Anthocyanins from red wine – Their stability under simulated gastrointestinal digestion

G.J. McDougall *, S. Fyffe [1], P. Dobson, D. Stewart

Quality, Health and Nutrition Programme, Genes to Products Theme, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK

Abstract

The stability of anthocyanins from red wine was assessed using an in vitro digestion system that simulated the physiochemical changes that occur in the upper gastrointestinal tract. Anthocyanins in red wine were stable to gastric conditions whereas there was a small loss in total phenol content. After pancreatic digestion, the total anthocyanins were very poorly recovered compared to the bulk phenols in the IN sample, which was previously described as the "serum-available" fraction, and the majority of the anthocyanins and phenols were recovered in the OUT fraction, previously described as the "colon-available" fraction. Removing alcohol from the wine samples prior to the procedure did not markedly affect this pattern.

The composition of anthocyanins in the post gastric, IN and OUT samples was analysed using liquid chromatography mass-spectrometry. The red wine used contained over 20 identifiable anthocyanins of which the main components were 3-O-glucosides of malvidin, peonidin, petundin, delphidin and cyanidin. Coumaroylated-glucoside derivatives of malvidin, petundin, peonidin, and delphinidin were observed and acetylated glucosides of peonidin, petundin and malvidin were also identified. Anthocyanins with modified aglycones similar to vitisin A derivatives of delphinidin, peonidin, petunidin and malvidin were also identified.

After the in vitro digestion procedure, only five anthocyanins could be detected in the IN (serum-available) and the OUT (colon-available) fractions, which were confirmed as malvidin-3-O-glucoside and the vitisin A adducts of malvidin-3-O-glucoside, malvidin-3-O-acetylglucoside, malvidin-3-O-coumaroylglucoside and peonidin-3-O-glucoside. Malvidin-3-O-glucoside was recovered at 0.2% in the IN fraction and 0.9% in the OUT fraction. However, the vitisin derivatives were much more stable to pancreatic digestion. Assuming that the vitisin A derivatives display similar biological properties to their parent anthocyanins, their enhanced gastrointestinal stability could lead to enhanced bioavailability and bio-effectiveness in vivo.

1. Introduction

Anthocyanins belong to the flavonoid group of polyphenolic compounds and are responsible for the red and blue colours of plant organs such as fruits, flowers and leaves (Strack and Wray, 1993). Naturally occurring anthocyanins are composed of six anthocyanidin aglycones linked to sugar groups at positions 3 and/or 5 (Diagram 1). They are industrially important natural food colourings (Stinzing and Carle, 2004).

Dietary consumption, mainly from red fruits, certain vegetables and red wine (Macheix et al., 1990), can be as high as 200 mg/day and their consumption in red wine has been proposed as part of the reason for the "French Paradox" (Clifford, 2000) in which a diet rich in saturated fats and moderate alcohol consumption does not lead to elevated levels of heart disease, cancers and strokes found in other countries. Anthocyanins are effective antioxidants

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* Corresponding author. Tel.: +44 1382 562731; fax: +44 1382 562426.
  E-mail address: gmcdou@scri.sari.ac.uk (G.J. McDougall).
[1] Present address: Division of Biological Chemistry and Molecular Microbiology, School of Life Sciences, University of Dundee, Dundee DD1 5EH, UK.

Malvidin-3-O-glucoside

vitisinAMalvidin-3-O-glucoside

vitisinBMalvidin-3-O-glucoside

R1	R2	
H	H	Pelargonidin
H	OH	Cyanidin
OH	OH	Delphinidin
OCH$_3$	H	Peonidin
OCH$_3$	OH	Petunidin
OCH$_3$	OCH$_3$	Malvidin

