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Food Chemistry 86 (2004) 441–448
Food Chemistry
www.elsevier.com/locate/foodchem

Identification of anthocyanins of pinta boca (*Solanum stenotomum*) tubers

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Received 23 May 2003; received in revised form 11 September 2003; accepted 11 September 2003

Abstract

The coloured tubers of *Solanum stenotomum*, a native and very common crop in the Bolivian and Peruvian Altiplano, were analysed to determine their anthocyanin composition. Tubers were boiled, ground and freeze-dried, in a pre-treatment step, in order to inhibit enzymatic reactions and to obtain a stable dried powder or "concentrate". After dissolving the tuber concentrate and the subsequent purification, the anthocyanin composition was characterised by HPLC-DAD-MS. Eleven different compounds were found, all of them acylated with hydroxycinnamic acids and most of them having the same substitution pattern, anthocyanidin 3-acylrutinoside-5-glucoside. The principal anthocyanin was petunidin 3-O-*p*-coumaroylrutinoside-5-O-glucoside. Other anthocyanins found were 3-O-*p*-coumaroylrutinoside-5-O-glucoside of peonidin, malvidin and delphinidin, 3-O-feruloylrutinoside-5-O-glucoside of petunidin, peonidin and malvidin, 3-O-caffeoylrutinoside-5-O-glucoside of petunidin and peonidin and 3-O-*p*-coumaroylrutinoside of petunidin. The presence of acylation in all the anthocyanins found in the sample would improve their stability against some degrading factors and makes the pinta boca tubers a potential source of more stable anthocyanins.

Keywords: Coloured potato tubers; Anthocyanins; LC-MS; Pinta boca; *Solanum stenotomum*

1. Introduction

Nowadays, there is an increasing interest in the development of colorants from natural sources, especially in the food industry, basically favoured by one circumstance: the social trend toward the consumption of natural products instead of synthetic ones.

Among these natural pigments, the anthocyanins are the target of numerous studies, due to their characteristic colorant properties, and since they provide colours ranging from red to blue, including orange, purple and violet. They are responsible for pigmentation of different parts of the flowering plants (not only flowers and fruits, but also stems, leaves, roots and storage organs (Harborne & Grayer, 1988)). Moreover, anthocyanins are supposed to have some beneficial effects on human health, due to their antioxidant activity (Bridle & Timberlake, 1997). Grapes and by-products from the wine industry, as well as some other sources (*Sambucus nigra*, *Prunus* spp.), have traditionally been used as important and inexpensive sources of anthocyanins, but the anthocyanins obtained from them lack stability against some degrading factors (light, SO_2, temperature) (Markakis, 1982; Shenoy, 1993). It has been demonstrated that acylation of the anthocyanin sugar moieties, especially with long acylation groups, can improve their stability, because the acyl group allows the anthocyanin to adopt a folded conformation, which protects the basic anthocyanin structure against degrading factors (Delgado-Vargas, Jiménez, & Paredes-López, 2000; George et al., 2001). Thus, new sources of anthocyanins are being studied, especially sources that can provide acylated anthocyanins.

The pinta boca (*Solanum stenotomum*) is the most common diploid species in the Bolivian and Peruvian

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doi:10.1016/j.foodchem.2003.09.019

Altiplano. It belongs to the solanaceae family. This species includes several cultivars. In the tubers, the peel has a bright colour and the flesh shows a coloured zone just below the peel that spreads to the centre of the tuber. Peel and flesh colours are due to the presence of anthocyanins. These tubers are one of the principal foodstuffs in the countries in which they are cultivated. In addition to this nutritional use, they were used in the past for their medical properties (Duke, 1983). To our knowledge, the anthocyanin composition of these tubers has yet to be described. Nevertheless, anthocyanin compositions from other plants of the genus *Solanum* (*Solanum tuberosum*, *Solanum scabrum*, *Solanum americanum*) or belonging to the Solanaceae family (*Petunia* spp., *Cyphomandra betacea*) have been recorded by different authors (Timberlake & Bridle, 1982; Hrazdina, 1982; Strack & Wray, 1994; Mazza & Miniati, 1993). Some recent works (Rodríguez-Saona, Giusti, & Wrolstad, 1998; Naito et al., 1998; Lewis, Walker, Lancaster, & Sutton, 1998; Giusti, Rodríguez-Saona, Griffin, & Wrolstad, 1999), focussed on the coloured varieties of potatoes, report that red varieties contain pelargonidin derivatives as the principal anthocyanins, while purple ones contain petunidin, malvidin and delphinidin derivatives. It is notable that some of these compounds, described in the literature, show acylation in the sugar at position 3, which makes them interesting from the point of view of their stability.

