Rapid methods for extracting and quantifying phenolic compounds in citrus rinds

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Keywords
Extraction method, flavanone glycosides, phenolic acids, phenolics

Abstract
Conventional methods for extracting and quantifying phenolic compounds in citrus rinds are time consuming. Rapid methods for extracting and quantifying phenolic compounds were developed by comparing three extraction solvent combinations (80:20 v/v ethanol:H₂O; 70:29.5:0.5 v/v/v methanol:H₂O:HCl; and 50:50 v/v dimethyl sulfoxide (DMSO):methanol) for effectiveness. Freeze-dried, rind powder was extracted in an ultrasonic water bath at 35°C for 10, 20, and 30 min. Phenolic compound quantification was done with a high-performance liquid chromatography (HPLC) equipped with diode array detector. Extracting with methanol:H₂O:HCl for 30 min resulted in the optimum yield of targeted phenolic acids. Seven phenolic acids and three flavanone glycosides (FGs) were quantified. The dominant phenolic compound was hesperidin, with concentrations ranging from 7500 to 32,000 µg/g DW. The highest yield of FGs was observed in samples extracted, using DMSO:methanol for 10 min. Compared to other extraction methods, methanol:H₂O:HCl was efficient in optimum extraction of phenolic acids. The limit of detection and quantification for all analytes were small, ranging from 1.35 to 5.02 and 4.51 to 16.72 µg/g DW, respectively, demonstrating HPLC quantification method sensitivity. The extraction and quantification methods developed in this study are faster and more efficient. Where speed and effectiveness are required, these methods are recommended.

Introduction
Citrus fruit has a high concentration of natural bioactive compounds with a positive influence on antioxidant capacity (Xu et al. 2008a,b; Tomas-Barberan and Andres-Lacueva 2012). As an effective bioactive compound source, rinds of citrus fruit can be explored for health promoting food product values. The phenolic compound profile and concentration in citrus fruit rind has received scientific interest in recent years, due to antioxidant capacity (Manthey and Grohmann 1996; Li et al. 2006; Xu et al. 2008b; Khan et al. 2010; Sun et al. 2010).

The phenolic profile of citrus fruit rinds consists of numerous compounds such as coumarins, psoralens, phenolic acids, and flavonoids (Benavente-García et al. 1998; Bocco et al. 1998). Flavonoids in citrus rinds are
represented by two classes of compounds referred to as flavanone glycosides (FGs) and polymethoxylated flavones (Benavente-García et al. 1998). These two classes of flavonoids are found only in citrus fruit, and their presence or absence is specific for each species and therefore could be used as taxonomic markers and be related to postharvest physiology (Manthey and Grohmann 1996; Tomnomic markers and be related to postharvest physiology (Manthey and Grohmann 1996; Tomán 2003; Mathur et al. 2011). The polymethoxylated flavones occur in relatively lower concentrations but exhibit higher biological activity than phenolic acids and FGs, which are the main primary groups of phenolic compounds in citrus rinds (Benavente-García et al. 1998; Ma et al. 2008; Simonne and Ritenour 2011; Ye et al. 2011).

An abundant flavonoid group found in different parts of citrus fruit are FGs including hesperidin, neohesperidin, narirutin, and didymin (Khan et al. 2010; Fabri-Karoui and Marzouk 2013). FGs are unique to citrus and are characteristic of some species and varieties (Tomás-Barberán et al. 2003). A classic example is hesperidin which is a major component in rind tissues of oranges and mandarins. Naritin on the other hand is a predominant FG in grapefruit (Kalt et al. 1999). The concentrations of FGs may differ due to differences in fruit maturity, environmental conditions during growth and development, postharvest treatments, and storage conditions (Abad-García et al. 2012). Thus, these compounds have a potential to be used as biochemical indicators of fruit origin, species and cultivar.