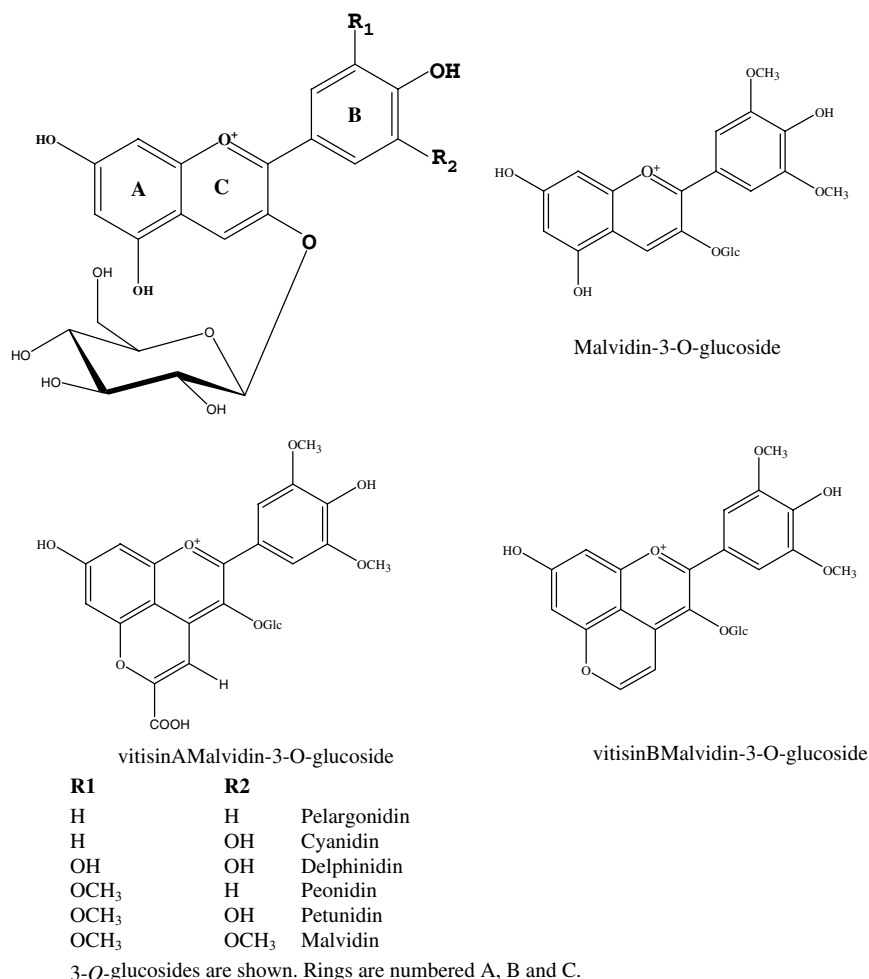3-O-glucosides are shown. Rings are numbered A, B and C.

Diagram 1. Structure of anthocyanins and vitisin derivatives.

(Stinzing and Carle, 2004) but they have also been proposed to have other biological activities independent of their antioxidant capacities that produce health benefits; examples range from inhibition of cancer cell growth in vitro (Zhang et al., 2005), induction of insulin production in isolated pancreatic cells (Jayaprakasam et al., 2005), reduction of starch digestion through inhibition of α-glucosidase activity (Matsui et al., 2001), suppression of inflammatory responses (Tall et al., 2004) to protection against age-related declines in cognitive behaviour and neuronal dysfunction in the central nervous system (Joseph et al., 1999).

A major part of our research efforts into the genetic improvement of soft fruit, such as blackcurrant (Brennan, 1996) and raspberry (Graham et al., 2004), is to ensure that we select varieties with optimal nutritional and health benefits. Increased anthocyanin content has been a breeding target (Brennan, 1996). However, to achieve any biological effect in a specific tissue or organ, anthocyanins must be bioavailable; i.e. effectively absorbed from the gastrointestinal tract (GIT) into the circulation and delivered to the appropriate location within the body. Oral intake of anthocyanin-rich fruits, extracts or pure anthocyanins has

proved to have beneficial effects in preventing or suppressing diseased states in vivo (Ramirez-Tortosa et al., 2001; Mazza et al., 2002). Studies of oral administration of anthocyanins have confirmed increased antioxidant status of the serum (Serafini et al., 1998; Ramirez-Tortosa et al., 2001; Mazza et al., 2002; Bitsch et al., 2004) but this is usually accompanied by very low uptake of anthocyanins into the serum (<1% of dose) (Lapidot et al., 1998; Bub et al., 2001; Frank et al., 2003) and correspondingly low levels of urinary excretion as intact or conjugated forms. The apparent low bioavailability of anthocyanins seems to cast doubt on their ability to exert their proposed beneficial effects throughout the body.

Assessment of true bioavailability of any class of phytochemicals requires data concerning their absorption, metabolism, tissue and organ distribution and excretion (van de Waterbeemd et al., 2004). Such studies carried out in animals or human subjects are complex, expensive and raise moral and ethical questions. In this study, we assess the stability of anthocyanins from red wine using an in vitro digestion procedure that mimics the physiochemical and biochemical conditions encountered in the upper GIT. We monitor the recovery of individual anthocyanins using

liquid chromatography–mass-spectrometry (LC–MS^n). The relative stability of the anthocyanins under GIT conditions is crucial for the bioavailability of these compounds, as it will determine the pool size for whatever active mechanisms are present in the stomach (Passamonti et al., 2003) or the small intestine (Gee et al., 1998) that can transport anthocyanins into the blood stream. Information on the relationship between the structure and gastrointestinal stability of anthocyanins will be fed-back into traditional or marker-assisted breeding programs to facilitate the generation of fruit with enhanced health benefits.

