Evaluation of Main Anthocyanins in Skin of Forest Pear (*Pyrus pyraster*) (Sange Khouj) Fruit in Guilan Province of Iran

Ahmad Dadashpour[1]*, Hassan MohammadAmooie[2], Mohammad Jouki[1], Naimeh Khazaei[1]
[1]Young Researchers Club, Shahre-Qods Branch, Islamic Azad University, Tehran, Iran.
[2]Shahr-e-Qods Branch, Islamic Azad University, Tehran, Iran.

Abstract

The anthocyanins of the fruit skin of Iranian forest pear (Sange Khouj) were separated, 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 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 was identified for the first time in ordinary pear (*P. communis* L). DPPH (2, 2-diphenyl-1-picrylhydrazyl) and FRAP (ferric reducing antioxidant power) assays showed that the extract from the fruit peel of 'Sange Khouj' contained anthocyanins and possessed high antioxidant capacity.

INTRODUCTION

Color is an important factor determining fruit external/visual quality, which has been used successfully in the characterization of fruits (Gautier-Hion *et al.*, 1985). This characteristic 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 reactions. In addition, it is difficult to measure independently the anthocyanins from other flavonoids because they have similar reactionary characterisics. 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 analysis and identification of anthocyanins were developed by using HPLC with mass spectrometry or with tandem mass spectrometry (Bakker *et al.*, 1986; Hong and Wrolstad, 1990). In recent years, HPLC coupled with MS have been become the standard methods for identification and separation in most laboratories (Desai *et al.*, 2010; Huang *et al.*, 2009). HPLC can provide a faster and improved separation of complex compounds and allows their tentative identification based on their retention characteristics. However, it is still difficult to obtain each reference compound for the same spectrum that represents the anthocyanins. Therefore, HPLC coupled with MS, especially ESI/MS are possibly the most powerful methods for identifying the structure of anthocyanins. These methods allow the 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 anthocyanin by means of HPLC-DAD-MS. Using the HPLC-DAD-ESI-MS method, Lopes-da-Silva et al (Lopes-da-Silva *et al.*, 2002) discovered that strawberry extract contained three major anthocyanins (Pg3-gluc, Pg3-rut and Cy3-gluc) and 12 minor anthocyanins, although identity could be only assigned to five of them as Pg-3-acetylglucoside, Cy-3-rutinoside, Pg-3-malylglucoside, Pgdiglucoside, and Cy3-malonylglucosy-l-5-glucoside which the three later had been discovered in strawberry for the first time. So this technique has been shown to be appropriate for isolation and identification of complex mixtures. Many studies focused on the composition of anthocyanin 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). However, few studies have been conducted on pear phenolic compounds, especially anthocyanins. Pear is one of the most important temperate fruits. Fruit skin color is a significant factor that affects the quality and customer acceptance.

The objective of this study was to isolate and identify the anthocyanin molecules responsible for fruit skin coloration in Iranian forest pear (Sange Khouj).

MATERIALS AND METHODS
Plant Samples, Chemicals, Extraction of Anthocyanins, HPLC-MS Analysis

The sample fruits were picked at commercial maturity from forests of Guilan province by local named "Sange Khouj" in Roudbaneh rigion, Lahijan, Guilan, in the north of Iran during 2009-2010 and transported to laboratory of postharvest technology of University of Tehran immediately. Peel external color of fruits was red. Fruits free of defects and without evidence of mechanical damage were selected. Fruits were peeled and frozen in liquid nitrogen and kept at -70ºC until analysis. HPLC-grade methanol and aceonitrile were obtained from Merck KGaA, Darmany, Germany. Formic acid, cyanidin-3-glucoside, cyanidin-3-galactoside, cyanidin-3-arabinoside, peonidin-3-glucoside, peonidin-3-galactoside, DPPH, and 2,4,6-tripyridyl-s-triazine (TPTZ) were obtained from Sigma Chemical co. All other chemicals used in this study were analytical grade.

The separation of anthocyanins was performed according to Wu (Wu *et al.*, 2006), with some modification. 20 mL methanol with 1% (v/v) formic acid was added into 100 mL Erlenmeyer flask containing 1g peel powder. Anthocyanins were extracted at 4°C for 60 min in dark chamber; this procedure was repeated three times. The extract was concentrated under vacuum at 30°C using a rotary evaporator until dryness. The dried extract was resolved in 5 mL solvent of 1% formic acid in distilled water. About 1 mL of extracted solution was strained through a 0.45 μm millipore filter. The solvents for chromatography 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 volume was 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 150-1000. All analyses were replicated twice.

