Food Research International 44 (2011) 2088–2099

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Polyphenol composition, vitamin C content and antioxidant capacity of Mauritian citrus fruit pulps

Deena Ramful [a], Evelyne Tarnus [b], Okezie I. Aruoma [c], Emmanuel Bourdon [b], Theeshan Bahorun [d,*]

[a] Department of Agricultural and Food Science, Faculty of Agriculture, University of Mauritius, Réduit, Mauritius
[b] Laboratoire de Biochimie et Génétique Moléculaire (LBGM), Groupe d'Etude sur l'Inflammation Chronique et l'Obésité (GEICO), Université de Saint Denis de La Réunion, France
[c] Department of Pharmaceutical and Biomedical Sciences, Touro College of Pharmacy, NY, USA
[d] Department of Biosciences, Faculty of Science, University of Mauritius, Réduit, Mauritius

ARTICLE INFO

Article history:
Received 20 November 2010
Accepted 30 March 2011

Keywords:
Citrus fruits
Pulp
Flavonoids
Vitamin C
TSS/TA ratio
Antioxidants

ABSTRACT

The pulp extracts of twenty-one varieties of citrus fruits (oranges, satsumah, clementine, mandarins, tangor, bergamot, lemon, tangelos, kumquat, calamondin and pamplemousses), commonly grown in Mauritius, were characterised in terms of their total soluble solids (TSS), titratable acidity (TA), polyphenol composition and vitamin C contents. Total phenolics ranged from 406.3 ± 14 to 1694 ± 19 μg g^{-1} fresh weight (FW). Total flavonoids varied between 133 ± 6 and 965 ± 7 μg g^{-1} FW and vitamin C contents were from 166 ± 19 μg/mL to 677 ± 22 μg/mL. The pulp of a pamplemousse variety had the highest TSS/TA ratio whereas lemon pulps had lowest TSS/TA ratios. The antioxidant activities of the pulp extracts were assessed and total phenolics correlated strongly with the trolox equivalent antioxidant capacity (TEAC), ferric reducing antioxidant capacity (FRAP) and hypochlorous acid (HOCl) scavenging activity assays. Based on their antioxidant activities, nine citrus fruits namely, one orange, tangor, kumquat, calamondin and pamplemousse variety and two mandarin and tangelo varieties were further characterised for their flavanone, flavonol and flavone levels by HPLC. Hesperidin (6.89 ± 0.06 to 26.98 ± 0.07 mg/g FW) and narirutin (0.27 ± 0.01 and 20.91 ± 0.10 mg/g FW) were present at high concentrations compared to the other flavonoid glycosides in the pulp extracts. Naringin was detected only in pulp extracts of pamplemousses. In the light of the data obtained, citrus fruit pulps represent an important source of phytochemicals with potent antioxidant capacity.

1. Introduction

Nutritional studies are now laying more emphasis on the protective and health-promoting effects of fresh fruits and vegetables. According to the United States Department of Agriculture (USDA) dietary guidelines (Dietary Guidelines Advisory Committee (DGAC), 2005), a range of 2½ to 6½ cups (5 to 13 servings) of fruits and vegetables is recommended each day for the 1200- to 3200-calorie level diet. Epidemiological studies have demonstrated that there is a significant positive relationship between fruit and vegetable consumption and reduced risk of chronic diseases (Liu, Manson, Lee, Cole, Hennekens, Willet et al., 2000; John, Ziebland, Yudkin, Roe, & Neil, 2002; Dauchet, Amouyel, Hercberg, & Dallongeville, 2006). An optimal mix of essential vitamins, minerals, fibres and bioactive phytochemicals such as alkaloids, carotenoids, nitrogenous compounds and polyphenolics are reported to contribute to these beneficial effects. Antioxidant polyphenols, by virtue of their hydrogen and electron donating abilities and metal chelating effects (Lindsay & Astley, 2002; Valko, Rhodes, Moncol, Izakovic, & Mazur 2006), exhibit a wide range of biological properties including anti-allergenicity, anti-atherogenicity, anti-inflammatory, anti-microbial, anti-thrombotic, cardioprotective and vasodilatory actions (Middleton, Kandaswami, & Theoharides, 2000; Puupponen-Pimiä, Nohynek, Meier, Kähkönen, Heinonen, Hopia et al., 2001; Samman, Lyons Wall, & Cook, 1998).

