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Profiling and quantifying quercetin glucosides in onion (*Allium cepa* L.) varieties using capillary zone electrophoresis and high performance liquid chromatography

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Abstract

There is increasing evidence that flavonols demonstrate beneficial properties for human health. Quercetin is the major flavonol present in onion (*Allium cepa cv*) and is present predominantly as quercetin 3,4′-diglucoside and quercetin 4′-monoglucoside. These compounds are known to be potent free radical scavengers and antioxidants, and are considered to be protective against cardiovascular disease. Analysis for the presence of these compounds has therefore become more important. Robust capillary zone electrophoresis and high performance liquid chromatography procedures were developed for profiling and quantifying the levels of quercetin 3,4′-diglucoside and quercetin 4′-monoglucoside in 70% methanol/water extracts of six different onion varieties available in Victoria, Australia. Quercetin 3,4′-diglucoside, which is not commercially available as a reference standard, was isolated from freeze-dried onion powder by preparative high performance liquid chromatography and used to quantify the levels in the onion extracts. Significant differences in the levels and ratios of the two compounds were seen between red, brown and white onion varieties (e.g. 'Redwing'; quercetin 3,4′-diglucoside 191 mg/100 g DW, quercetin 4′-monoglucoside 85 mg/100 g DW; 'Cream Gold', quercetin 3,4′-diglucoside 153 mg/100 g DW, quercetin 4′-monoglucoside 58 mg/100 g DW, 'Spanish white'; quercetin 3,4′-diglucoside <1 mg/100 g DW, quercetin 4′-monoglucoside <1 mg/100 g DW).

Keywords: Profiling; Quantification; Quercetin glucosides; Onion; Capillary electrophoresis; High performance liquid chromatography

1. Introduction

Phytochemicals are biologically active, non-nutrient plant chemicals found in many commonly consumed fruits and vegetables (Schreiner & Huyskens-Keil, 2006). Flavonoids are the most commonly found phytochemicals, and typically these chemicals help protect the plant against UV light, fungal parasites, herbivores, pathogens and oxidative cell injury (Cook & Samman, 1996). When consumed regularly by humans, flavonoids have been linked to a reduction in the incidence of diseases such as cancer and heart disease (Beecher, 2003; Cook & Samman, 1996; Lako, Wattanapenpaiboon, Wahlqvist, & Trenerry, 2006; Liu, 2004). There is currently great interest in flavonoid research due to the possibility of improved public health through diet, where preventative health care can be promoted through the consumption of fruit and vegetables. Flavonols are a class of flavonoid commonly found in many fruits and vegetables, and content varies widely, depending on variety and

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environmental factors, such as growing conditions, growing climate, storage and cooking conditions (Lachman et al., 2003; Yang, Meyers, van der Heide, & Liu, 2004). Studies have shown that quercetin, which is the major flavonol present in onions, capers, apples, tea and berries (USDA, 2003), exhibits anti-cancer, anti-inflammatory, antiviral activity, and may also prevent cardiovascular disease in humans (Gil, Ferreres, & Tomas-Barberan, 1999). Quercetin occurs naturally in plants as conjugated glycosides, with the most common glycosides being quercetin 3,4′-diglucoside, quercetin 4′-monoglucoside and quercetin 3-monoglucoside. Furthermore, studies have shown that different flavonoid glycosides are preferentially absorbed in the small intestine through various uptake mechanisms, suggesting that certain glycosides may be more bioeffective (Hollman et al., 1997).

High-performance liquid chromatography (HPLC) with UV detection is the method of choice for the separation and quantification of flavonols in plant extracts (Merken & Beecher, 2000; Price & Rhodes, 1997). More recently, HPLC-mass spectrometry has been used to aid in the separation, quantification and structure elucidation of flavonoids found in many plants, vegetables and fruits (Cuyckens & Claeys, 2004; De Rijke, Zappey, Ariese, Gooijer, & Brinkman, 2003; Rauha, Vuorela, & Kostiainen, 2001; Rochfort, Imsic, Jones, Trenerry, & Tomkins, 2006; Tolonen & Uusitalo, 2004; Vallejo, Tomas-Barberan, & Ferreres, 2004). This technique was used by Bonaccorsi et al. (2005) to identify seven flavonols in samples of Southern Italian Red Onion (*Allium cepa* L.). Quercetin 4′-monoglucoside and quercetin 3,4′-diglucoside were the most abundant flavonols in the samples. Five minor flavonols were also identified and this provided a characteristic profile for the red onions used in this study. The individual flavonols were quantified and expressed as quercetin 4′-monoglucoside.

Capillary electrophoresis (CE), which is an alternative separation technique to HPLC, is especially suited for the separation and quantification of low to medium molecular weight polar and charged compounds and the resultant separations are often faster and more efficient than the corresponding HPLC separations (Frazier & Papadopoulou, 2003; Trenerry, 1998, 2001). However, there are very few examples of CE being used to separate and determine the levels of naturally occurring flavonols in plant material (Chen, Zhang, & Ye, 2000; Dadakova, Prochazkova, & Krizek, 2001; Pietta, Mauri, Rava, & Sabatini, 1991; Pietta, Bruno, Mauri, & Rava, 1992; Vaher & Koel, 2003; Wang & Huang, 2004). Capillary electrophoresis is also a powerful tool in the emerging discipline of metabolomics (Ramautar, Demirci, & de Jong, 2006).