This work is focussed on the identification of the anthocyanins from pinta boca tubers and the results obtained could be used in further research to determine whether the pinta boca tubers can be considered as a good and cost-effective source of more stable anthocyanins.

2. Materials and methods

2.1. Tuber pre-treatment

Tubers of pinta boca were obtained from the region of Candelaria (Department of Cochabamba, Bolivia). In the country of origin, tubers underwent a pre-treatment in order to obtain a concentrate from them; first, the tubers were boiled (15 min) to inhibit enzymatic reactions, and then the coloured zones were separated from the rest and ground to obtain a ''pre-concentrate''. This ''pre-concentrate'' was freeze-dried and the concentrate of the tubers was finally obtained and sent to Spain for analysis.

2.2. Sample preparation

Forty milligrams of the concentrate were dissolved in ultra-pure water acidified with HCl to pH 0.5 and this solution was purified by solid phase extraction (SPE) in C18 cartridges (Sep pak®, Waters). The cartridges were activated by passing an MeOH solution acidified with HCl (0.1%) and equilibrated with water. The concentrate solution was carefully put into the cartridge, and then washed with water to remove sugars and substances which could interfere with the analysis. The elution of the anthocyanins was performed by the addition of acidified MeOH. The eluate was concentrated under vacuum and re-dissolved in water to a final volume of 5 ml.

2.3. Alkaline hydrolysis

One millilitre of the purified aqueous solution of the tuber concentrate was placed in a screw-cap test tube. Then, an aqueous solution of 10% KOH was added until the colour of the sample solution changed from red to blue and immediately flushed with nitrogen and capped. The test tube was kept in darkness for 10 min at room temperature and, afterwards, concentrated HCl was added until the recovery of the initial red colour. The separation between phenolic acids and anthocyanins was performed with ethyl ether. The organic phase, containing the phenolic acids, was concentrated under vacuum and re-dissolved in an MeOH:H_2O (50:50) solution. The aqueous phase, containing the de-acylated anthocyanins, was also concentrated under vacuum and re-dissolved in acidic water (HCl, pH 0.5). Both final solutions had to be filtered before HPLC-DAD-MS analysis.

2.4. HPLC-DAD analysis

HPLC-DAD analysis was performed in a Hewlett-Packard 1100 series liquid chromatograph and detection was carried out using a photodiode detector. An AQUA C18 reverse phase, 5 μm, 150×4.6 mm column (Phenomenex®, Torrance, CA, USA), thermostatted at 35 °C, was used.

The HPLC conditions employed in this work had been previously optimised in our laboratory by Lopes-Da-Silva, de Pascual-Teresa, Rivas-Gonzalo, and Santos-Buelga (2002). The solvents used were: (A) an aqueous solution (0.1%) of trifluoracetic acid (TFA) and (B) 100% HPLC-grade acetonitrile, establishing the following gradient: isocratic 10% B for 5 min, from 10% to 15% B over 15 min, isocratic 15% B for 5 min, from 15% to 18% B over 5 min, and from 18% to 35% B over 20 min at a flow rate of 0.5 ml/min. Detection was carried out at 520 nm as the preferred wavelength.

2.5. LC/MS analysis

LC/MS analyses were performed using a Finnigan LCQ MS detector (Thermoquest, San Jose, USA) equipped with an API source, using an ESI probe. The

HPLC system was connected to the probe of the mass spectrometer via the UV cell outlet. Both the sheath and the auxiliary gas were a mixture of nitrogen and helium. The sheath gas flow was 1.2 l/min and the auxiliary gas flow, 6 l/min. The capillary voltage was 4 V and the capillary temperature 195 °C. Spectra were recorded in positive ion mode between *m/z* 120 and *m/z* 1500. The mass spectrometer was programmed to do a series of three consecutive scans: a full mass, an MS^2 scan of the most abundant ion in the full mass, and an MS^3 of the most abundant ion in the MS^2. The normalised energy of collision was 45%.