Tropical countries like Mauritius enjoy the right balance of sunshine and rainfall for the growth of a wide range of exotic fruits, delicious in taste and refreshing in flavour and aroma. Citrus genus is one of the most important fruit tree crop in the world and in Mauritius, citrus fruits are the second most consumed fruits, after bananas (Central Statistics Office (CSO), 2008), as fresh produce, juice, marmalades, jams and paste. Consumption of citrus fruit or juice appears to be associated with improved blood lipid profiles (Kurowska, Spence, Jordan, Wetmore, Freeman, Piche et al., 2000), survival in the elderly (Fortes, Forastiere, Farchi, Rapiti, Pastori & Perucci, 2000), lower risk of cancers (Benavente-Garcia & Castillo, 2008), lowering of blood pressure (Adibelli, Dilek, & Akpolat, 2009), reduced risks of stroke (Chen, Ward, Graubard, Heineman, Markin, Potischman et al., 2002), cardiovascular and coronary heart diseases and obesity (González-Molina, Domínguez-Perles, Moreno, & García-Viguera,

* Corresponding author at: Faculty of Science, University of Mauritius, Réduit, Mauritius. Tel.: +230 4037471; fax: +230 4656928.
E-mail address: tbahorun@uom.ac.mu (T. Bahorun).

0963-9969/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodres.2011.03.056

2010). The health promoting effects of citrus have been mainly associated with its antioxidant vitamin C and flavonoid contents. More than sixty flavonoid compounds have so far been identified in *Citrus* sp. and a majority of them can be regrouped into flavanones, flavones and flavonols existing as glycoside or aglycone forms (Benavente-Garcia, Castillo, Marin, Ortuno, & Del Rio, 1997).

The polyphenolic and vitamin C composition and *in vitro* antioxidant activities of flavedo extracts of 21 citrus fruit varieties grown in Mauritius have been recently investigated by our group (Ramful, Bahorun, Bourdon, Tarnus, & Aruoma, 2010; Ramful, Tarnus, Rondeau, Robert da Silva, Bahorun, & Bourdon, 2010). In this present study, we report the polyphenolic composition, total soluble solids/titratable acidity ratio (TSS/TA) and *in vitro* antioxidant potencies of the pulp extracts of these 21 citrus fruit varieties.

2. Methods and materials

2.1. Standards and chemicals

2,2′-Azino-bis(3-ethylbenzthiozoline-6)-sulfonic acid (ABTS) and Folin and Ciocalteu's phenol reagent were purchased from Sigma (St. Louis, MO, USA). 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) was from Analytical Rasayan, s.d. fiNe-CHEM Limited (Mumbai, India). Metaphosphoric acid was from Sigma Chemical Co. (St. Louis, MO). 2,6-Dichloroindophenol indophenol sodium salt was from Alpha Chemika (Mumbai, India). L-ascorbic acid was from BHD Laboratory Supplies (Poole, England). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, 97% (Trolox) was from Sigma-Aldrich Chemie (Steinheim, Germany). Gallic acid, quercetin, rutin, diosmin, rhoifolin, isorhoifolin, neoeriocitrin, poncirin, narirutin, neohesperidin, didymin, hesperidin and naringin (HPLC grade) were from Extrasynthèse (Genay, France). HPLC-grade acetonitrile and methanol were obtained from Merck (Darmstadt, Germany). All other reagents used were of analytical grade.

2.2. Plant material

Citrus fruits (Table 1) were obtained from "La Compagnie Agricole de Labourdonnais" situated at Mapou, in the north of Mauritius. Fruits were harvested at the mature stage when they were ready for sale or ready for processing. Some varieties were sampled twice at different periods of the harvest season to determine the effect of harvest time on tested parameters. After harvest, the fruits were rapidly processed on the same day. At least 10 fruits of each variety were carefully washed under running tap water and patted dry. The peel was carefully removed with a manual peeler and the pulp obtained was homogenised in a Waring Commercial blender (Dynamics Corporation, CT, USA). Weighed portions of the pulp of pooled samples of each variety were lyophilised for 48 h and the freeze-dried weight was determined. Samples were ground into a fine powder in a coffee grinder and stored in airtight containers at −4 °C until analysed.