2. Materials and methods

2.1. In vitro digestion procedure

Red wine (Echo Falls, a Merlot variety wine from Mission Bell Winery, Madera, California, USA) was purchased from a local supermarket and was concentrated on a rotary evaporator to remove alcohol then diluted with ultra pure water to the original volume. The in vitro digestion procedure was carried out on both the de-alcoholised and the original wine. The procedure was adapted from a published method (Gil-Izquierdo et al., 2002) which itself was adapted from previous work (Miller et al., 1981) which showed significant correlation between in vitro and in vivo measurements of iron bioavailability. The method consists of two sequential steps; an initial pepsin/HCl digestion for 2 h at 37 °C to simulate gastric conditions followed by a digestion with bile salts/pancreatin for 2 h at 37 °C to simulate small intestine conditions. The 2-h steps represent an average time for gastrointestinal transit.

The extract (20 ml) was adjusted to pH 1.7 with 5 N HCl and pepsin (Sigma Chem. Co Ltd product number P6887) was added at 315 units/ml and incubated at 37 °C in a heated water bath for 2 h with shaking at 100 rpm. After 2 h, aliquots (2 ml) of the post-gastric digestion were removed and frozen. The remainder was placed in a 250 ml glass beaker and 4.5 ml of 4 mg/ml pancreatin, 25 mg/ml bile salts mixture added. A segment of cellulose dialysis tubing (molecular weight cut-off 12 kDa) containing sufficient NaHCO$_3$ to neutralise the sample's titratable acidity was added and the beaker sealed with parafilm. After 2-h incubation at 37 °C, the solution outside the dialysis tubing was taken as the OUT sample representing material that remained in the gastrointestinal tract (=colon available) and the solution that had diffused into the dialysis tubing was taken as the IN sample (=serum-available material). Diffusion of NaHCO$_3$ out of the dialysis tubing represents the simplest and most convenient means to mimic the gradual rise in pH that occurs as the stomach contents enter the small intestine. The pH of the OUT sample reached neutrality within 30 min.

The amount of 1 M NaHCO$_3$ required to neutralise an 18 ml aliquot of the post gastric digest plus 4.5 ml bile salts/pancreatin was defined as the titratable acidity. The post gastric, IN and OUT samples were thawed when required, centrifuged at 16,000 rpm in an Eppendorff microfuge and the supernatants assayed for anthocyanin and phenol content.

2.2. Anthocyanin and phenol assays

The total anthocyanin concentration was estimated by a pH differential absorbance method (Ribereau-Gayon and Stonestreet, 1965). The absorbance value was related to anthocyanin content using the molar extinction coefficient calculated in-house for cyanidin-3-O-glucoside (purchased from ExtraSynthese Ltd, Genay, France). Samples with low anthocyanin content masked by absorbance from the pancreatin/bile salts mixture were analysed by a direct absorption method that had been validated for a range of samples of anthocyanin content recorded by the pH differential method. Briefly, 50 μl 5 N HCl was added to (up to) 200 μl of sample then made to 1 ml with distilled water. After centrifugation, the absorbance at 510 nm was recorded. The A_{510} value was related to anthocyanin content using the equation; AC content (mg/100 ml) = A_{510} value/0.2429. Phenol content was measured using a modified Folin-Ciocalteau method (Singleton and Rossi, 1965). Phenol contents were estimated from a standard curve of gallic acid. All results have been corrected for the presence of phenols in the pancreatin/bile salts mixture.

Samples from the in vitro digestion procedure were acidified to 0.5% (v/v) trifluoroacetic acid (TFA) by slow addition of 10% TFA and mixed well. After centrifugation, insoluble material was retained for further analysis. The soluble material was passed through C18 solid phase extraction (SPE) columns (1000 mg capacity, Phenomenex Ltd) which had been pre-equilibrated in ultra pure water (UPW) containing 0.5% (v/v) trifluoroacetic acid (TFA). After a wash with 2 volumes of 0.5% TFA, the bound material was eluted by the addition of 0.5% (v/v) TFA in 25% (v/v) acetonitrile. This concentration of acetonitrile afforded the best recovery of total phenolics and their separation from the bile salts present in the IN and OUT samples. Recovery of cyanidin-3-O-glucoside from the SPE procedure was ~90%. The phenol and anthocyanin contents of the bound fractions and the "insoluble" material, which was soluble in methanol, were assayed. The fractions were then dried in a speed-vac (Thermo-Finnegan Ltd, Macclesfield, UK) to suitable phenol concentrations for LC–MS.