The aim of this study was to develop robust CE and HPLC procedures for profiling and quantifying the major quercetin glycosides present in a range of onion varieties available in Victoria, Australia, and to demonstrate how these data could be used to differentiate between onion varieties.

2. Materials and methods

2.1. Reagents

Quercetin dihydrate, kaempferol, 0.1% formic acid in acetonitrile (HPLC–MS grade) and 0.1% formic acid in water (HPLC–MS grade) were obtained from Sigma Chemical Co, Sydney, Australia. Quercetin 4′-monoglucoside was obtained from Extrasynthese, France. Isorhamnetin was purchased from Carl Roth, Germany. All other chemicals and reagents were AR or HPLC grade. C18 Bondelut SPE cartridges (1 g) and bulk 40 μm C18 packing material were obtained from Varian Inc., Mulgrave, Australia, and Alltech Chromatography, Baulkham Hills, Australia, respectively.

2.2. Preparation of standards

Stock and working standards of quercetin, quercetin 4′-monoglucoside, quercetin 3,4′-diglucoside and sorbic acid were prepared in methanol. The solutions were stable for at least 1 month when stored at −20 °C. Sorbic acid was added as the internal standard for CE assays at a final concentration of 40 μg/ml.

2.3. Sampling and sample preparation

2.3.1. Sampling, preparation and storage

Samples of onion varieties were purchased from commercial outlets in Werribee, Victoria. Five medium onions of the same variety were cleaned, peeled and the inedible parts removed. The onions were then sliced into thin (5 mm) rings, frozen in liquid nitrogen and placed in a freeze drier and dried to a constant weight (72 h). The freeze-dried material was ground to a fine powder in a coffee grinder and stored in an airtight container at −20 °C. The powder was stable in this form for at least 4 months.

2.3.2. Sample extraction

2.3.2.1. Quercetin glucosides. Approximately 2 g of freeze dried onion powder was mixed with 50 ml of 70% v/v methanol/water. The mixture was homogenised for 5 min with a Bamix blender. The solution was filtered through a Whatman No. 4 filter paper under reduced pressure. The filter paper and residue were re-extracted with another 50 ml of 70% v/v methanol/water for 5 min. This was filtered through a Whatman No. 4 filter paper and the filtrates combined and made to 100 ml with methanol. For HPLC analysis, the solution was filtered through a 0.45 μm nylon filter disc prior to analysis. For CE analysis, the solution was reduced in volume to approx. 20 ml *in vacuo*, 1 ml of 1000 μg/ml sorbic acid solution (internal standard) added and the volume made to 25 ml with methanol. The solution was filtered through a 0.45 μm nylon filter disc before analysis. The solutions were stable at room temperature for 14 days.

2.3.2.2. Onion autolysis (Price & Rhodes, 1997). Autolysis of a sample of freeze dried 'Cream Gold' onion powder was carried out as described by Price and Rhodes (1997).

2.3.2.3. Total quercetin (Hertog, Hollman, & Katan, 1992). Freeze dried onion powder (0.5 g) was mixed with 40 ml 37.5% v/v aqueous methanol containing 2 g/l tetrabutyl hydroxy quinone (TBHQ) and 10 ml of 6 N HCl solution. The mixture was stirred and heated under reflux for 2 h. The solution was cooled and made to 100 ml with methanol. A portion of the extract was filtered through a 0.45 μm nylon filter disc and the levels of quercetin determined by HPLC with UV detection at 370 nm.

2.3.2.4. Preparative HPLC. Extraction of quercetin glucosides from freeze dried onion powder.

Freeze dried 'Cream Gold' onion powder (10 g) was extracted with 200 ml 70% v/v methanol/water for 5 min with a Bamix blender. The mixture was sonicated for 5 min before filtering through a Whatman No. 540 filter paper under vacuum. The combined residue and filter paper were re-extracted with 200 ml 70% v/v methanol/water for a further 5 min and filtered. The residue was re-extracted with a further 200 ml 70% v/v methanol/water and filtered. The filtrates were combined and the solvent removed *in vacuo* to reveal approx. 10 g of a wet gummy residue. This was dissolved in a minimum of 80% v/v methanol/water and loaded (under vacuum) onto a 7 g 40 μm C18 reverse phase flash chromatography column that had been previously activated with methanol and water. The column was washed with water (50 ml) and the quercetin glucoside fraction removed from the column with methanol (50 ml). The solvent was removed *in vacuo* and the residue re dissolved in 5 ml of 80% v/v methanol/water. This was mixed with 1 g of C18 powder and the solvent removed *in vacuo*. Approximately 1 g of acid washed sand was placed inside a stainless steel precolumn (45 × 25 mm i.d.). The dried residue-C18 powder was then loaded onto the sand. The precolumn was then filled with acid washed sand and connected to the inlet side of the preparative HPLC column. Pure quercetin 3,4′-diglucoside (17 mg) and pure quercetin 4′-monoglucoside (12 mg) were isolated from the residue-C18 powder using the conditions described in Section 2.4.3. The structures were confirmed from the UV, mass and NMR spectral data (Fossen, Pedersen, & Andersen, 1998; Price & Rhodes, 1997).

