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Assessment of phenolic contributors to antioxidant activity of new kiwifruit cultivars using cyclic voltammetry combined with HPLC

Yang Jiao[a], Paul A. Kilmartin[b], Mingtao Fan[a,*], Siew Young Quek[b,c,*]

[a] College of Food Science and Engineering, Northwest Agriculture and Forestry University, Yangling, Shaanxi 712100, China
[b] School of Chemical Sciences, The University of Auckland, Private Bag 92019, Auckland 1142, New Zealand
[c] Riddet Institute, Palmerston North, New Zealand

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Epicatechin gallate (PubChem CID: 107905)
Epigallocatechin gallate (PubChem CID: 65064)
Rutin (PubChem CID: 5280805)
Quercetin (PubChem CID: 5280343)
Gallic acid (PubChem CID: 370)
Vanillic acid (PubChem CID: 8468)
Protocatechuic acid (PubChem CID: 72)
Syringic acid (PubChem CID: 10742)
p-Coumaric acid (PubChem CID: 637542)
Ferulic acid (PubChem CID: 445858)
Caffeic acid (PubChem CID: 689043)
trans-Cinnamic acid (PubChem CID: 444539)

ABSTRACT

The phenolics profile of two new kiwifruit cultivars, Zespri® SunGold and Zespri® Sweet Green, were characterized and quantified for the first time using cyclic voltammetry, an electrochemical method, combined with HPLC. Results from the cyclic voltammetry revealed high correlations with those obtained from the spectrophotometry and HPLC methods, providing evidence to support the application of cyclic voltammetry as a rapid method in determining the phenolic profile and reducing power of kiwifruit extracts. Catechol-containing phenolics were identified as the major phenolic sub-class in the skins while flavonoids and phenolic acids were abundant in flesh of the tested cultivars. Epicatechin was the predominant phenolic compound and contributor to antioxidant capacity in all samples. Results also showed that SunGold and Sweet Green (both flesh and skin) exhibited significantly higher phenolic contents and antioxidant activities comparing with the well-established commercial 'Hayward' cultivar, indicating their commercial value and potential applications in food and nutraceuticals.

1. Introduction

Consumption of diets rich in fruits and vegetables has been identified as having positive effects on preventing or delaying the occurrence of certain chronic degenerative diseases related to aging, such as cardiovascular malfunction and common cancers (Dower, Geleijnse, Hollman, Soedamah-Muthu, & Kromhout, 2016; Hung et al., 2004; Liu et al., 2000). The antioxidant properties of fruits and vegetables are considered as a possible cause of their health-promoting effects (Dai & Mumper, 2010; Kaur & Kapoor, 2001). With this in mind, compounds associated with antioxidant ability have widely been investigated in food and plant materials in order to evaluate their beneficial effects on health, and to discover new sources of natural antioxidant as dietary supplements (Abeywickrama, Debnath, Ambigaipalan, & Shahidi, 2016; Huber & Rupasinghe, 2009; Wojdyło, Nowicka, Oszmiański, & Golis, 2017; Xi et al., 2014).

Kiwifruit is a nutrient-dense fruit derived from the woody vines of *Actinidia* species. The gold-fleshed Zespri® SunGold Kiwifruit (SunGold, *A. chinensis* 'Zesy002', commonly known as Gold3) and green-fleshed Zespri® Sweet Green Kiwifruit (Sweet Green, *A. chinensis x A. deliciosa* 'Zesh004', commonly known as Green14) are two new kiwifruit cultivars that have been firstly introduced to the New Zealand market in 2012 (Sivakumaran, Huffman, Sivakumaran, & Drummond, 2018).

Besides the basic nutrients, the functional properties of kiwifruit is

* Corresponding authors at: Building 302, Science Centre, University of Auckland, 23 Symonds Street, Auckland 1010, New Zealand (S.Y. Quek); College of Food Science and Engineering, Northwest Agriculture and Forestry University, Yangling, Shaanxi 712100, China (M. Fan).
E-mail addresses: fanmt@nwafu.edu.cn (M. Fan), sy.quek@auckland.ac.nz (S.Y. Quek).

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largely related to its valuable natural antioxidants (Giangrieco et al., 2016; Hunter, Greenwood, Zhang, & Skinner, 2011; Kim, Beppu, & Kataoka, 2009; Latocha, Łata, & Stasiak, 2015) with both polyphenols and vitamin C being confirmed as highly correlated with the antioxidant capacity (AOC) in kiwifruit (D'evoli et al., 2015; Du, Li, Ma, & Liang, 2009). Nutritional composition analysis of Zespri® SunGold and Sweet Green revealed considerably higher levels of vitamin C, compared to the well-known kiwifruit cultivar of 'Hayward' (Sivakumaran et al., 2018). However, little information is available concerning their phenolic profiles and AOC. As the abundant and excellent electron donors in kiwifruit, phenolic compounds provide plant and animal cells with strong defense against the damaging effects of free radicals (Ghasemzadeh & Ghasemzadeh, 2011; Hunter et al., 2011; Pal & Verma, 2013). An accurate analysis of phenolic compounds and antioxidant capacity of Zespri® SunGold and Sweet Green is seen as crucial in order to fully understand their functional properties.

Antioxidant compounds tend to be oxidized easily at an inert electrode due to their ability to act as reductants in solution (Kilmartin, Zou, & Waterhouse, 2001). Specific antioxidant classes can be characterized by interpreting the redox behaviour of samples (Kilmartin & Hsu, 2003; Sordoń, Salachna, & Jakubowska, 2016). Electrochemical methods, with the inherent redox correlation, upsurge as fast and less expensive alternatives to the conventional spectrophotometric analysis, combining simplicity and sensitivity in predicting antioxidant content and capacities of plants and plant-derived products (Arteaga et al., 2012; Lino et al., 2014). The detection limit of flavonoid was reported to be 1000 times lower using an electrochemical detector compared to that of photodiode array detection, and even colorful and turbid samples can be used for antioxidant analysis by electrochemical methods (Gomes, Ghica, Rodrigues, de Souza Gil, & Oliveira-Brett, 2016; Sordoń et al., 2016).