Antioxidant Capacity Determined by FRAP

This procedure involved the reduction of ferric ion (Fe^{3+}) to the ferrous ion (Fe^{2+}) to a blue colored complex Fe^{2+}/TPTZ in the presence of bioactive compounds (antioxidants), which was revealed as the increase of absorption at 593nm (Benzie and Strain, 1999). Extracts were added to the FRAP reagent. The FRAP reagent contained 10 mM TPTZ in 40 mM HCl, 20 mM $FeCl_3$ and 300 mM acetate buffer (pH=3.6), that was freshly prepared in the ratio of 1:1:10 and warmed 30 min at 37.8⁰C before and after adding of sample extract. The $FeSO_4 \cdot 7H_2O$ compound was used to prepare the increasing concentrations of standard solutions. Results were expressed as ascorbic acid equivalent antioxidant capacity (mg ascorbic acid /100g fresh weight).

Antioxidant Capacity Determined by DPPH-radical Scavenging Activity (DPPH assay)

The ability of DPPH free radical scavenging was determined based on the method of Brand Williams (Brand-Williams *et al.,* 1995), with minor modification. Briefly, reaction mixtures containing 25μL extracts and 2 ml 6.25×10^{-5}M DPPH solution, 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 DPPH absorbance. The absorbance at 517 nm was recorded to determine the concentration of remaining DPPH. Results were expressed as ascorbic acid equivalent antioxidant capacity (mg ascorbic acid /100g fresh weight).

RESULTS AND DISCUSSIONS
Anthocyanins Measurement

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 via the shikimic acid pathway which are widespread in the plant kingdom. Betalains, a group of pigments that includes the betacyanins are indole-derived alkaloids and contain nitrogen. Pear peel extracts (99 % methanol with 1 % HCl) were tested for the presence of anthocyanins by observing pigment color under acidic or alkaline conditions by adding HCl or NaOHsodium 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 color was observed when adding sodium hydroxide to the extract, which indicated the presence of anthocyanins in the extracts.

Identification of Pear Anthocyanins

Table 1 shows the anthocyanin profile of the extract from fruit skin of 'Sange Khouj' using the HPLC-DAD chromatograms at 516 nm. A total of eight anthocyanin compounds were identified

by their elution order, UV / vis and by comparing the m / z of each anthocyanin molecule and its fragmentation to prepared standards and previous reports (Table 1). Five peaks (peak 1, 2, 3, 4 and 8) showed fragment ions at m / z 287 in MS2 analyses that could be tentatively identified as cyanidin derivatives. Peak 1 and 2 showed identical molecular ions at m / z 499 and fragmentation patterns, but their retention times in the HPLC system were 28 and 32 min. for peak 1 and 2, respectively. Their elution order and mss characteristics suggest that they should be cyaniding-3-galactoside and cyanidin-3-glucoside, respectively, which concurs with findings in previous study (Cerezo *et al.*, 2010), and also coincides with the available standard used for 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 416 and 549, respectively. Peak 3 produced a 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. Peak 4 produced a fragment pattern matching cyanidin-3-rutinoside. Peak 6 and peak 7 were assigned as peonidin-3-galactoside 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 $[M+H]^+$ at 579, which gave MS^2 fragments that were at m / z 271 and 433 and also was identified as pelargonidin-3-rutinoside. Due to the lack of correspondent reference compounds for cyanidin-3-rutinoside, pelargonidin-3-rutinoside and the compound representing peak 8, the anthocyanins identified were quantitated via a calibration curve determined using cyanidin-3-glucoside. Other identified anthocyanins were quantitated via the calibration curves of corresponding reference compounds. Table 2 shows the individual concentration of the anthocyanins in the extracts of 'Sange Khouj' peel. cyanidin-3-galactoside, the major compound, accounts for 93.7% of total anthocyanin, which confirms what was found in previous study (Sanchez *et al.*, 2003). 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-galactoside (0.75%). Percentages of 94.2% for cyanidin-3-galactoside and 4.5% for cyanidin-3-rutinoside, were found by Andrea *et al.* (2003) in Red D'Anjou. pelargonidin-3-rutinoside has also been previously reported in Concord grape (Baublis *et al.*, 1994), cranberry (Heinonen, 2007), strawberry (da Silva *et al.,* 2007), blackberry (Wrolstad, 1993), sweet cherry (Gao & 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 anthocyanins obtained from fruit peel of 'Khouj' was evaluated with the DPPH and FRAP test. The free radical scavenging activity determined by DPPH was 165.23 ± 11.6 mg / 100 fresh weight and the value of total antioxidant capacity determined by FRAP was 87.88 ± 9.8 mg / 100 fresh weight (ascorbic acid equivalent antioxidant capacity). The extract possessed higher antioxidant capacity than that founds in previous studies. This difference might be due to the differing maturation rate of individual fruit and different methods of extraction and analysis. HPLC coupled with mass spectrometry has been become the standard and most powerful methods 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 are delphini-