The health-related beneficial characteristics of some phenolic compounds have led to a number of studies to develop better extraction, identification, and quantification methods. Many analytical methods are widely used to determine and quantify phenolic compounds in citrus fruit (Ahmad et al. 2006). High-performance liquid chromatography (HPLC) is the most used technique for analysis of individual compounds (Li et al. 2006).

Extraction of compounds from plant materials is one of the most important steps prior to their determination by HPLC. Conventional extractions are usually time consuming and require relatively large quantities of solvents. It is also well known that the complexity of phenolic compounds in plant matrices makes extraction difficult (Manthey and Grohmann 1996). In recent years, some novel extraction methods of phenolic compounds have been developed including enzyme-assisted extraction methods (Li et al. 2006), ultrasound-assisted extraction (Khan et al. 2010), ultrasonic extraction (Ma et al. 2008), microwave-assisted extraction (Ahmad et al. 2006), and the use of solvents like dimethyl sulfoxide (DMSO), methanol-DMSO mixtures, and dimethylformamide (Manthey and Grohmann 1996).

A major trend in modern HPLC is the reduction in particle size and column length to allow very fast separations with greater resolution (Gritti and Guiochon 2012). The use of smaller particles in packed-column LC to provide increased efficiencies is currently the most prevalent method employed in liquid phase separation (de Villiers et al. 2006). As a result of this new leap forward in column technology, manufacturers began to produce and commercialize shorter columns, down to between 50 and 150 mm, which are as or more efficient than longer columns (Omamogho et al. 2011). This study was therefore conducted to develop a fast faster extraction method and rapid HPLC method for the quantification of phenolic compounds in ‘Nules Clementine’ mandarin rind tissues.

**Materials and Methods**

**Chemicals**

All chemicals were of analytical grade. Polyphenols (p-hydroxybenzoic acid, chlorogenic acid, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid, sinapic acid, naringin, and hesperidin) standards were purchased from Sigma Aldrich (Dorset, UK). Narirutin and didymin standards were purchased from Extrasynthese (Lyon, France). Acetonitrile, methanol, and formic acid were all of HPLC grade, dimethyl sulfoxide (DMSO) was analytical grade, and purchased from Fisher Scientific Chemicals (Leics., UK). Solutions and solvents were prepared with Milli-Q water (Milipore Inc. (Molsheim, France); $\sigma = 18$ mol/L $\Omega/$cm).

**Plant material and sample preparation**

A total of 20 “Nules Clementine” mandarin (Citrus reticulata Blanco) fruit were harvested in 2012 from an orchard at Stellenbosch University experimental farm, Western Cape Province, South Africa ($33^\circ 53'04.56"$S, $18^\circ 37'36.84"$E). These fruit were selected, weighed, peeled, and the rind snap-frozen in liquid nitrogen and stored at ultralow temperature of $-80^\circ$C. Fresh frozen samples were then freeze-dried in a Labogene ScanVac CoolSafe Freeze Dryer System (CS55-4, Lynge, Denmark) for 7 days at 0.015 kPA and $-55^\circ$C. Lyophilized samples were ground using a pestle and mortar into fine powder. To achieve standard particle size, the ground material was sieved through a 1-mm metal sieve. Large particles remaining on the sieve were further ground until all the material passed through the sieve. Ground samples were returned into the freezer until extraction and further analysis.

**Polyphenol extraction method**

Three different extraction solvent combinations and three extraction times were compared for effectiveness. The extraction solvents included aqueous ethanol [80:20; v/v, ethanol:H$_2$O] (Xu et al. 2008a), acidic aqueous methanol
ratios were as follows: 0–10% B during 5 min, 5–10% B up to 10 min; 10–12% B up to 16 min, 12–15% up to 25 min, 15–100% B up to 27 min. For FGs, the solvent gradient conditions were 0–5% B during 5 min, 5–10% B up to 10 min, 10–12% B up to 16 min, 12–15% up to 25 min, 15–100% B up to 27 min. For naringin, the concentration of phenolic compounds was 94.3% to 103.7%.