3. Results and discussion

The anthocyanin composition of pinta boca concentrate was determined by means of HPLC-DAD-MS analysis. The chromatogram of this sample, recorded at 520 nm is shown in Fig. 1. As can be seen, there are eight peaks in the chromatogram, whose chromatographic and spectral characteristics are compiled in Table 1. The MS, MS^2 and MS^3 analyses revealed the existence of 11 different compounds, which indicates that some individual peaks correspond to several compounds that co-elute under the conditions employed in this work. This is the case for peaks 2, 6 and 7, which each include two different compounds.

Peaks 1 and 4 showed the same molecular ion and the same fragment ions, but different retention times and a visible absorbance maximum at different wavelengths, the UV maximum wavelengths being identical (Table 1 and Fig. 2). Both UV–Vis spectra have an absorbance maximum at 309 nm, which is characteristic of the acylation of the sugar moiety of the anthocyanin with *p*-coumaric acid. The visible maximum wavelength of peak 1 (527 nm) had a hypsochromic shift compared with that corresponding to peak 4 (531 nm), and the ratios between absorbance at λ_{max} (visible) and absorbance at λ_{max} (UV) were different in both cases.

These two compounds presented, in their mass spectra, a major signal at *m/z* 933, corresponding to the molecular ion. In the MS^2 analysis, this molecular ion was broken into three major fragments, yielding signals at *m/z* 771, *m/z* 317 and *m/z* 479 (in order of relative intensity). The first fragment ion (*m/z* 771) was generated by the loss of one hexose moiety (loss of 162 amu). The second ion generated in the MS^2 analysis (*m/z* 317) corresponds to the anthocyanidin petunidin and it resulted from the loss of 616 amu from the molecular ion. The third ion (*m/z* 479) had the same *m/z* ratio as the monohexoside of petunidin and originated from the molecular ion by loss of 454 amu. Thus, the compounds of peaks 1 and 4 were petunidin derivatives containing two moieties of hexose (probably glucose, according to Harborne, cited in Lewis et al., 1998) and other substituents possessing a total mass of 292. The MS^3 analysis and the UV–Vis spectra data allowed us to confirm the identity of these other substituents: the fragmentation of the ion of *m/z* 771 led to the formation of two ions with signals at *m/z* 317 and *m/z* 479, corresponding to the anthocyanidin and monoglucoside of petunidin, respectively. As previously mentioned, the maximum at 309 nm in the UV–Vis spectrum was indicative of the acylation of the moiety with *p*-coumaric acid. The molecular weight of the residue of this acid is 146. The 146 amu to complete the total value of 292 should be attributed to a rhamnose moiety. This fragmentation pattern and some bibliographic data (Ribéreau-Gayon, 1968; Brouillard, 1982; Lewis et al.,

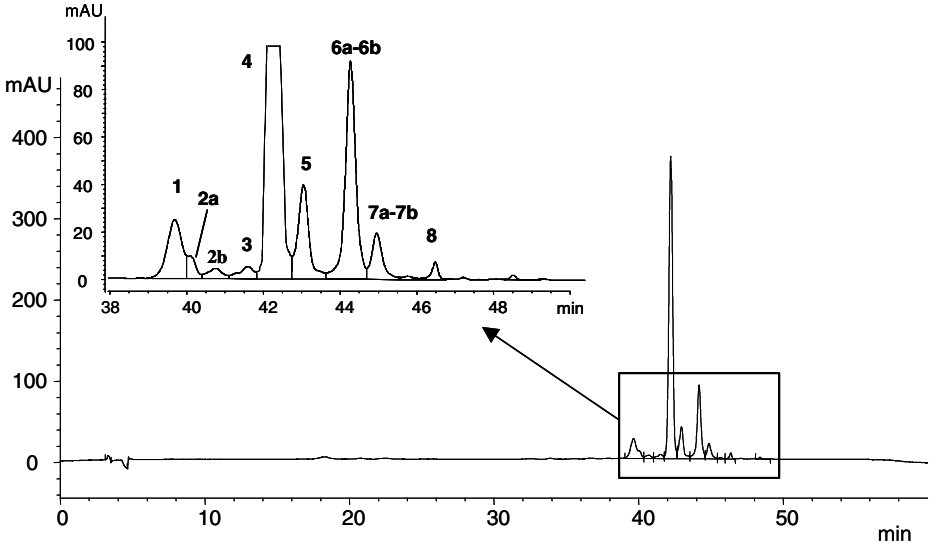

Fig. 1. Chromatogram recorded at 520 nm corresponding to the pinta boca tuber concentrate.