It is noteworthy that there is a general tendency to mix the terms 'pamplemousses' and 'grapefruits' even though they are two distinct citrus varieties. The variety analysed in this study, is the pamplemousses(*Citrus grandis* or *Citrus maxima*) which originates from South East Asia and has an extremely thick peel covering its pulp which is either yellow, pale pink or pale green in colour. Graprefruit (citrus × paradise) is a hybrid of the pamplemousses and sweet orange (*Citrus maxima* × *Citrus sinensis*). Its peel is thinner than the pamplemousses and its pulp is either yellow or deep pink in colour.

2.3. Extraction

The extraction procedure used was adapted from Franke, Custer, Arakaki, and Murphy (2004) and Chun et al. (2003). 500 mg of freeze-dried citrus pulp powder was weighed in a plastic screw-capped tube and 5 mL of 80% methanol was added. The tube contents were vortexed and left to macerate overnight at 4 °C. Centrifugation was then carried out at 4500 rpm for 15 min and the clear supernatant obtained was decanted into a clean vial and stored at −20 °C. 5 mL of 80% methanol was added to the residue and the same procedure was repeated. 2 mL of 80% methanol was subsequently added to the residue to obtain a final volume of 12 mL. Supernatants of all three extractions were pooled and stored at −20 °C until used for the determination of total phenol and total flavonoids and for the antioxidant assays.

Table 1
Scientific and common names, variety and harvest dates of citrus fruits analysed.

Scientific name	Common name	Variety	Harvest month	Variety and harvest code
Citrus sinensis	Orange	Valencia late	Aug	1
		Washington	Mar	2A
		Navel	May	2B
Citrus unshiu	Satsumah	Owari	Mar	A
			May	B
Citrus clementina	Clementine	Commune	Mar	A
			May	B
Citrus reticulata	Mandarin	Fairchild	Apr	1A
			May	1B
		Dancy	May	2A
			Jun	2B
		Beauty	Jun	3A
			Aug	3B
		Suhugan	Aug	4
		Fizu	Aug	5
C. reticulata × *C. sinensis*	Tangor	Elendale	Jun	A
			Aug	B
Citrus aurantium ssp. bergamia	Bergamot	–	Apr	–
Citrus meyeri	Lemon	Meyer	Apr	A
			May	B
C. reticulata × *C. paradisis*	Tangelo	Mineola	Jun	1A
			Aug	1B
		Orlando	Aug	2
		Ugli	Jun	3A
			Aug	3B
Fortunella margarita	Kumquat	Nagami	Apr	A
			Jun	B
Citrus mitis	Calamondin	–	Jun	A
			Aug	B
Citrus maxima	Pamplemousses (Pummelo)	Rainking	May	1A
			Aug	1B
		Kaopan	May	2A
			Aug	2B
		Pink	May	3A
			Aug	3B
		Chandler	Aug	4

2.4. Total phenolic content

The Folin–Ciocalteu assay, adapted from Singleton and Rossi (1965), was used for the determination of total phenolics present in the citrus fruit extracts. To 0.25 mL of diluted extract, 3.5 mL of distilled water was added followed by 0.25 mL of Folin–Ciocalteu reagent (Merck). A blank was prepared using 0.25 mL of 80% methanol instead of plant extract. After 3 min, 1 mL of 20% sodium carbonate was added. Tube contents were vortexed before being incubated for 40 min in a water-bath set at 40 °C. The absorbance of the blue coloration formed was read at 685 nm against the blank standard. Total phenolics were calculated with respect to gallic acid standard curve (concentration range: 0–12 μg mL^{-1}). Results are expressed in μg of gallic acid g^{-1} FW of plant material.

2.5. Total flavonoid content

Total flavonoids were measured using a colorimetric assay adapted from Zhishen, Mengcheng, and Jianming (1999). 150 μL of 5% aqueous