**HPLC quantification of polyphenols**

Quantification of phenolic compounds was executed on an Agilent 1200 series HPLC equipped with an Agilent DA G1315B/G1365G diode array detector. (DAD) with multiple wavelength detector, degasser and cooled autosampler (Agilent Technologies, Berks, UK). The system was operated by Windows NT-based ChemStation© software (Agilent Technologies), which was also used for data processing. Citrus rind extracts (20 μL) were injected into a Poroshell 120 column (4.6 × 150 mm and 2.7 μm particle size, Agilent), which was held at 40°C. The flow rate of the mobile phase was set at 1 mL/min. The mobile phases consisted of two solvents, 0.1% (v/v) formic acid: water (A) and 80% (v/v) acetonitrile:water (B). The mobile phase was filtered through a 0.2 μm syringe-driven filter (Millipore corporation, Billerica, MA).

**Extraction recovery and preparation of standard solution**

The recovery of different phenolic compounds was evaluated using a pooled rind sample extracted as above. Briefly, freeze-dried samples were prepared, spiked with specific concentration of naringin and cinnamic acid (16 μg/mL) in triplicates. The recoveries were calculated based on a method described elsewhere (Chang et al. 1997). The recovery of these phenolic compounds ranged from 94.3% to 103.7%. A mixed standard solution (5 mg/mL) was prepared by transferring all measured phenolic compounds into the extraction solvent. Eight concentration levels of the mixed standard solution were prepared by serial dilution of the stock solution. Concentrations of phenolic acids were determined from linear standard calibration curves (R² = 0.99).

**Limit of detection and limit of quantification**

The limit of detection (LOD) and limit of quantification (LOQ) for phenolic compounds were calculated by repeatedly (n = 10) injecting known concentration of a mixture of standard solution. The LOD and LOQ values were calculated as the amount of each individual phenolic compound required to give the signal to noise ratio of 3:1 and 10:1, respectively (Bressolle et al. 1996).

**Statistical analysis**

Statistical analyses were carried out using SPSS 10.0 for Windows (SPSS Inc. Chicago, IL). Data were subjected to analysis of variance (ANOVA). Duncan’s multiple-range tests were used to compare the significant differences in the mean values (P ≤ 0.05).

**Results and Discussion**

**Development and Discussion**

Dry powder samples of mandarin rind were extracted with 80:20 (v/v) aqueous ethanol compared to acidic aqueous methanol 70:29.5:0.5 (v/v/v; methanol:H₂O:HCl) and 50:50 (v/v; DMSO:methanol) to determine the efficacy of the extraction procedure for optimum phenolic acid and flavanones yield. Extraction solvent and extraction time were the two main parameters that affected the yield of phenolic compounds (Table 1). The concentration of phenolic acids increased with an increase in ultrasonic extraction time, while flavanones stayed the same. Results showed that an extraction period of 30 min using 70:29.5:0.5 (v/v/v; methanol:H₂O:HCl) and 50:50 (v/v; DMSO:methanol) was sufficient to extract phenolic acids. For example, the concentration of ferulic acid after extraction using acidic methanol for 10, 20, and 30 min, gradually increased (12.43, 13.37, 25.19 μg/g DM), respectively. The same trend was observed for sinapic acid, where the corresponding concentrations were 41.35, 61.23, and 64.87 μg/g DM. In general, phenolic acids yield was higher in samples extracted for 30 min using aqueous methanol. For flavanones, the highest yield was observed in samples extracted using 50:50 (v/v; DMSO:methanol) for 10 min. However,
phenolic acids yield was lower using this extraction combination. The concentrations of phenolic acids are similar to those reported by Xu et al. (2008a,b). Therefore, acidic aqueous methanol extraction in ultrasonic bath for 30 min is suitable to extract phenolic acids and 50:50 (v/v; DMSO: methanol) for 10 min was ideal to extract flavanones. By using these methods, extraction time was reduced significantly from 1, 3, 24, and 72 h reported by Xu et al. (2008a), Li et al. (2006), Manthey and Grohmann (1996), and Mathur et al. (2011), respectively.