Table 1
Chromatographic and spectral characteristics of the compounds found in pinta boca tuber concentrate

Peak number	Rt (min)	Molecular ion: M^+ (m/z)	Fragment ions in MS^2 (m/z)[a]	Fragment ions in MS^3 (m/z)[a]	Absorbance maxima (nm)
1	36,2	933	771, 317, 479	317, 479, 302	280, 299, 309, 527
2a	37,3	949	787, 479, 317	317, 534, 479, 625	280, 334, 531
2b	37,5	919	757, 303, 465	303, 465, 257	
3	39,3	933	771, 301, 463	301, 463, 317, 479	
4	40,1	933	771, 317, 479, 302	317, 479, 609, 302	280, 299, 309, 531
5	40,9	963	801, 317, 479	317, 479, 625, 302	280, 323, 532
6a	42,0	917	755, 301, 463	301, 463	280, 292, 309, 523
6b	42,4	947	785, 331, 493	331, 493, 463, 301	280, 313, 527
7a	42,7	947	785, 301, 463	301, 463, 623	281, 322, 427, 525
7b	43,2	977	815, 331	331, 493, 273	296, 320, 526
8	44,4	771	317, 479, 302, 609, 625	317, 302	280, 309, 531

[a] The fragment ions are shown in this table in order of their relative abundance: the first ion, in each case, is the most abundant.

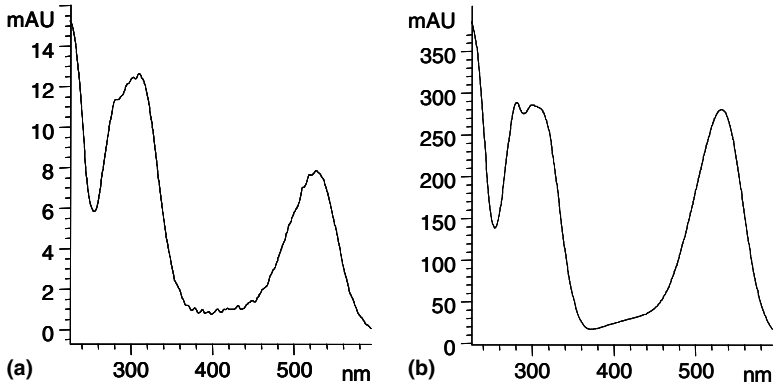

Fig. 2. UV–Vis spectra corresponding to peak 1 (a) and peak 4 (b).

1998; Giusti et al., 1999; Oliveira, Esperança, & Ferreira, 2001; Favretto & Flamini, 2000) allowed us to propose the following identity for the compounds of peaks 1 and 4: Petunidin 3-O-*p*-coumaroylrutinoside-5-O-glucoside. In accordance with the possibility of *p*-coumaric acid forming *trans-cis* isomers and taking into account the identification carried out by George et al. (2001) of *trans-cis* isomers from acylated anthocyanins in *Petunia integrifolia* samples, based on their spectral characteristics, it seemed possible that compounds 1 and 4 should be the *trans* (peak 1) and the *cis* isomer (peak 4). Nevertheless, the fact that the supposed *trans* isomer eluted earlier than the *cis* one in our sample did not coincide with the elution order of these types of isomers described earlier (Tatsuzawa et al., 2000). In reverse phase, the *cis* isomers of acylated anthocyanins have lower retention times than their corresponding *trans* isomers.