2.4. Apparatus

2.4.1. CE

2.4.1.1. Profiling and quantification of quercetin 3,4′-diglucoside and quercetin 4′-monoglucoside. The extracts were analysed with a 57 cm × 75 μm uncoated fused silica capillary column (SGE, Ringwood, Australia) with an effective length of 49.4 cm fitted to a Beckman P/ACE model 5010 Capillary Electropherograph (Beckman Instruments, Inc. Ca, USA). The separations were performed at 25 kV and at 25 °C using a buffer consisting of 10 mM boric acid, 10 mM sodium tetraborate and 15 mM EDTA dissolved in 15% v/v methanol/water. The pH was adjusted to 10.2 with 1.0 M sodium hydroxide solution. The capillary was conditioned by washing with 0.1 M sodium hydroxide solution (15 min), water (15 min) and running buffer (15 min) on a daily basis and then rinsed with running buffer for 1 min between analyses. The electropherograms were recorded with a fixed UV–Vis wavelength detector set at 280 nm. Data processing was carried out by P/ACE system software 5000 series on IBM personal computer 340. Solutions were loaded onto the column under "high pressure" for 2 s. The detector response for quercetin 3,4′-diglucoside and quercetin 4′-monoglucoside was linear to at least 200 μg/ml with these conditions. Peak areas were used in the calculations. A "standard addition" procedure was used to quantify quercetin 3,4′-diglucoside, and an "external standard" procedure was used to quantify quercetin 4′-monoglucoside.

2.4.2. Analytical HPLC

Profiling and quantifying quercetin 3,4′-diglucoside and quercetin 4′-monoglucoside.

The analyses were performed with an Agilent series 1100 quaternary solvent delivery system, cooled autosampler (4 °C) and photodiode array detector (Agilent, Waldron Germany) connected to a Thermo Electron LTQ ion trap mass spectrometer operating in the negative ion electrospray mode (Thermo Electron, San Jose, USA). For profiling, the compounds were separated on a 2.1 × 150 mm 3 μm Alltech Prevail C18 column (Alltech Chromatography, Baulkham Hills, Australia) fitted with a C18 guard column. The column was maintained at 30 °C. The mobile phase consisted of (A) 0.1% formic acid in water and (B) 0.1% formic acid in methanol with the following gradient: 20–80% B (0–10 min), 80% B (10–20 min), 80–20% B (20–20.5 min), 20% B (20.5–25 min) with a flow rate of 0.2 ml/min. For quantification, the compounds were separated on a 2.1 × 150 mm 3 μm BDS C18 Hypersil HPLC column fitted with a C18 guard column maintained at 30 °C and a mobile phase of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile with the following gradient: 5% B (0–5 min), 5–80% B (5–25 min), 80% B (25–30 min), 80–5% B (30–31 min), 5% B (31–35 min) with a flow rate of 0.2 ml/min. All chromatograms were monitored at 280, 346, 364 and 370 nm. Excalibur V2.1 software was used to process the chromatographic data.

2.4.3. Preparative HPLC

Preparative HPLC was performed with a Waters 600 E solvent delivery system connected to a Waters 717 plus autosampler. The column effluent was monitored with a Waters 996 photodiode array detector and the fractions were collected with a Waters Fraction Collector (Waters Chromatography, Sydney, Australia). Waters Empower software was used to monitor the chromatography. The compounds were separated using a Varian Polaris 5 μm

C18–A 250 × 21.2 mm preparative HPLC column (Varian Inc., Mulgrave, Australia) and a mobile phase consisting of (A) 0.1% formic acid in water and (B) 0.1% formic acid in methanol with the following gradient: 5% B (0–5 min), 5–45% B (5–45 min), 45–80% B (45–55 min), 80% B (55–56 min), 80–5% B (56–58 min), with a flow rate of 10 ml/min.

2.4.4. NMR

NMR spectra (^{1}H and 2D spectra) were obtained on a Bruker 800 MHz spectrometer, equipped with a cryoprobe. Spectra were acquired at 25 °C in d_6-DMSO and referenced to residual ^{1}H signals in the deuterated solvent (δ 2.54).

3. Results and discussion

3.1. Capillary electrophoresis

Capillary electrophoresis (CE) is a very powerful technique and has been used extensively for the separation and determination of a wide range of low molecular weight compounds in food and plant material, however, there are limited applications for the determination of quercetin and its metabolites in plant material (Chen et al., 2000; Wang & Huang, 2004). Morin, Vallard, and Dreus (1993a, 1993b) used differently substituted flavonols as model compounds to demonstrate the impact of borate-sugar complexation during electro driven separations. Price and Rhodes (1997), using a buffer consisting of 20% methanol, 25 mM borate pH 9.2, were able to separate quercetin and its major metabolites, quercetin 4′-monoglucoside and quercetin 3,4′-diglucoside, after onion autolysis, whilst Dadakova et al. (2001) used micellar capillary electrokinetic chromatography to quantify the levels of quercetin in a variety of samples.