**Development of HPLC quantification for polyphenols**

A typical chromatogram with phenolic compounds separation obtained using conditions described earlier is portrayed in Figure 1. A total of seven phenolic acids, including three hydroxybenzoic acids (p-hydroxybenzoic and vanillic), and five hydroxycinnamnic acids (chlorogenic, caffeic, p-coumaric, ferulic, and sinapic) as well as three flavanones (narirutin, hesperidin and didymin) were identified and quantified. The method separated 10 phenolic compounds faster (50 min) than 120 min previously reported (Li et al. 2006; Kelebek 2010; Kelebek and Selli 2011). Hesperidin was the dominant compound previously reported (Li et al. 2006; Kelebek 2010; Kelebek and Selli 2011). Hesperidin was the dominant compound previously reported (Li et al. 2006; Kelebek 2010; Kelebek and Selli 2011). Hesperidin was the dominant compound previously reported (Li et al. 2006; Kelebek 2010; Kelebek and Selli 2011). Hesperidin was the dominant compound previously reported (Li et al. 2006; Kelebek 2010; Kelebek and Selli 2011).

Table 1. Composition of phenolic compounds in rind extracts using different extraction solvents and time combination. Means with different letters in the three rows (solvent) and three columns (extraction times) corresponding to the same compound are significantly different (P < 0.05).