2.3. Liquid chromatography–mass spectroscopy (LC–MS)

Samples (containing 40 μg gallic acid equivalents by Folin assay) were analysed on a LCQ-DECA system, comprising Surveyor autosampler, pump and photo diode array detector (PDAD) and a ThermoFinnigan mass spectrometer iontrap. The PDAD scanned three discrete channels at 280, 365 and 520 nm. Samples were eluted over a gradient of 5% acetonitrile (0.5% formic acid) to 25%

acetonitrile (0.5% formic acid) on a C18 column (Synergi Hydro C18 with polar end capping, 4.6 mm × 150 mm, Phenomenex Ltd) over 60 min at a rate of 400 μl/min. The LCQ-Deca LC–MS was fitted with an ESI (electrospray ionisation) interface and analysed the samples in positive ion mode. There were 2 scan events; full scan analysis followed by data dependent MS/MS of the most intense ions. The data dependant MS/MS used collision energies (source voltage) of 45% in wideband activation mode. The MS detector was tuned against cyanidin-3-O-glucoside (positive mode) then retuned against malvidin-3-O-glucoside by direct infusion of the red wine sample.

The % recovery of the anthocyanins in the IN and OUT samples was calculated as the percentage of the amount of that anthocyanin that was present in the post-gastric sample in MS detector peak areas. This comparison overcomes potential problems in differential ionization of different anthocyanins. As an equal amount of phenols were applied to the LC–MS, a factor was used to correct for the degree of concentration of the sample required to give 40 μg phenols/injection.

3. Results and discussion

The total anthocyanin content was stable under the gastric conditions of the first part of the in vitro digestion procedure (Table 1), probably due to the well documented acid stability of the flavylium cation form of anthocyanins (Clifford, 2000). However, they were more poorly recovered in the IN and the OUT fractions following pancreatic digestion than the phenols. Only ∼4% of red wine anthocyanins were recovered in the IN fraction when measured by the direct absorbance method. The low levels of anthocyanins in the IN sample were confirmed by measuring anthocyanin content after solid phase extraction to remove bile salts (results not shown) prior to LC–MS. The original wine sample and the de-alcoholised wine sample gave near identical recoveries (results not shown). Other studies of the stability of anthocyanins from pomegranates, strawberries and raspberries (Perez-Vicente et al., 2002; Gil-Izquierdo et al., 2002; McDougall et al., 2005) gave similar patterns of low anthocyanin recovery in the IN fraction. The in vitro digestion procedure was validated as iron content in the IN or "serum-available" fraction was closely correlated to serum iron bioavailability in vivo (Miller et al., 1981) but it has not been validated for any class of polyphenol. The partition between the IN and OUT fractions could be ignored and the recoveries in both fractions combined as

a measure of survival under simulated pancreatic digestion. Nevertheless, it is intriguing that the low recovery of anthocyanins in the IN or "serum-available" fraction from the in vitro digestion procedure matched the low bioavailability of serum anthocyanins noted in animal and human feeding trials (Lapidot et al., 1998; Bub et al., 2001; Frank et al., 2003).

The in vitro digestion method cannot mimic the unidentified active transport processes that occur in the stomach (Passamonti et al., 2003) or the transport of flavonoids through interaction with the sodium dependent glucose transporter (SGLT1) in the small intestine (Gee et al., 1998). Nevertheless, stability under gastrointestinal conditions is crucial to potential bioavailability as it will limit the pool of anthocyanins available to any effective transport mechanism.

Anthocyanins could be altered during transport through the gut mucosa as transport across sections of rat small intestine (Kuhnle et al., 2000) was combined with methylation or glucuronidation of the flavanols, catechin and epicatechin. Also, β-glucosidase enzymes capable of deglycosylating certain flavonol glycosides have been identified in cell free extracts of intestinal mucosa cells (Day et al., 2000; Nemeth et al., 2003). After removal of the hydrophilic glycoside, the aglycones can be passively absorbed through the gut lumen. However, it must be stressed that although anthocyanins are structurally similar to these other flavonoids, whether they can be transported by SGLT1 or are liable to attack by the glycosidases has not been confirmed.