In order to determine the identity of this pair of compounds, and to verify whether isomerisation between them was possible, the sample was irradiated with UV light (360 nm). We hypothesised that if the compounds of peaks 1 and 4 had the same anthocyanidin and the same substitution pattern and they only differed in the conformation of the *p*-coumaroyl moiety double bond, irradiation with UV light would transform one isomer into the other and this should be seen in the chromatogram at 520 nm. Thus, an aliquot of the sample was irradiated with UV light for five hours, then analysed by HPLC-DAD-MS and the chromatogram obtained was compared to the chromatogram of another aliquot of the sample which had not been exposed to UV light (Figs. 1 and 3). The formation of a new peak (peak 9 in Fig. 3), with a retention time higher than that of peak 4, was observed. The molecular ion produced a major signal in the MS spectrum at m/z 933 and the major fragments, originating in the MS^2 and MS^3 analyses, were the same as those formed in the cases of peaks 1 and 4. The UV–Vis spectra of the new peak had characteristics associated with *trans* isomers. All these data, allowed us to conclude that peak 9 should be the corresponding *trans* isomer to peak 4. Consequently, peak 1 should not be the *trans* isomer of petunidin 3-O-*p*-coumaroylrutinoside-5-O-glucoside and we propose that peak 1 might be a petunidin derivative containing the same substituents as peak 4, but with different substitution positions, probably 3 and 7, taking into account the fact that 3,7-diglycosides elute earlier than

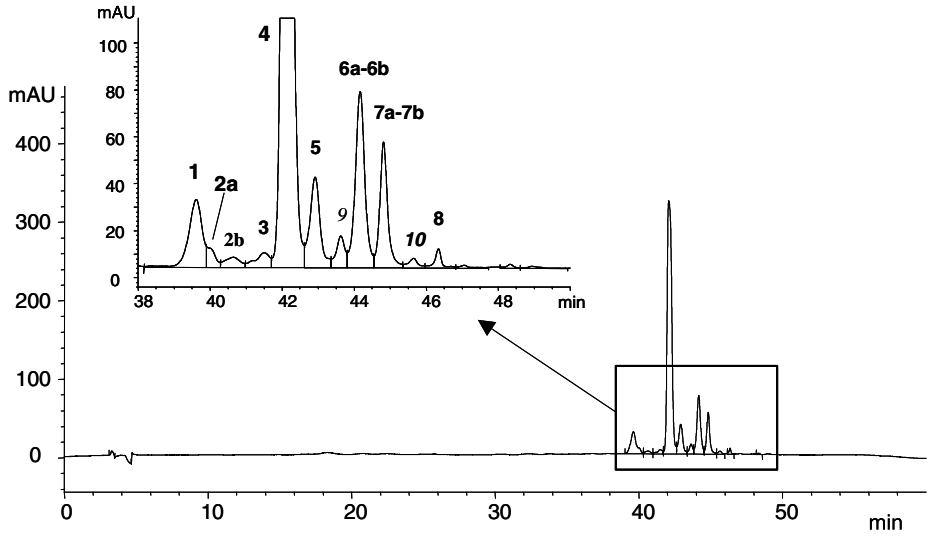

Fig. 3. Chromatogram recorded at 520 nm corresponding to the sample of pinta boca tuber after irradiation with UV light (360 nm).

3,5-diglucosides (Strack & Wray, 1994). However, further analysis will be needed to confirm this proposition.

Peak 3, corresponded to a compound whose molecular ion, when analysed, also showed a major signal in the mass spectrum at m/z 933, but, by means of the MS^2 and MS^3 analyses, it could be identified as a different compound from 1 and 4. In the MS^2 spectrum, there were three major signals at m/z 771, m/z 301 and m/z 463. The first originated from the loss of 162 amu, the second corresponded to the molecular weight of the anthocyanidin peonidin and was formed by loss of all the substituents (632 amu), and the last corresponded to the molecular weight of the monoglucoside of peonidin and originated from loss of 470 amu. The MS^3 spectrum again contained the signal corresponding to the anthocyanidin and the signal of the monoglucoside of peonidin. This means that, once the molecular ion has lost one sugar moiety, it still has another glucose linked to it, i.e., the compound is a peonidin derivative that contains two glucose moieties and other susbtituents whose total weight is 308 and can be attributed to a caffeoylrhamnosyl residue (146 + 162 amu). The substitution pattern should be similar to that of compound 4, and the only differences were the anthocyanidins (peonidin instead of petunidin) and the acylating acids (caffeic acid instead of *p*-coumaric acid). The identity ascribed to this compound was peonidin 3-O-caffeoylrutinoside-5-O-glucoside, a structure that can explain all the fragmentations observed in the MS^2 and MS^3 analyses, as well as its UV–Vis spectrum.