Considering the advantages, electro-analysis has proven to be highly practical and efficient in determining the phenolic composition and reducing power of complex matrices, including fruit extracts, honey, wine, tea and coffee (Arteaga et al., 2012; Gomes et al., 2016; Lino et al., 2014; de Oliveira Neto et al., 2017). Methods involving cyclic voltammetry (CV) have also been applied to characterize a series of reducing substances such as flavonoids, phenolic acids, ascorbic acid and synthetic antioxidants (Arteaga et al., 2012; Kilmartin et al., 2001). However, the feasibility of electrochemical methods in determining antioxidant in kiwifruit samples are yet to be studied.

The objective of this study was to investigate the antioxidant properties and the major phenolic contributors present in the flesh and skin extracts of two kiwifruit cultivars, namely the Zespri® SunGold and Sweet Green kiwifruits. Based on the advantages of electrochemical method as mentioned above, we have attempted a novel approach by investigating the feasibility of applying cyclic voltammetry (CV) to determine the major phenolic classes and to predict the AOC of kiwifruit extracts. This was conducted through correlation analyses of results obtained from the CV method with those collected from the spectrophotometric and HPLC methods. From current study, we had demonstrated that CV could be established as a faster, efficient, economic and environmental friendly way to identify and evaluate the AOC of the new kiwifruit cultivars.

2. Material and methods

2.1. Chemicals and reagents

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)-diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ), p-dimethylaminocinnamaldehyde (DMAC), Folin-Ciocalteu reagent, ascorbic acid and all phenolic compound standards (catechin, epicatechin, epicatechin gallate, epigallocatechin gallate, rutin, quercetin, gallic acid, vanillic acid, protocatechuic acid, syringic acid, *p*-coumaric acid, ferulic acid, caffeic acid, *trans*-cinnamic acid) with a purity of ≥96% were purchased from Sigma-Aldrich (St. Louis, USA). Other HPLC grade chemicals used were formic acid from BDH Chemical Ltd., Co. (Poole, England) and acetonitrile from Romil Ltd., Co. (Cambridge, England). All other solvents and reagents used in this study were of analytical grade, and Milli-Q grade water was used in the experiments.

2.2. Sample and standard preparations

About 5 kg of the ready-to-eat fruits of three kiwifruit cultivars, Zespri® SunGold, Sweet Green, and 'Hayward', were sourced from the Plant and Food research orchard in Te Puke, Bay of Plenty, New Zealand (Te Puke: 37°49′S, 176°19′E) in August 2017. Kiwifruits with similar size and firmness were washed with distilled water to remove foreign substances and manually separated into two parts (flesh and skin). Then, samples were dried using a freeze-drier (Labconco® FreeZone® Plus™ 12L, USA) and homogenized by a homogenizer. The powders obtained were stored at −80 °C until the extraction was undertaken.

One gram of freeze-dried sample was extracted with 10 mL of 70% methanol (v/v, pH 2.0) in an ultrasonic bath for 30 min, and centrifuged at 5000 rpm for 15 min at room temperature (Ma et al., 2017). The supernatant was collected after centrifugation and the residue was re-extracted two more times under the same conditions. Three supernatants were combined and stored at −80 °C for further use. Stock solutions of each standard compound were also dissolved in 70% methanol (v/v, pH 2.0) and stored at −80 °C. Extraction medium was added into the samples where a constant steam of N_2 was supplied, and aluminum foil was used to cover the glassware during the processes of sample drying, extraction and storage.

2.3. Spectrophotometric determinations of total phenolic, flavonoid and flavanol contents

All the spectrophotometric measurements were carried out using a UV/Vis microplate reader (PerkinElmer 2300 EnSpire multilabel reader). Folin-Ciocalteu method was used for total phenolic content (TPC) measurement with some adaptations (Singleton & Rossi, 1965; Tang et al., 2015). Results were expressed as mg gallic acid equivalents per gram of freeze-dried sample (mg GAE/g FDW). The total flavonoid content (TFC) was determined according to the method described by Tang et al. (2015) with modifications. Results were expressed as mg catechin equivalents per gram of freeze-dried sample (mg CE/g FDW). The slightly modified DMACA-HCl method was used to evaluate the total flavanol content (TAC) (Li, Tanner, & Larkin, 1996; Ma et al., 2017). Results were also expressed as mg CE/g FDW.

2.4. Spectrophotometric determinations of antioxidant capacity (AOC)

ABTS assay of kiwifruit extracts was measured following the method of Re's group with modifications (Du et al., 2009; Re et al., 1999). The ability to scavenge DPPH free radicals was assessed using modified DPPH method established by Brandwilliams's group (Brandwilliams, Cuvelier, & Berset, 1995; Tang et al., 2015). The ferric reducing ability of plasma (FRAP) assay was carried out based on the method of Benzie's group with a few adaptations (Benzie & Strain, 1996; Tang et al., 2015). All results were expressed as micromole trolox equivalents per gram of freeze-dried sample (μmol TE/g FDW).

2.5. Electrochemical determinations of antioxidant and antioxidant capacity

Samples were prepared for cyclic voltammetry (CV) analysis by consistently diluting the extracts 5, 10, 20, 40, 80, 160 and 320 times with methanol containing 0.1 M $LiClO_4$ to reach a linear range between