din, 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-galactosid, cyanidin 3-sambubioside, cyanidin 3-glucoside, cyanidin 3-arabinoside, cyanidin 3-rutinsode and cyanidin 3-(maloyl) 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 'Khouj' 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 *et al.,* (Muchuweti and Chikwambi, 2008). Anthocyanin compositions vary greatly with genotype. For example, pelargonidin 3-glucoside was the major anthocyanin in strawberry (*Fragaria× 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. Highbush has more polar anthocyanins than rabbiteye 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

Literature Cited

Aaby, K., Ekeberg, D. and Skrede, G. 2007. Characterization of phenolic compounds in strawberry (Fragaria$\frac{3}{2}$ananassa) fruits by different HPLC detectors and contribution of individual compounds to total antioxidant capacity. J. Agric. Food Chem. 55: 4395-4406.

Aneersen, M. 1985. Chromatographic separation of anthocyanins in cowberry (Lingonberry) Vaccinium vites-idaea L. J. Food Sci. 50: 1230-1232.

Bakker, J., Preston, N. W. and Timberlake, C. F. 1986. The determination of anthocyanins in aging red wines: comparison of HPLC and spectral methods. Am. J. Eno. Viti. 37: 121.

Baublis, A., Spomer, A. and Berer-Jimenez, M. 1994. Anthocyanin pigments: comparison of extract stability. J. Food Sci. 59: 1219-1221.

Benzie, I. F. F. and Strain, J. J. 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. Meth. Enzym. 299: 15-27.

Brand-Williams, W., Cuvelier, M. E. and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. Lwt-Food Sci. Tech. 28: 25-30.

Cerezo, A. B., Cuevas, E., Winterhalter, P., Garcia-Parrilla, M. C. and Troncoso, A. M. 2010. Isolation, identification, and antioxidant activity of anthocyanin compounds in camarosa strawberry. Food Chem. 123: 574-582.

Da Silva, F. L., Escribano-Bailn, M. T., Perez Alonso, J. J., Rivas-Gonzalo, J. C. and Santos-Buelga, C. 2007. Anthocyanin pigments in strawberry. Lwt-Food Sci. Tech. 40: 374-382.

Desai, K. G. H., Olsen, K. F., Mallery, S. R., Stoner, G. D. and Schwendeman, S. P. 2010. Formulation and in vitro-in vivo evaluation of black raspberry extract-loaded PLGA/PLA injectable millicylindrical implants for sustained delivery of chemopreventive anthocyanins. Pharma. Res. 27: 628-643.

DuPont, M. S., Bennett, R. N., Mellon, F. A. and Williamson, G. 2002. Polyphenols from alcoholic apple cider are absorbed, metabolized and excreted by humans. J. Nutr. 132: 172.

Gamble, J., Jaeger, S. R. and Harke, F. R. 2006. Preferences in pear appearance and response to novelty among australian and new zealand consumers. Post. Bio. Tech. 41: 38-47.

Gao, L. and 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. and Revilla, E. 2003. Analysis of grape and wine anthocyanins by HPLC-MS. J. Agric. Food Chem. 51: 5622-5629.

Gautier-Hion, A., Duplantier, J. M., Quris, R., Feer, F., Sourd, C., Decoux, J. P., Dubost, G., Emmons, L., Erard, C. and Hecketsweiler, P. 1985. Fruit characters as a basis of fruit choice and seed dispersal in a tropical forest vertebrate community. Oeco. 65: 324-337.

Gil, M. I., Holcroft, D. M. and Kader, A. A. 1997. Changes in strawberry anthocyanins and other polyphenols in response to carbon dioxide treatments. J. Agric. Food Chem. 45: 1662-1667.

Gould, K. S., McKelvie, J. and Markham, K. R. 2002. Do anthocyanins function as antioxidants in leaves? Imaging of H2O2 in red and green leaves after mechanical injury. Plant. Cell. Enviro. 25: 1261-1269.