Peak 6, as previously mentioned, contained two different substances, denominated respectively, 6a and 6b. The UV–Vis spectrum of the compound 6a showed an absorbance maximum at 309 nm (indicative of acylation with *p*-coumaric acid). The mass spectrum displayed a major signal at m/z 917, corresponding to the molecular ion. This ion was broken, in the MS^2 analysis, into three major fragments, originating signals in the MS^2 spectrum at m/z's 755, 301, and 463. The first was the most abundant ion and was formed by the loss of one glucose moiety (162 amu). The second corresponded to the molecular weight of peonidin and the third was the monoglucoside of peonidin and originated from the loss of 454 amu. As seen with peaks 1 and 4, the loss of 454 amu in the m/z signal can be attributed to the loss of one glucose moiety (162 amu), one rhamnose moiety (146 amu) linked to the glucose, and a *p*-coumaroyl residue (146 amu), whose existence was confirmed by the absorbance maximum in the UV–Vis spectrum at 309 nm. The fragmentation observed in the MS^3 analysis supported the former conclusions: two major fragments were formed from the ion of m/z 755, with signals in the spectrum at m/z 301 (anthocyanidin) and m/z 463 (monoglucoside of peonidin). The latter originated from the loss of 292 amu in the m/z signal and this loss can be attributed to the loss of the *p*-coumaroylramnosyl residue. Again, the substitution pattern is similar to the pattern of the compounds described previously and the identity assigned to the compound was peonidin 3-O-(*p*-coumaroylrutinoside)-5-O-glucoside.

Compound 6b showed, not only different UV–Vis spectrum features from compound 6a, but also different mass spectral features. The visible λ_{max} of compound 6b was higher than that of 6a, probably due to differences in the nature of the anthocyanidin. The mass spectrum of compound 6b showed an intense signal at m/z 947. The signals that appeared in the MS^2 spectrum (at m/z 785, m/z 331 and m/z 493) corresponded to fragment ions originating from losses of 162, 616 and 454 amu, respectively, losses which are the same as in the cases of peaks 1, 4, and 6a. Thus, the substituents should be the same in all four cases. The signal at m/z 331

corresponded to the molecular weight of malvidin, and the fragment ion of m/z 493 corresponded to the monoglucoside of malvidin. Again, the signals observed in the MS^3 spectrum (at m/z 331 and m/z 493) corresponded to malvidin and the monoglucoside of malvidin. The identity proposed for this compound was malvidin 3-O-(*p*-coumaroylrutinoside)-5-O-glucoside.

Another important peak was peak 5. Its UV–Vis spectrum showed three absorbance maxima. The UV maximum at 323 nm could be indicative of the acylation of the anthocyanin with ferulic acid. The MS spectrum presented a major signal at m/z 963 (molecular ion). The MS^2 spectrum displayed three major signals at m/z 801, m/z 317 and m/z 479, the first being the most intense and originating from loss of one glucose moiety and the other corresponding to petunidin and the monoglucoside of petunidin. The MS^3 spectrum showed two major signals, at m/z 317 and m/z 479, the latter originating from the loss of 322 amu. Taking into account that the molecular weight of a feruloyl residue is 176, the loss of 322 amu can be attributed to the loss of a feruloylrhamnoside residue. In the light of these data, the identity assigned to this compound was petunidin 3-O-feruloylrutinoside-5-O-glucoside.

Peak 7, when analysed by mass spectrometry, showed the presence of two different compounds (7a and 7b). Both compounds had, in their UV–Vis spectra, an absorbance maximum at 320–322 nm, as did peak 5, indicative of an acylation of compounds 7a and 7b with ferulic acid. The visible λ_{max} of compounds 7a and 7b were different, that corresponding to 7b being higher. The molecular ions of these two compounds were also different: peak 7a had a major signal in its MS spectrum at m/z 947 and 7b at m/z 977. The anthocyanidin of each compound could be determined by the fragment ions (see Table 1) originating in the MS^2 and MS^3 analyses. Compound 7a was a peonidin derivative, since the ion of m/z 301 was present, not only in the MS^2 spectrum, but also in the MS^3 spectrum, as in previous cases, and compound 7b was a malvidin derivative (m/z 331). The losses in the m/z signal, from the molecular ion to the fragment ions, as well as the losses observed in the MS^3 analysis, were the same in both compounds and identical to the ones observed in compound 5. This fact allowed us to propose that compound 7a should be peonidin 3-O-feruloylrutinoside-5-O-glucoside and compound 7b should be malvidin 3-O-feruloylrutinoside-5-O-glucoside.