Feeding trials in human or animal subjects (Lapidot et al., 1998; Bub et al., 2001; Frank et al., 2003) or model studies using intestinal sections (e.g. Kuhnle et al., 2000) are technically difficult, expensive and, by their nature, low through-put. The in vitro digestion method is simple, cheap, and reproducible and can be used to screen large numbers of samples or to study a wide range of experimental conditions. For example, using a similar in vitro digestion procedure, Argyri et al. (2005) found that the co-digestion of red wine (fortified with iron) with vitamin C and meat resulted in an increase and a decrease, respectively, in antioxidant capacity and total phenol content. Similarly, co-digestion of raspberry extracts with meat, bread and cereals decreased the recovery of total phenols (McDougall et al., 2005) but not the recovery of anthocyanins in the IN fraction.

The composition of anthocyanins in the post gastric, IN (serum-available) and OUT (colon-available) samples was analysed using liquid chromatography–mass-spectrometry (Fig. 1). The red wine used contained 20 peaks at A_{520} which could be confirmed as anthocyanins by their distinctive MS and MS^2 data (Table 2). The identification of anthocyanins was based on their retention on C18, their MS data and PAD spectra and was supported by previous work (Cho et al., 2004). The main components were the 3-O-glucosides of malvidin, peonidin, petundin, delphidin and cyanidin (Table 2) and the 3-O-glucosides of vitisin

Table 1
Recovery of phenols and anthocyanins in red wine after in vitro digestion

Sample	% Recovery phenols	% Recovery anthocyanins
Post gastric	66.4 ± 2.5	99.1 ± 1.6
Serum-available	7.2 ± 0.3	3.7 ± 0.3
Colon-available	39.7 ± 1.8	34.1 ± 1.9

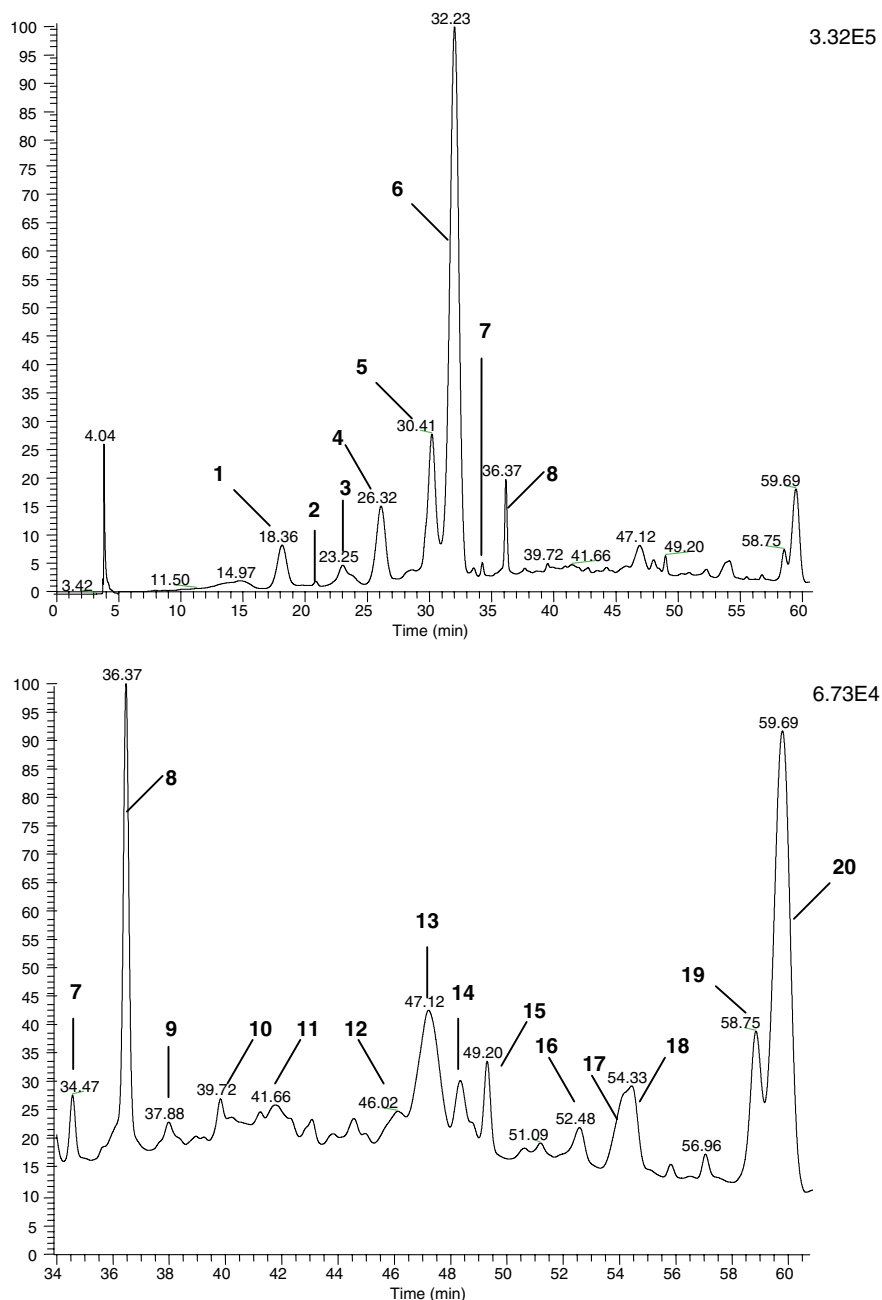All values are averages of six replicate experiments with standard errors.