We were unable to successfully reproduce the CE separation of quercetin, quercetin 4′-monoglucoside and quercetin 3,4′-diglucoside in a sample of freeze dried "Cream Gold" onion powder after autolysis using the conditions described by Price and Rhodes (1997). The autolysis experiment was conducted to provide a 'natural' sample containing quercetin and the two glucosides as this best represented samples that would be routinely assayed. Even though onions contain very little free quercetin, it is important to successfully electrophorese quercetin as it is an indicator of quercetin glucoside breakdown either through autolysis or through hydrolysis during extraction. The quercetin peak became quite broad and diffuse after successive injections of the extract. The poor peak shapes were seen with different batches of silica columns from three different suppliers and extensive column conditioning with either 1 M NaOH or 1 M HCl, or the use of high ionic strength buffers, had no effect on the peak shape. A broad peak, which also deteriorated after successive injections and column cleaning, was also recorded with the MECC conditions described by Dadakova et al. (2001). One possible explanation for the poor peak shape is the complexation of the quercetin by metal ions present either in the buffer reagents or in the silica capillary column. Poor chromatography of flavonols has previously been reported in liquid chromatography when the silica packing material contained high levels of metal ions (Jones, Lim, Ferry, &

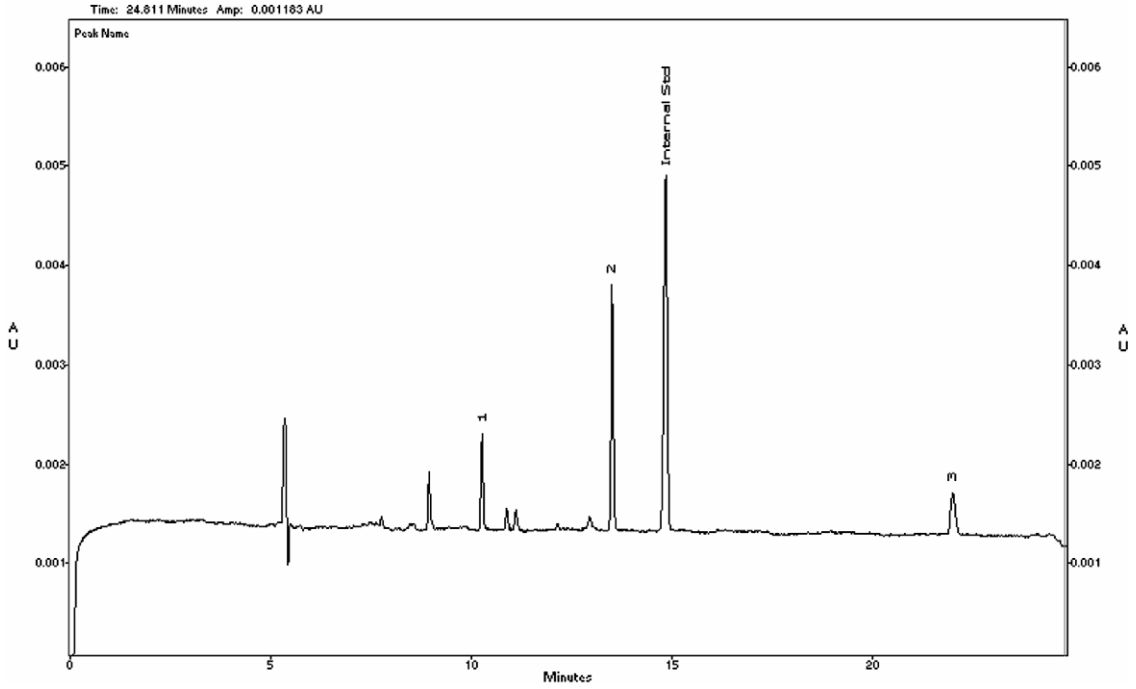

Fig. 1. Electropherogram showing the separation of: (1) quercetin 3,4′-diglucoside; (2) quercetin 4′-monoglucoside, the internal standard and (3) quercetin using the conditions described in Section 2.4.1.

Gescher, 1998). The addition of 1 M EDTA to the mobile phase, which complexes with the metal ions in the HPLC silica, produced sharp reproducible peaks. The addition of 1 M EDTA to the CE running buffer also produced a sharp peak for quercetin, and this peak shape was maintained with successive injections.

