioch.*, 59, 1157-1158
- Timberlake CF, Bridle P (1976). Interactions between anthocyanins, phenolic compounds, and acetaldehyde and their significance in red wines. *Am. J. Enol. Vitic.*, 27: 97.
- Wilson ID, Plumb R, Granger J, Major H, Williams R, Lenz EM (2005). HPLC-MS-based methods for the study of metabonomics. *J. Chromatogr. B.*, 817: 67-76.
- Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL (2006). Concentrations of anthocyanins in common foods in the United States and estimation of normal consumption. *J. Agric. Food Chem.*, 54: 4069-4075.
- Wu X, Ronald L (2005). Systematic identification and characterization of anthocyanins by HPLC-ESI-MS/MS in common foods in the United States: fruits and berries. *J. Agric. Food Chem.*, 53: 2589-2599.
- Zheng J, Ding C, Wang L, Li G, Shi J, Li H, Wang H, Suo Y (2010). Anthocyanins Composition and Antioxidant Activity of wild *Lycium ruthenicum* Murr. from Qinghai-Tibet Plateau. *Food Chem.*, 126: 859-865.