| Phenolic compound       | Extraction solvent | 10 min   | 20 min   | 30 min   |
|-------------------------|--------------------|---------|---------|---------|
| Hydroxybenzoic acids    |                    |         |         |         |
| p-Hydroxybenzoic acid   | Methanol           | 22.08 ± 0.6<sup>ab</sup> | 19.78 ± 0.7<sup>b</sup> | 21.02 ± 3.1<sup>ab</sup> |
|                         | DMSO               | 92.85 ± 1.5<sup>a</sup> | 87.75 ± 5.6<sup>d</sup> | 86.66 ± 1.9<sup>d</sup> |
|                         | Ethanol            | 29.26 ± 3.5<sup>c</sup> | 25.32 ± 1.5<sup>bc</sup> | 29.29 ± 0.3<sup>c</sup> |
| Vanillic acid           | Methanol           | 17.82 ± 0.2<sup>c</sup> | 12.69 ± 0.6<sup>b</sup> | 24.47 ± 2.7<sup>d</sup> |
|                         | DMSO               | nd      | nd      | nd      |
|                         | Ethanol            | 8.86 ± 0.8<sup>a</sup> | 7.11 ± 1.1<sup>a</sup> | 17.25 ± 1.9<sup>c</sup> |
| Hydroxycinnamic acids   |                    |         |         |         |
| Chlorogenic acid        | Methanol           | 15.37 ± 0.4<sup>c</sup> | 25.91 ± 0.5<sup>e</sup> | 43.25 ± 1.2<sup>h</sup> |
|                         | DMSO               | 5.98 ± 1.1<sup>a</sup> | 11.89 ± 0.3<sup>b</sup> | 33.89 ± 4.4<sup>g</sup> |
|                         | Ethanol            | 18.76 ± 0.9<sup>d</sup> | 11.06 ± 0.7<sup>c</sup> | 29.85 ± 0.4<sup>h</sup> |
| Caffeic acid            | Methanol           | 28.21 ± 0.7<sup>c</sup> | 23.57 ± 0.5<sup>e</sup> | 39.81 ± 3.9<sup>g</sup> |
|                         | DMSO               | 11.95 ± 0.9<sup>c</sup> | 12.44 ± 0.2<sup>e</sup> | 23.33 ± 1.9<sup>d</sup> |
|                         | Ethanol            | 15.49 ± 1.7<sup>b</sup> | 11.94 ± 0.6<sup>e</sup> | 19.70 ± 0.8<sup>c</sup> |
| p-Coumaric acid         | Methanol           | 9.63 ± 0.1<sup>c</sup> | 14.55 ± 1.8<sup>c</sup> | 6.94 ± 0.1<sup>b</sup> |
|                         | DMSO               | 5.63 ± 0.4<sup>ab</sup> | 5.35 ± 1.1<sup>c</sup> | 9.80 ± 1.3<sup>c</sup> |
|                         | Ethanol            | 4.49 ± 0.2<sup>e</sup> | 10.43 ± 0.1<sup>c</sup> | 12.53 ± 0.2<sup>d</sup> |
| Ferulic acid            | Methanol           | 12.43 ± 0.8<sup>b</sup> | 13.37 ± 0.8<sup>h</sup> | 25.19 ± 4.9<sup>g</sup> |
|                         | DMSO               | 7.92 ± 1.2<sup>b</sup> | 6.28 ± 2.5<sup>b</sup> | 13.50 ± 1.1<sup>b</sup> |
|                         | Ethanol            | 17.81 ± 0.3<sup>c</sup> | 15.81 ± 0.1<sup>c</sup> | 40.55 ± 0.5<sup>c</sup> |
| Sinapic acid            | Methanol           | 41.35 ± 0.5<sup>c</sup> | 61.23 ± 3.8<sup>e</sup> | 64.87 ± 2.8<sup>b</sup> |
|                         | DMSO               | 15.19 ± 1.6<sup>bc</sup> | 23.52 ± 2.4<sup>c</sup> | 23.58 ± 2.8<sup>c</sup> |
|                         | Ethanol            | 19.45 ± 4.3<sup>b</sup> | 24.99 ± 0.8<sup>b</sup> | 39.45 ± 1.1<sup>d</sup> |
| Flavanones              | Narirutin          | 737 ± 1.4<sup>b</sup> | 738 ± 7.9<sup>b</sup> | 690 ± 14.4<sup>b</sup> |
|                         | DMSO               | 1370 ± 29.6<sup>d</sup> | 1299 ± 140<sup>d</sup> | 1151 ± 23.1<sup>c</sup> |
|                         | Ethanol            | 396 ± 30.7<sup>a</sup> | 355 ± 11.2<sup>a</sup> | 408 ± 12.6<sup>a</sup> |
|                         | Hesperidin         | 8005 ± 529<sup>cd</sup> | 8628 ± 269<sup>d</sup> | 7553 ± 290<sup>c</sup> |
|                         | DMSO               | 32,008 ± 373<sup>a</sup> | 31,179 ± 1181<sup>e</sup> | 32,019 ± 866<sup>a</sup> |
|                         | Ethanol            | 5456 ± 389<sup>ab</sup> | 4329 ± 439<sup>a</sup> | 3966 ± 161<sup>a</sup> |
|                         | Didymin            | 268 ± 4.6<sup>d</sup> | 246 ± 23.6<sup>bc</sup> | 257 ± 11.1<sup>cd</sup> |
|                         | DMSO               | 402 ± 7.2<sup>b</sup> | 402 ± 9.5<sup>b</sup> | 404 ± 4.5<sup>b</sup> |
|                         | Ethanol            | 238 ± 5.1<sup>ab</sup> | 224 ± 7.4<sup>b</sup> | 232 ± 3.2<sup>ab</sup> |

nd, non detectable; *Mean ± SD of three samples.
Table 2 summarizes the concentration range, retention times, regression equation \((y = mx)\), coefficient of determination \((R^2)\), LOD, LOQ, and the relative standard deviation (RSD) for each compound. The reproducibility of the retention time of phenolic compounds under selected HPLC conditions was executed by doing repeated injections \((n = 10)\) of the mixture of the 10 standards at the concentration of 10.0 \(\mu g/mL\). The regression equation, LOD, LOQ, and RSD were calculated for each identified phenolic compound using only the best extraction method, which in this case was acidic methanol. The LOD, defined as the smallest concentration that the analytical procedure can reliably distinguish from the noise levels and LOQ for all analytes were very small, ranging from 1.35 to 5.02 and 4.51–16.72 \(\mu g/mL\), respectively. The RSD values for all retention times ranged from 0.45 to 1.67 indicating good stability and adequate performance of the method investigated.