The buffer was further optimised with respect to the borate and EDTA concentrations, pH and type of organic modifier. Fig. 1 shows the separation of quercetin, quercetin 4′-monoglucoside and quercetin 3,4′-diglucoside using a buffer consisting of 15% methanol, 15 mM EDTA, 10 mM boric acid, 10 mM borate pH 10.2 operating at 25 kV. Flavonols and their metabolites absorb strongly between 340 and 370 nm, however, it was not possible to monitor the electropherograms at these wavelengths due to the lack of a suitable UV–Vis filter. Therefore, the electropherograms were

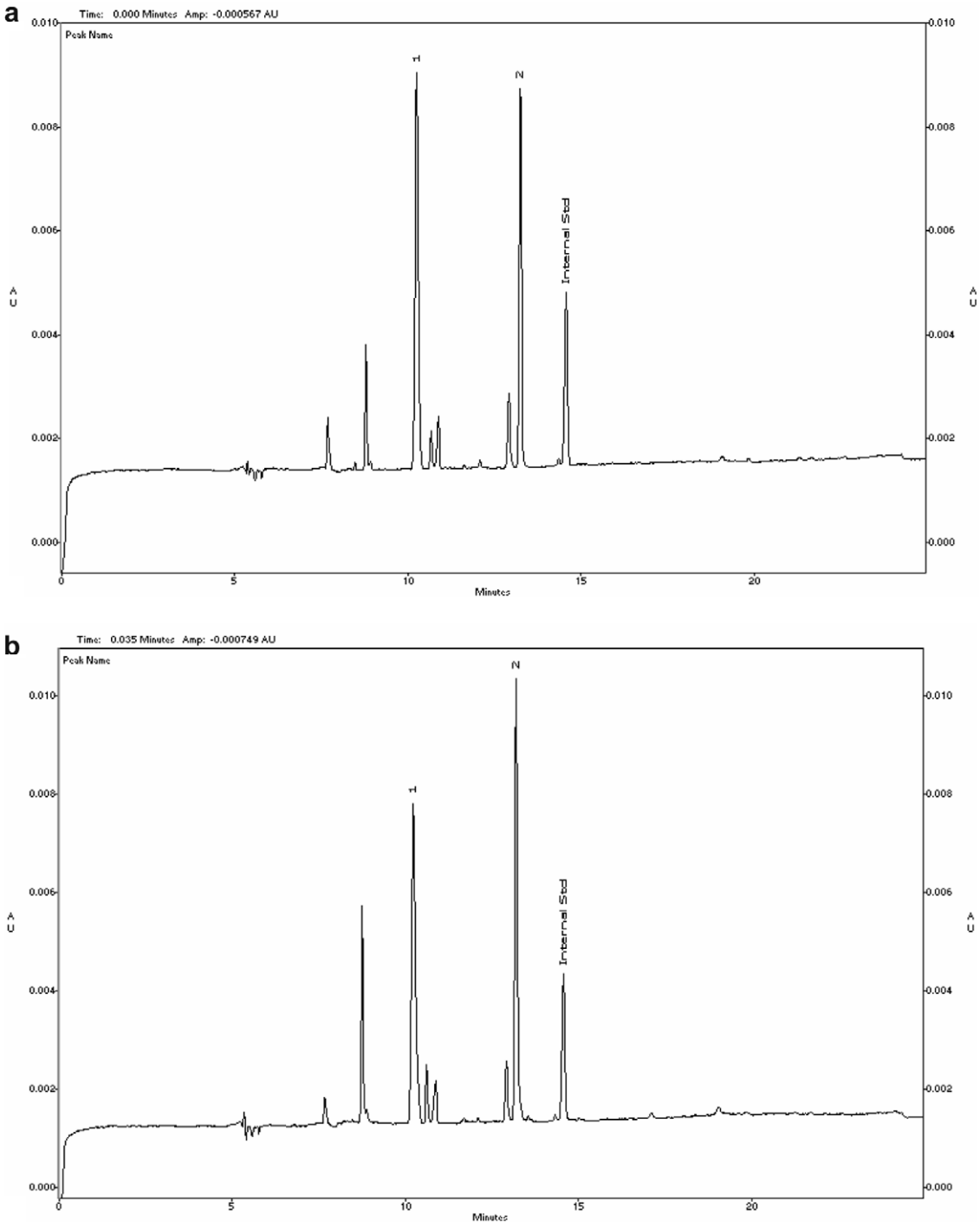

Fig. 2. Electropherograms showing: (a) 'Cream Gold' onion extract; (b) 'Redwing' onion extract and (c) 'Spanish white' onion extract using the conditions described in Section 2.4.1. See Fig. 1 for peak assignments.