Peak 2 was formed by the elution of two compounds (2a and 2b). The UV–Vis spectrum of compound 2a showed an absorbance maximum at 334 nm, which is characteristic of acylation with caffeic acid. All the compounds found in the sample seemed to have the same substitution pattern, anthocyanidin 3-O-acylrutinoside-5-O-glucoside, so it was possible that this one also had it. The MS spectrum of this compound presented a major signal at m/z 949 and the MS^2 spectrum showed three signals (at m/z 787, m/z 317 and m/z 479), corresponding to fragment ions originating from the molecular ion by losses of 162, 632 and 470 amu, respectively. The MS^3 analysis yielded two major fragment ions (at m/z 317 and m/z 479) originating from loss of 470 and 308 amu, respectively. All these losses were the same as those observed in the fragmentation of compound 3. With these data we concluded that compound 2a should be petunidin 3-O-caffeoylrutinoside-5-O-glucoside.

Compound 2b was present in the sample in very small amounts, so it was difficult to observe its UV–Vis spectrum. The mass spectrum showed a major signal at m/z 919. The MS^2 and MS^3 analyses revealed the identity of the anthocyanidin (delphinidin, since there was a signal at m/z 303 in the MS^2 and MS^3 spectra) and the identity of the substituents. The losses observed in all the fragmentations were the same as those observed in the fragmentation of peaks 1, 4, 6a and 6b, so we concluded that the substituents should be the same as in the other cases. Thus, the identity proposed for compound 2b was delphinidin 3-O-*p*-coumaroylrutinoside-5-O-glucoside.

The last peak in the chromatogram was peak 8. The UV–Vis spectrum showed an absorbance maximum in the region of 309 nm, which indicated acylation with *p*-coumaric acid. The mass spectrum displayed a major signal at m/z 771, and the fragment ions originating in the MS^2 analysis gave rise to signals in the spectrum at m/z's 317, 479 and 302. The molecular ion of compound 8 had the same m/z ratio as the most intense fragment ion in the MS^2 spectrum of compounds 1 and 4 and the fragment ions obtained in the MS^2 analysis of the former are the same as those obtained in MS^3 analysis of compounds 1 and 4. The difference between the m/z ratio of the molecular ions of compounds 1 and 4 and the m/z ratio of the molecular ion of compound 8 was 162 amu, which meant that compound 8 had one less glucose moiety than compounds 1 and 4. In accordance with these data the identity proposed for this compound was petunidin 3-O-*p*-cumaroylrutinoside.

In order to confirm the nature of the acylating acids in the compounds present in the sample, an alkaline hydrolysis was carried out in the sample solution, as described in the Material and methods section.

In the chromatogram of the organic phase, three peaks appeared and their identities were established in accordance with their retention times, elution order and above all with their UV–Vis spectra in comparison to the spectra of frequent acylating acids. The first peak corresponded to caffeic acid, the second to *p*-coumaric acid and the third to ferulic acid. The UV–Vis spectra of these peaks are shown in Fig. 4.

In the chromatogram of the aqueous phase, we found five major peaks, identified, above all, by means of the