### Conclusions

Rapid and efficient methods for extracting and quantifying phenolic compounds in citrus rinds were successfully developed. Aqueous acidic methanol and 50:50 (v/v; DMSO:methanol, respectively) extract phenolic acids and flavanone glycosides rapidly and efficiently. The HPLC method developed in this study separated faster than methods previously described. Phenolic compounds can be extracted rapidly and efficiently from citrus rind tissue.
Acknowledgments
This work is supported by the Postharvest Innovation Fund of Department of Science and Technology, Citrus Research International, National Research Foundation and the Flanders Research Cooperation Programme (Project UID: 73936). We are grateful to contributions made by Dr Katherine Cools, Dr Maria Del Carmen Alamar Gavidia, Dr Jose Ordaz Ortiz, and Ms Rosemary Burns, for technical research support.

Conflict of Interest
None declared.

References
Abad-García, B., L. A. Berrueta, S. Garmón-Lobato, A. Urkaregi, B. Gallo, and F. Vicente. 2012. Chemometric characterization of fruit juices from Spanish cultivars according to their phenolic compound contents: I. citrus fruits. J. Agric. Food Chem. 60:3635–3644.
Ahmad, M. M., S.-R. Rehman, F. M. Anjum, and E. E. Bajwa. 2006. Comparative physical examination of various citrus peel essential oils. Int. J. Agric. and Biol. 8:186–190.
Benavente-García, O., J. Castillo, F. R. Marin, A. Ortuño, and J. A. Del Río. 1998. Uses and properties of citrus flavonoids. J. Agric. Food Chem. 45:4505–4515.
Bocco, A., M. Cuvelier, H. Richard, and C. Berset. 1996. Antioxidant activity and phenolic composition of citrus peel and seed extracts. J. Agric. Food Chem. 46:2123–2129.
Bressolle, F., M. Bromet-Petit, and M. Audran. 1996. Validation of liquid chromatographic and gas chromatographic methods, applications to pharmacokinetics. J. Chromatogr. B 686:3–10.
Chang, C. W., S. L. Hsiu, P. P. Wu, S. C. Kuo, and P. D. L. Chao. 1997. HPLC Assay of naringin and hesperidin in Chinese herbs and serum. J. Food Drug Anal. 5:111–120.
Crespo, P., J. Giné Bordonaba, L. A. Terry, and C. Carlen. 2010. Characterisation of major taste and health-related compounds of four strawberry genotypes grown at different Swiss production sites. Food Chem. 122:16–24.
Gritti, F., and G. Guiochon. 2012. The current revolution in column technology: how it began, where is it going? J. Chromatogr. A 1228:2–19.
Jabri-Karoui, I., and B. Marzouk. 2013. Characterization of bioactive compounds in Tunisian bitter orange (Citrus aurantium L.) peel and juice and determination of their antioxidant activities. BioMed. Res. Int. http://dx.doi.org/10.1155/2013/345415.
Kalt, W., C. F. Forney, A. Martin, and R. Prior. 1999. Antioxidant capacity, vitamin C, phenolics, and anthocyanin after fresh storage of small fruit. J. Agric. Food Chem. 47:4638–4644.
Kelebek, H. 2010. Sugars, organic acids, phenolic compositions and antioxidant activity of Grapefruit (Citrus paradisi) cultivars grown in Turkey. Ind. Crops Prod. 32:269–274.
Kelebek, H., and S. Selli. 2011. Determination of volatile, phenolic, organic acid and sugar components in a Turkish cv. Dortyol (Citrus Sinesis L. Osbeck). J. Sci. Food Agric. 91:1855–1862.