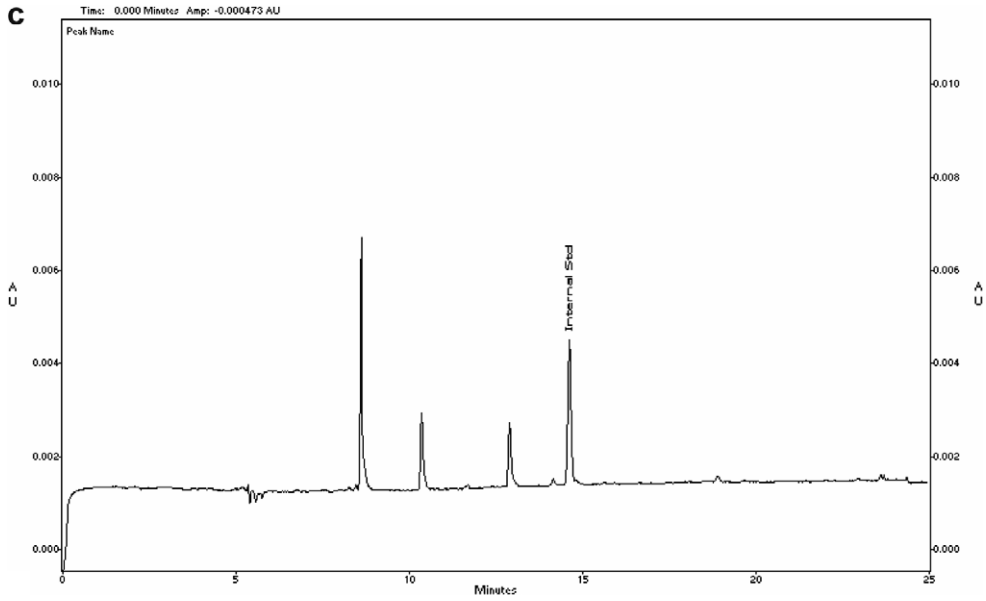

Fig. 2 (*continued*)

monitored at 280 nm, as this was the best compromise between sensitivity and electropherogram complexity.

Electropherograms of the onion varieties "Cream Gold" (brown), "Redwing" (red) and "Spanish white" (white) onion extracts are displayed in Fig. 2a–c. Considerable levels of the two quercetin glucosides are seen in the 'Cream Gold' and 'Redwing' varieties, however, the compounds were not present in the 'Spanish white' variety. The separation deteriorated slightly after six repeated analyses, with the co-migration of quercetin 3,4′-diglucoside and quercetin 4′-monoglucoside with other compounds of similar migration times. The separation reverted to normal after column cleaning (1 M NaOH) and buffer replenishment. Sorbic acid, which absorbs strongly at 280 nm, was added to the extract as an internal standard to compensate for peculiarities in the injection procedure and for the gradual change in migration times that are often seen due to buffer degradation and column fouling (Weinberger, 1993).

Suitable peak area and migration time repeatability data were recorded for this separation ($n = 6$, %CV peak area, quercetin 3,4′-diglucoside 2.0%, quercetin 4′-monoglucoside, 2.8%; %CV migration time, quercetin 3,4′-diglucoside 0.4%, quercetin 4′-monoglucoside 0.6%).

3.2. High performance liquid chromatography

The separation of quercetin, quercetin 4′-monoglucoside and quercetin 3,4′-diglucoside was based on a HPLC-UV procedure outlined by Fossen et al. (1998). The authors achieved a suitable separation with a 200 × 5 mm stainless steel C18 column and a mobile phase consisting of methanol/10% formic acid/water gradient and a flow rate of 1.2 ml/min. The separation parameters were modified to suit standard LC–MS conditions: i.e. a 150 × 2 mm HPLC column was used with the amount of formic acid in the mobile phase reduced to 0.1% and the flow rate lowered to 0.2 ml/min (see Section 2.4.2). The separations were monitored with a photodiode array detector operating in the full scan mode between 190 and 600 nm and a linear ion trap mass spectrometer operating in the (−)ve ion ESI mode. Chromatograms were extracted at 280 nm (for comparison with the CE data), 370 nm and at the peak maxima for the two quercetin glucosides (346 nm and 364 nm). Fig. 3 shows the chromatogram of a 'Redwing' onion extract recorded at the spectral maxima for each compound using the conditions described in Section 2.4.2. The identities of quercetin 4′-monoglucoside and quercetin 3,4′-diglucoside were confirmed from the UV and mass spectral data. Two minor components in the extract were identified as isorhamnetin 3,4′-diglucoside and isorhramnetin 4′-monoglucoside from the UV and mass spectral data (Price & Rhodes, 1997). Milligram quantities of quercetin 4′-monoglucoside and quercetin 3,4′-diglucoside were also isolated by preparative HPLC and further characterised by H^1 NMR spectroscopy (Fossen et al., 1998). Peak area and retention time repeatability were acceptable ($n = 20$, %CV peak area quercetin 3,4′-diglucoside 2%; quercetin 4′-monoglucoside 1.8%; %CV retention time, quercetin 3,4′-diglucoside 2.7%; quercetin 4′-monoglucoside 2.3%).

3.3. Profiling of freeze dried onion extracts

Profiling provides a rapid assessment tool to gauge the levels of the two major quercetin metabolites during various stages of the growing cycle/storage if quantification, through the non availability of pure standard compounds, is not possible.