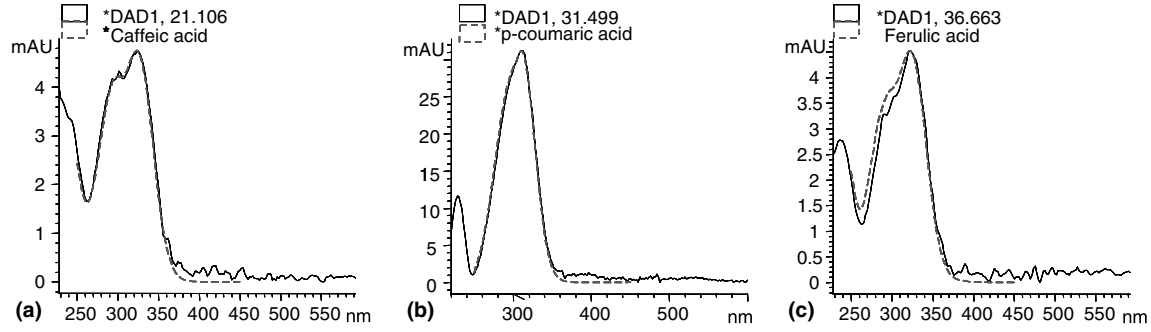

Fig. 4. UV–Vis spectra of the acids found in the organic phase (a, caffeic acid; b, *p*-coumaric acid; c, ferulic acid) of the alkaline hydrolysis. UV–Vis spectra of the acids in the sample (——); UV–Vis spectra of the standard acids (- - - - -).

Table 2
Identity proposed for all the compounds found in the concentrate of pinta boca tubers

Peak number	Proposed identity
1	Petunidin 3-O-*p*-coumaroylrutinoside-7-O-glucoside
2a	Petunidin 3-O-caffeoylrutinoside-5-O-glucoside
2b	Delphinidin 3-O-*p*-coumaroylrutinoside-5-O-glucoside
3	Peonidin 3-O-caffeoylrutinoside-5-O-glucoside
4	Petunidin 3-O-(*cis*-*p*-coumaroyl)rutinoside-5-O-glucoside
5	Petunidin 3-O-feruloylrutinoside-5-O-glucoside
6a	Peonidin 3-O-(*cis*-*p*-coumaroyl)rutinoside-5-O-glucoside
6b	Malvidin 3-O-*p*-coumaroylrutinoside-5-O-glucoside
7a	Peonidin 3-O-feruloylrutinoside-5-O-glucoside
7b	Malvidin 3-O-feruloylrutinoside-5-O-glucoside
8	Petunidin 3-O-*p*-coumaroylrutinoside

mass analysis (peak 1, delphinidin 3-O-rutinoside-5-O-glucoside, peak 2 petunidin 3-O-rutinoside-5-O-glucoside, peak 3, peonidin 3-O-rutinoside-5-O-glucoside, peak 4, malvidin 3-O-rutinoside-5-O-glucoside and peak 5, petunidin 3-O-rutinoside). Thus, all the compounds that were expected to appear in this aqueous phase were found and the identities of the compounds found in the sample were confirmed.

Table 2 contains the list of all the compounds found in the pinta boca tuber concentrate.

4. Conclusions

To our knowledge, this is the first time that the anthocyanin composition of the pinta boca tubers has been described. All the anthocyanins found in this tuber are acylated with hydroxycinnamic acids and their structures (except in the case of compound 8) follow the same pattern (anthocyanindin 3-O-acylrutinoside-5-O-glucoside). Some of the anthocyanins reported in this work have been described previously in other varieties of *Solanum tuberosum*, or in other plants belonging to the Solanaceae family while others, as far as we know, have not yet been reported in this family. This is the case for the compounds delphinidin 3-O-*p*-coumaroylrutinoside-5-O-glucoside, petunidin 3-O-feruloylrutinoside-5-O-glucoside and malvidin 3-O-feruloylrutinoside-5-O-glucoside. Furthermore, it is important to emphasize the presence of *cis* isomers in a plant belonging to the genus *Solanum* because, to date, all the acylated anthocyanins reported in the genus *Solanum* had a *trans* configuration. The *cis* configuration is also very rare in the Solanaceae family. Taking into account that the acylation of the anthocyanins with hydroxycinnamic acids protects them against some colour-damaging factors, due to their ability to form folded structures, and that this protective effect is higher when the configuration of the acid is *cis* (George et al., 2001), we can conclude that the tubers of pinta boca (*Solanum stenotomum*) should be a good source of colorants to be used in the food industry.

Acknowledgements

We would like to thank Mr. G.H. Jenkins for revising the English version of the manuscript. This work is a part of the Programa Iberoamericano de Ciencia y Tecnología para el desarrollo (CYTED, Proyecto IV.10), and it has been financially supported by the Spanish Comisión Interministerial de Ciencia y Tecnología (CICYT, ALI1997-0773).

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