Gu, L., Kelm, M., Hammerstone, J. F., Beecher, G., Cunningham, D., Vannozzi, S. and Prior, R. L. 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.l and Food Chem. 50: 4852-4860.

Hamauzu, Y., Yasui, H., Inno, T., Kume, C. and Omanyuda, M. 2005. Phenolic profile, antioxidant property, and anti-influenza viral activity of chinese quince (Pseudocydonia 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. Molec. Nutr. Food Res. 51: 684-691.

Hong, V. and Wrolstad, R. E. 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. and Pace, R. D. 2009. Identification of anthocyanins in muscadine grapes with HPLC-ESI-MS. Lwt-Food Sci. Tech. 42: 819-824.

Lin, L. Z. and Harnly, J. M. 2008. Phenolic compounds and chromatographic profiles of pear skin (*Pryus* spp.). J. Agric. Food Chem. 56: 9094-9101.

Lohachoompol, V., Mulholland, M., Srzednicki, G. and 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. and Santos-Buelga, C. 2002. Identification of anthocyanin pigments in strawberry (cv. Camarosa) by LC using DAD and ESI-MS detection. Europ. Food Res. Tech. 214: 248-253.

Manetas, Y. 2006. Why some leaves are anthocyanic and why most anthocyanic leaves are red? Flora-morphology, distribution, functional ecology of plants. 201: 163-177.

Matysik, G. and Benesz, M. 1991. Thin-layer chromatography and densitometry of anthocyanins in the petals of red poppy during development of the flowers. Chroma. 32: 19-22.

Muchuweti, M. and 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 Tech. 3: 1-12.

Oliveira, J., Azevedo, J., Silva, A. M. S., Teixeira, N., Cruz, L., Mateus, N. and 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, R. L., Lazarus, S. A., Cao, G., Muccitelli, H. and Hammerstone, J. F. 2001. identification of procyanidins and anthocyanins in blueberries an cranberries (*Vaccinium* spp.) using High-Performance Liquid Chromatography/Mass Spectrometry. J. Agric. Food Chem. 49: 1270-1276.

Sanchez, A., Galvis, C., Gil-Lzquierdo, A. and Gil, M. I. 2003. comparative study of six pear cultivars in term of their phenolic and vitamin c conterns and antioxidant capacity. J. Sci. Food.Agric. 83: 995-1003.

Sherm, J. 2000. Thin-layer chromatography in food and agricultural analysis. J. Chromatography A. 880: 129-147.

Shoji, T., Masumoto, S., Moriichim N., Kanda, T. and 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. Chromatography A. 1102: 206-213.

Steyn, W. J., Wand, S. J. E., Holcroft, D. M. and Jacobs, G. 2002. Anthocyanins in vegetative tissues: A proposed unified function in photoprotection. New Phytolog. 155: 349-361.

Stintzing, F. C. and Carle, R. 2004. Functional properties of anthocyanins and betalains in plants, food, and in human nutrition. Trends. Food Sci. Tech. 15: 19-38.

Timberlake, C. F. and Bridle, P. 1976. Interactions between anthocyanins, phenolic compounds, and acetaldehyde and their significance in red wines. Am. J. Eno. Viti. 27: 97.

Wilson, I. D., Plumb, R., Granger, J., Major, H., Williams, R. and Lenz, E. M. 2005. HPLC-MS-based methods for the study of metabonomics. J. Chromatography B. 817: 67-76.

Wrolstad, R. E. 1993. Color and pigment analyses in fruit products. Agricultural Experiment Station Oregon State University.

Wu, X., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E. and Prior, R. L. 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. and 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.

Tables

Table 1. HPLC/DAD and HPLC/ESI -MS of anthocyanins in fruit skin of 'Sange Khouj'

Peak number	t_R(min)	[M+] m/z	MS² 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-methylmalonylgluside?
standard	28.76	449	287	Cyanidin-3-succinylglucoside?
standard	32.33	449	287	Cyanidin-malylrhamnoside?
standard	34.30	419	287	Cyanidin-3-galactoside
standard	38.43	463	301	Cyanidin-3-glucoside
standard	40.1	463	301	Cyanidin-3-arabinoside
				Peonidin-3- galactoside
				Peonidin-3- glucoside

Table 2. Anthocyanins content and percentage in the extract of 'Sange khouj' peel

Compounds	content[a](mg/100g fresh weight)	%[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 quanitified in cyaniding equivalents (mg/100g fresh weight).
[b] Percentage of the total content.