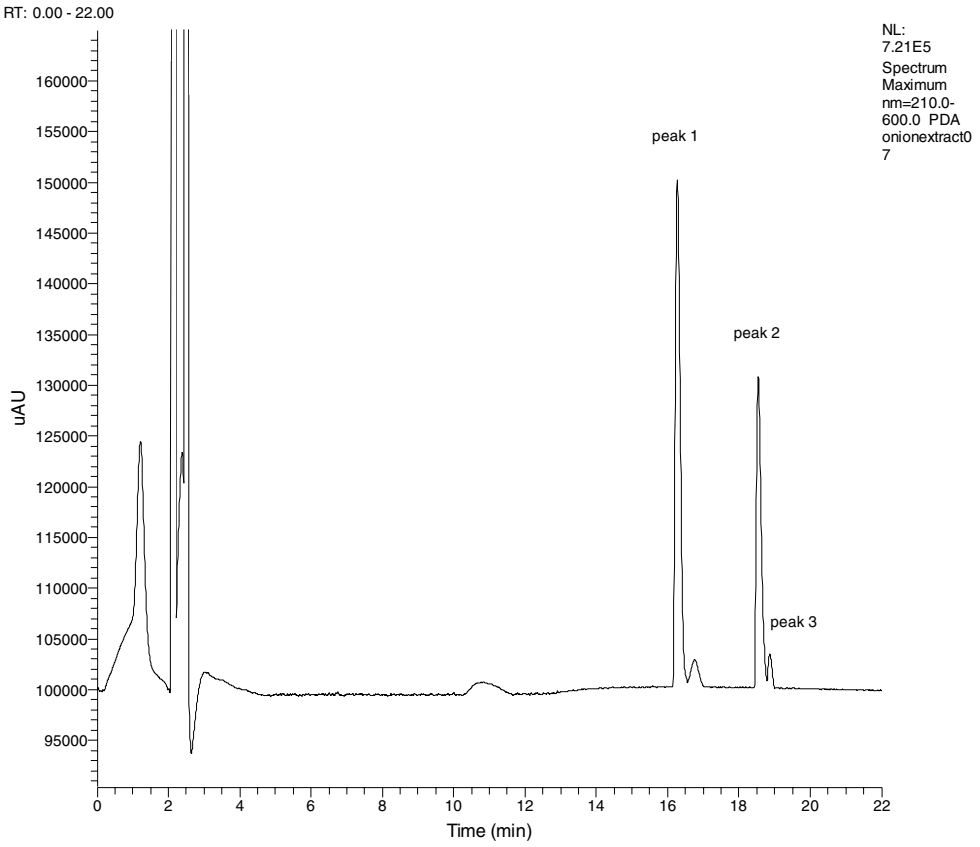

Fig. 3. Chromatogram of a 'Redwing' onion extract showing the separation of quercetin 3,4′-diglucoside (peak 1), quercetin 4′-monoglucoside (peak 2) and isorhamnetin 4′-monoglucoside (peak 3) using the conditions described in Section 2.4.2.

Freeze dried onion extracts were prepared and profiled with the CE and HPLC conditions described in Sections 2.4.1 and 2.4.2 respectively. The relative ratios of the peak areas for quercetin 3,4′-diglucoside and quercetin 4′-monoglucoside were measured at 280 nm for CE and 280 nm, 370 nm and at the UV maxima (346 nm and 364 nm) for HPLC and are displayed in Table 1.

The different ratios measured at 280 nm for CE and HPLC are due to a number of factors which include detector configuration (CE, fixed wavelength UV; HPLC diode array) different solvents in the running buffer/mobile phase and the pH of the CE buffer (alkaline) and HPLC mobile phase (acid). The two red onion varieties ('Tango' and 'Redwing') had similar profiles by CE and by HPLC, as did two of the three brown onions (''Murray" and ''Patrick"). The profiles for the 'Cream Gold' (brown) variety were significantly different from the other two brown onions. The ''Spanish white" onion contained only trace amounts of the two glucosides (measured by LC-diode array-ms).

The similarities/differences between the ratios of quercetin 3,4′-diglucoside and quercetin 4′-monoglucoside in onion varieties can also represented graphically with excel graphs. The ratios determined by CE are shown in Fig. 4.

Table 1
Relative ratios of the peak areas, measured at 280 nm for CE and at 280 nm, 370 nm and at the UV maxima for quercetin 3,4′-diglucoside (346 nm) and quercetin 4′-monoglucoside (364 nm) for HPLC

Onion variety	CE (280 nm)		HPLC (280 nm)		HPLC (370 nm)		HPLC (UV maxima)	
							346 nm	364 nm
	Q 3,4′-dig	Q 4′-monog	Q 3,4′-dig	Q 4′-monog	Q 3,4′-dig	Q 4′ monog	Q 3,4′-dig	Q 4′-monog
Tango (red)	61	39	70	30	49	51	57	43
Redwing (red)	60	40	68	32	47	53	54	46
Murray (brown)	54	46	65	35	43	57	50	50
Patrick (brown)	53	47	63	37	41	59	48	53
Cream gold (brown)	66	33	74	26	54	46	62	38
Spanish white (white)	nd	nd	<1	<1	<1	<1	<1	<1

Q 3,4′-dig = quercetin 3,4′-diglucoside; Q 4′-monog = quercetin 4′-monoglucoside; nd = not detected.