Fig. 1. HPLC chromatograph of red wine. The first panel shows the entire chromatograph and the second panel shows an expanded section from 34 to 60 min. The full scale for the detector (absorbance units at 520 nm) is shown in the top right corner. The peaks are numbered and assignments are given in Table 2. Peaks without numbers are unknown compounds.

A derivatives of malvidin, peonidin and delphinidin (Table 2; Diagram 1). The vitisin derivatives are formed during fermentation by reaction with pyruvate and their identification was supported by previous data (Vivar-Quintana et al., 2002). Acetylated glucosides of malvidin, peonidin, petundin and the vitisin A derivative of malvidin were detected. The vitisin B pyruvyl derivative of malvidin-3-O-glucoside (peak 9) was also present. (Diagram 1). Coumaroylated-glucoside derivatives of malvidin, peonidin, petundin, delphinidin, cyanidin and the vitisin A derivative of malvidin were also observed. As well as enhanced reten-

tion on C18, the coumaroylated glucosides absorbed more strongly around 320 nm, which is indicative of acylation by cinnamic acids (Strack and Wray, 1993). The presence of two peaks for the coumaroylated-glucoside of malvidin (peaks 18 and 20) was probably due to *cis* and *trans*-isomers of *p*-coumaric acid, respectively (Nunez et al., 2004). As noted previously, the *cis*-isomer was less retained on C18 and absorbed less intensely at 320 nm compared to the *trans* form (results not shown). Caffeoylated glucosides of malvidin have been reported in some red wines (Perez-Magarino and Gonzalez-San Jose, 2004) but these were

Table 2
Peak assignments, retention times and mass spectral data of anthocyanins in red wine

Peak	RT	m/z (+ve)	MS^2	Identification
1	18.36	465.0	303.2	Delphinidin-3-O-glucoside
2	21.09	533.0	371.3	Vitisin A delphinidin-3-O-glucoside
3	23.25	449.0	287.2	Cyanidin-3-O-glucoside
4	26.32	479.0	317.2	Petunidin-3-O-glucoside
5	30.41	463.0	301.2	Peonidin-3-O-glucoside
6	32.23	493.0	331.2	Malvidin-3-O-glucoside
7	34.47	531.0	369.2	Vitisin A peonidin-3-O-glucoside
8	36.37	561.0	399.2	Vitisin A malvidin-3-O-glucoside
9	37.88	517.0	355.2	Vitisin B malvidin-3-O-glucoside
10	39.72	603.0	399.2	Vitisin A malvidin-3-O-(6-acetyl)-glucoside
11	41.66	521.1	317.2	Petunidin-3-O-(6-acetyl)-glucoside
12	46.02	505.1	301.2	Peonidin-3-O-(6-acetyl)-glucoside
13	47.12	535.1	331.2	Malvidin-3-O-(6-acetyl)-glucoside
14	48.24	611.1	303.3	Delphinidin-3-O-(6-p-coumaroyl)-glucoside
15	49.20	707.1	399.2	Vitisin A malvidin-3-O-(6-p-coumaroyl)-glucoside
16	52.48	595.1	287.2	Cyanidin-3-O-(6-p-coumaroyl)-glucoside
17	53.77sh	625.1	317.2	Petunidin-3-O-(6-p-coumaroyl)-glucoside
18	54.33	639.1	331.2	Malvidin-3-O-(6-p-coumaroyl)-glucoside *cis*
19	58.75	609.1	301.2	Peonidin-3-O-(6-p-coumaroyl)-glucoside
20	59.69	639.1	331.2	Malvidin-3-O-(6-p-coumaroyl)-glucoside *trans*

All peak numbers refer to Figs. 1 and 2. Identifications are supported by previous work (Cho et al., 2004; Vivar-Quintana et al., 2002).

not present. Certain anthocyanins co-eluted with other anthocyanins and could be detected by using selected ion monitoring of the MS data at their respective m/z values and then confirmed by MS^2 data. The vitisin A derivative of petundin-3-O-glucoside was present as the forward shoulder of the petundin-3-O-glucoside peak (peak 5) and delphinidin acetyl-glucoside co-eluted with the vitisin A malvidin-3-O-glucoside peak (peak 8). The composition of anthocyanins is typical of a young red wine (Vivar-Quintana et al., 2002; Nunez et al., 2004; Perez-Magarino and Gonzalez-San Jose, 2004).