Khan, M. K., M. Albert-Vian, A. Fabiano-Tixier, O. Dangles, and F. Chemat. 2010. Ultrasound-assisted extraction of polyphenols (flavanone glycosides from orange (Citrus sinensis L.)) peel. Food Chem. 119:851–858.
Li, B. B., B. Smith, and M. M. Hossain. 2006. Extraction of polyphenols from citrus peels II. Enzyme-assisted extraction method. Sep. Purif. Technol. 48:189–196.
Ma, Y.-Q., X.-Q. Ye, Z.-X. Fang, J.-C. Chen, G.-H. Xu, and D. H. Liu. 2008. Phenolic compounds and antioxidant activity of extracts from ultrasonic treatment of Satsuma mandarin (Citrus unshiu Marc.) peels. J. Agric. Food Chem. 56:5682–5690.
Manthey, J. A., and K. Grohmann. 1996. Concentration of hesperidin and other orange peel flavonoids in citrus processing byproducts. J. Agric. Food Chem. 44:811–814.
Mathur, A., S. K. Verma, R. Purohit, V. Gupta, V. K. Dua, G. B. K. S. Prasad, et al. 2011. Evaluation of in vitro antimicrobial and antioxidant activities of peel and pulp of some citrus fruits. IJPT’s J. Biotechnol. Biotherapeutics 1:1–17.
Omamogho, J. O., J. P. Hanrahan, J. Tobin, and J. D. Glennon. 2011. Structural variation of solid core and thickness of porous shell of 1.7 m core–shell silica particles on chromatographic performance: narrow bore columns. J. Chromatogr. A 1218:1942–1953.
Simonne, A. H., and M. A. Ritenour. 2011. Citrus (Orange, Lemon, Mandarin, Grapefruit, Lime and Other citrus fruits). Pp. 90–117 in L. A. Terry, ed. Health-promoting properties of fruits and vegetables. CAB International, Oxfordshire, U.K.
Sun, Y., J. Wang, S. Gu, Z. Liu, Y. Zhang, and X. Zhang. 2010. Simultaneous determination of flavonoids in different parts of Citrus reticulate ‘Chachi’ fruit by high performance liquid chromatography–photodiode array detection. Molecules 15:5378–5388.
Tomas-Barberan, F. A., and C. Andres-Lacueva. 2012. Polyphenols and health: current state and progress. J. Agric. Food Chem. 60:8773–8775.
Tomás-Barberán, F. A., A. Gil-Izquierdo, F. Ferreres, and M. I. Gil. 2003. Analysis and purification of flavanones, chalcones and dihydrochalcones. Pp. 359–371 in C. Santos-Buelga and C. Williamson, eds. Methods in polyphenol analysis. The Royal Society of Chemistry, Cambridge.
de Villiers, A., H. Lauer, R. Szucs, S. Goodall, and P. Sandra. 2006. Influence of frictional heating on temperature gradients in ultra-high-pressure liquid chromatography on 2.1 mm I.D. columns. J. Chromatogr. A 1113:84–91.
Xu, G. H., J. C. Chen, D. H. Liu, Y. H. Zhang, P. Jiang, and X. Q. Ye. 2008a. Minerals, phenolic compounds, and antioxidant capacity of citrus peel extract by hot water. J. Food Sci. 73:C11–C18.
Xu, G. H., X. Q. Ye, D. H. Liu, Y. Q. Ma, and J. C. Chen. 2008b. Composition and distribution of phenolic acids in Ponkan (Citrus poonensis Hort. ex Tanaka) and Hoyou (Citrus paradisi Macf. Changshanhuou) during maturity. J. Food Comp. Anal. 21:382–389.
Ye, X. Q., J. C. Chen, D. H. Liu, P. Jiang, J. Shi, S. Xue, et al. 2011. Identification of bioactive composition and antioxidant activity in young mandarin fruits. Food Chem. 124:1561–1566.