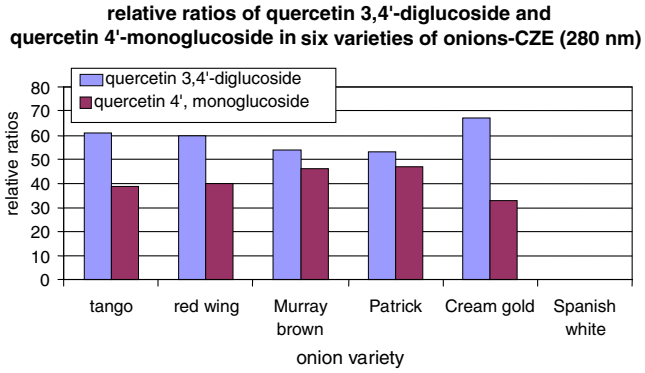

Fig. 4. Excel graph showing the similarities/differences between the ratios of quercetin 3,4′-diglucoside and quercetin 4′-monoglucoside in onion varieties determined by CE.

3.4. Quantification

The robust CE and HPLC separations afford the opportunity to quantify the major glucosides present in the onions. Quercetin 4′-monoglucoside is readily available commercially and milligram quantities of pure quercetin 3,4′-diglucoside were obtained by preparative HPLC-see Section 2.4.3. Similar levels of the respective compounds were obtained by either technique, e.g. 'Redwing' onion (quercetin 3,4′-diglucoside; CE 190 mg/100 g DW, HPLC 191 mg/100 g DW; quercetin 4′-monoglucoside; CE 96 mg/100 g DW, HPLC 86 mg/100 g DW). The levels of quercetin 4′-monoglucoside and quercetin 3,4′-diglucoside in the red onion varieties are similar to those reported by Bonaccorsi, Caristi, Gargiulli, and Leuzzi (2005) when corrected for estimated (90%) moisture content (Lewis & English, 1989; Ostrowska et al., 2004). For example, red onions, quercetin 3,4′-diglucoside; range 19–25 mg/100 g FW, literature, range 25–27 mg/100 g FW; quercetin 4′-monoglucoside; range 9–13 mg/100 g FW, literature, range 21–23 mg/100 g FW. It was also noted that the three brown onion varieties had lower amounts of quercetin 4′-monoglucoside compared to quercetin 3,4′-diglucoside.

Also, the sum of levels of the two major quercetin glucosides, obtained by solvent extraction and expressed as "quercetin equivalents", was similar to the level of total quercetin determined after acid hydrolysis (total quercetin, HPLC 158 mg/100 g DW; "quercetin equivalents", HPLC; 148 mg/100 g DW, CE 155 mg/100 g DW). The data are displayed in Table 2. The "quercetin equivalent" levels are similar to the total quercetin levels reported in the USDA database for the flavonoid content of selected foods (USDA data base, 2003) when corrected for the estimated moisture content. For example red onions-range 15–21 mg/100 g FW, USDA database 'mean' 19.3 mg/100 g FW. Summing the levels of quercetin 4′-monoglucoside and quercetin 3,4′-diglucoside and expressing the result as "quercetin equivalents" provides a faster alternative to estimating the total quercetin levels by acid hydrolysis (Hertog et al., 1992).

Table 2
Levels of quercetin 3,4′-diglucoside, quercetin 4′-monoglucoside and "quercetin equivalents" in the six onion varieties determined by HPLC-DAD

Onion variety	Quercetin 3,4′-diglycoside (mg/100 g DW)	Quercetin 4′-monoglycoside (mg/100 g DW)	Quercetin equivalents (mg/100 g DW)
Tango	252	126	205
Redwing	191	85	148
Murray	209	132	188
Patrick	404	286	383
Cream gold	153	58	112
Spanish white	<1	<1	<1

These results reflect the significant differences in quercetin content between onion varieties that have been reported previously. Patil, Pike, and Yoo (1995) reported similar quercetin content in red and yellow onions, but content in white onions was 10–20-fold lower. Total flavonoid content was reported to be approximately 10-fold lower in white onions than yellow varieties (Yang et al., 2004). Although Price and Rhodes (1997) reported quercetin contents approximately 10 times higher in 4 brown, red and white varieties than both Patil et al. (1995) and the present study, content in the white variety 'Albion' was approximately 20-fold lower than red varieties. Consumers wishing to maximise their quercetin intake should therefore choose brown and red onion varieties, rather than whites.

4. Conclusion

Rapid and robust CE and HPLC procedures have been developed for the profiling and quantification of quercetin 3,4′-diglucoside and quercetin 4′-monoglucoside in six varieties of onions available in Victoria, Australia. Profiling provides a rapid assessment tool to gauge the levels of the two major quercetin metabolites during various stages of the growing cycle if quantification is not possible. Significant differences in the levels of the two glucosides were seen between onion varieties; in particular, only trace amounts were present in the 'Spanish white' variety. Also, summing the amount of the two major glucosides provides a good estimate of the level of total quercetin in the sample.

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