After the in vitro digestion procedure, only five anthocyanins could be detected in the IN and the OUT fractions (Fig. 2), which were confirmed as malvidin-3-O-glucoside (peak 6), the vitisin A adduct of peonidin-3-O-glucoside (peak 7), the vitisin A adduct of malvidin-3-O-glucoside (peak 8), the vitisin A adduct of malvidin-3-O-acetylglucoside (peak 10) and the vitisin A adduct of malvidin-3-O-coumaroylglucoside (peak 15). Malvidin-3-O-glucoside was recovered at 0.2% (% of amount in the post-gastric sample) in the IN fraction and 0.9% in the OUT fraction. These figures are considerably lower than the recovery of bulk anthocyanins in the IN fraction (3.7%) and the OUT fraction (37%). Malvidin-3-O-glucoside was the most abundant anthocyanin in the original sample and it is likely that other anthocyanins dropped below the limit of detection if they had similar recoveries (<1%). On the other hand, the vitisin A adducts were substantially more stable and exhibited a significantly better recovery than the bulk anthocyanins. The vitisin A derivative of malvidin-3-O-glucoside was recovered at 9.1% in the IN fraction and at 27.8% in the OUT fraction. The vitisin A derivative of malvidin-3-O-acetyl glucoside was recovered at 16.4% and 58.5% in the IN and OUT samples, respectively.

A small portion of the "lost" anthocyanins (∼1% total) remained insoluble after the OUT fraction had been acidified prior to solid phase extraction. These anthocyanins bound to the precipitated proteins and if so bound in vivo would be released in the fermentative colon (Aura et al., 2005) where they may be subject to degradation by microflora (Rechner et al., 2002) or excreted in faeces (He et al., 2005). After recovery with acidified methanol, the same anthocyanins were noted as found in the IN and OUT fractions, which suggested that there was no selective precipitation of anthocyanins.

The pH shift to >7.5 in the pancreatic/small intestine digestion was the main factor in the irreversible breakdown of the anthocyanins. Anthocyanins exist in equilibrium of four molecular species; the coloured basic flavylium cation and three secondary structures; the quinoidal bases, the carbinol pseudobase and the chalcone pseudobase forms. At pH 2 or below, the flavylium cation form predominates but as the pH is raised towards neutrality the colourless chalcone pseudobase begins to dominate. Chalcone formation is also favoured by elevated temperatures and prolonged exposure may enhance degradation between the B and C rings resulting in the destruction of the anthocyanin chromophore (Strack and Wray, 1993; Clifford, 2000). The nature of the breakdown products, which may be bioavailable and bioactive themselves, remains to be fully elucidated. Hydroxybenzoic acids, presumably derived from the B-ring of the aglycones, have been identified as products after incubation of anthocyanins at neutral pH and room temperature (Seeram et al., 2001). Protocatechuic acid was also identified in the serum of rats fed cyanidin-3-O-glucoside (Tsuda et al., 2000). Further studies with pure anthocyanins in simple systems are required to identify other breakdown products.
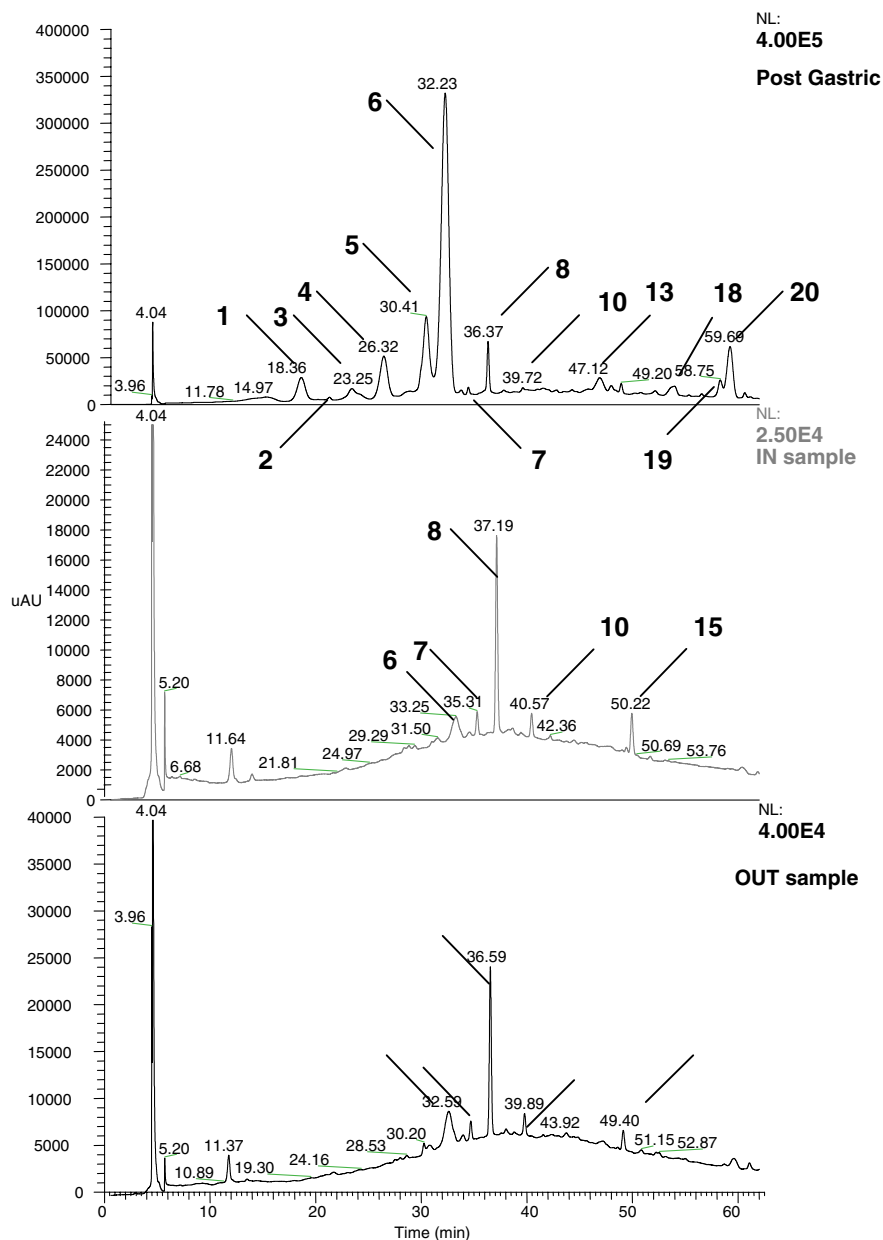
Fig. 2. HPLC chromatographs of samples from in vitro digestion procedure. The first panel shows the chromatograph for the post-gastric sample, the second panel, the serum-available sample and the third panel, the colon-available sample. The full scale for the detector is shown in the top right corner. The numbering of peaks follows Fig. 1 and assignments given in Table 2. For clarity, not all peaks are numbered.

Initial time-scale studies suggested that the difference in stability between vitisin A derivatives and their parent anthocyanins was apparent after 30 min of exposure to pH $\sim$ 7.5 (results not shown). The vitisin A adducts of anthocyanins are formed by reaction with pyruvate during fermentation (Vivar-Quintana et al., 2002). They are more stable to fermentation and ageing than their parent anthocyanins and accumulate to high levels in port wines (Bakker and Timberlake, 1997). The vitisin A derivatives have been found to be more stable to sulphur dioxide bleaching (Bakker and Timberlake, 1997) and presumably the addition of the pyruvyl group between the A and C rings of the anthocyanins (Diagram 1) protects the molecule from nucleophilic attack at position 4 (Timberlake and Bridle,

1967) thus preventing breakdown during pancreatic digestion. Anthocyanins have been assigned a number of biological activities which may bring health benefits (e.g. Joseph et al., 1999; Matsui et al., 2001; Tall et al., 2004; Jayaprakasam et al., 2005; Zhang et al., 2005). However, many of these experiments have been performed ex vivo on isolated organs, tissues, or cells and there is still little evidence that anthocyanins or their aglycones are sufficiently bioavailable (Serafini et al., 1998; Mazza et al., 2002; Bitsch et al., 2004) to have these effects in vivo when ingested. The vitisin A derivatives were substantially more stable to gastrointestinal conditions and may be more likely to become serum-available and exert biological effects at a cellular level. No data on the serum uptake of vitisin type

anthocyanins has been reported although there have been a number of studies on the bioavailability of red wine anthocyanins (Lapidot et al., 1998; Bub et al., 2001; Frank et al., 2003). Initial ex vivo experiments have suggested that vitisin A derivatives are slightly less biologically effective than their parent anthocyanins (Garcia-Alonso et al., 2004) but their enhanced stability warrants further research.

Acknowledgements

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