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Bioactive phenolics and antioxidant propensity of flavedo extracts of Mauritian citrus fruits: Potential prophylactic ingredients for functional foods application

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ABSTRACT

The flavedo extracts of twenty-one varieties of citrus fruits (oranges, satsumah, clementine, mandarins, tangor, bergamot, lemon, tangelos, kumquat, calamondin and pamplemousses) grown in Mauritius were examined for their total phenolic, flavonoid and vitamin C contents and antioxidant activities. Total phenolics correlated strongly with the trolox equivalent antioxidant capacity (TEAC), ferric reducing antioxidant capacity (FRAP) and hypochlorous acid (HOCl) scavenging activity assays ($r > 0.85$). Based on their antioxidant activities in these three assays nine citrus fruits namely, one orange, clementine, tangor and pamplemousse variety, two tangelo varieties and three mandarin varieties, were further characterized for their flavanone, flavonol and flavone levels by HPLC and their antioxidant activities were assessed by the copper-phenanthroline and iron chelation assays. The flavanone, hesperidin, was present at the highest concentrations in all flavedo extracts except for pamplemousses where it was not detected. Contents in hesperidin ranged from 83 ± 0.06 to 234 ± 1.73 mg/g FW. Poncirin, didymin, diosmin, isorhoifolin and narirutin were also present in all extracts whereas naringin was present only in one mandarin variety. The nine flavedo extracts exhibited good DNA protecting ability in the cuphen assay with IC_{50} values ranging from 6.3 ± 0.46 to 23.0 ± 0.48 mg FW/mL. Essentially the flavedos were able to chelate metal ions however, tangor was most effective with an IC_{50} value of 9.1 ± 0.08 mg FW/mL. The flavedo extracts of citrus fruits represent a significant source of phenolic antioxidants with potential prophylactic properties for the development of functional foods.

1. Introduction

The role played by dietary factors on health status has long been recognised but it has been only recently that epidemiological and clinical studies have provided a clearer insight on the chemical and physiological mechanisms of the effects of bioactive foods on human health (Shahidi, 2009). Phytophenolics play a crucial role in health promotion and disease prevention by mechanisms related to cell differentiation, deactivation of pro-carcinogenes, maintenance of DNA repair, inhibition of N-nitrosamine formation and change of estrogen metabolism, amongst others (Shahidi, 2004). Major mechanisms for the antioxidant effect of phenolics in functional foods include free radical scavenging and metal chelation activities. Reactive oxygen species (ROS), such as the superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and the hydroxyl radical ($HO^{\bullet}$) have been recognised to play a determining role in the pathogenesis of several human diseases (Halliwell, 1996; Halliwell et al., 1992; Aruoma, 1994, 2003). ROS-induced oxidation can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate or propagate the development of diseases including cancer (Huang et al., 2001), diabetes (Boynes, 1991), neurodegenerative diseases (Perry et al., 2000), the process of aging (Hensley and Floyd, 2002) and cardiovascular dysfunctions (Hool, 2006). Phenolic compounds such as phenolic acids, flavonoids, stilbenes, tannins and lignans can scavenge free radicals and quench ROS and therefore provide effective means for preventing and treating free radical-mediated diseases.

Mauritius is a tropical island in the Indian Ocean with a relatively high prevalence of cardiovascular diseases, cancers and diabetes (Central Statistic Office, 2007). This has triggered interest in the study of the phytochemistry and antioxidant capacity of the Mauritian diet, which comprises a wide variety of exotic fruits, vegetables and beverages (Luximon-Ramma et al., 2003; Bahorun et al., 2004, 2007, 2010). Citrus (*Citrus* L. from Rutaceae) is one of the most popular world fruit crops that, besides providing an ample sup-

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ply of vitamin C, folic acid, potassium and pectin, contains a host of active phytochemicals that can protect health. Citrus species of various origins have been assessed for their phenolic constituents and antioxidant activities (Proteggente et al., 2003; Gorinstein et al., 2004; Anagnostopoulou et al., 2006; Guimarães et al., 2009). Citrus fruits, citrus fruit extracts and citrus flavonoids exhibit a wide range of promising biological properties including antiatherogenic, anti-inflammatory and antitumor activity, inhibition of blood clots and strong antioxidant activity (Middleton and Kandaswami, 1994; Montanari et al., 1998; Samman et al., 1996). Citrus is consumed mostly as fresh produce and juice and most often the peel is discarded. This represents a huge waste as citrus peels are reported to possess highest amounts of flavonoids compared to other parts of the fruit (Manthey and Grohmann, 2001). Citrus peels are subdivided into the epicarp or flavedo and mesocarp or albedo. The flavedo is the colored peripheral surface of the peel while the albedo is the white soft middle layer of the peel (Fig. 1).

The phytophenolic composition and *in vitro* antioxidant activities of the flavedo extracts of 21 citrus fruit varieties (Table 1) grown in Mauritius were determined. From the initial results, nine of the flavedo extracts (Orange 2B, Clementine A, Mandarin 1A, 2A and 5, Tangor A, Tangelo 1A and 2 and Pamplemousses 2B) were further characterized for their flavanone, flavonol and flavone levels, their ability to protect DNA damage and their iron chelating activity. There has been so far no report on the nutritional and health-promoting values of Mauritian citrus varieties. Thus Mauritian citrus varieties could be important sources of dietary polyphenolic antioxidant compounds that may have potential benefits in health and disease management.

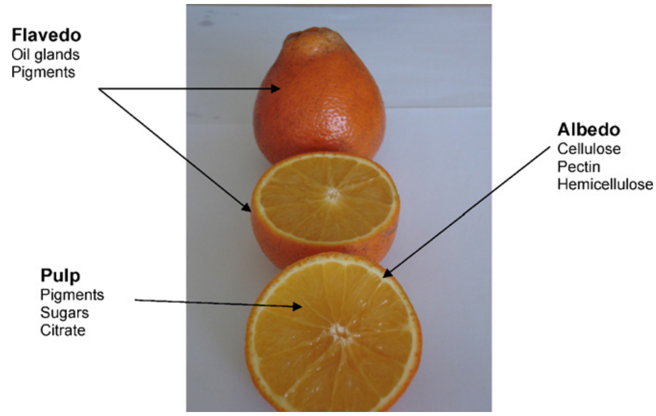

Fig. 1. Anatomy of citrus fruit showing the flavedo (the orange peripheral surface of the peel or epicarp), albedo (the white soft fibre middle layer of the peel or mesocarp) and the pulp (the inside layer of the fruit with juicy vesicles).

2. Materials and methods

2.1. Chemicals

2,2′-Azino-bis(3-ethylbenzthiozoline-6)-sulfonic acid (ABTS), Folin & Ciocalteu's phenol reagent and 2-Aminoethanesulfonic acid (Taurine) were purchased

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	August	1
		Washington Navel	March	2A
			May	2B
Citrus unshiu	Satsumah	Owari	March	A
			May	B
Citrus clementina	Clementine	Commune	March	A
			May	B
Citrus reticulata	Mandarin	Fairchild	April	1A
			May	1B
		Dancy	May	2A
			June	2B
		Beauty	June	3A
			August	3B
		Suhugan	August	4
		Fizu	August	5
C. reticulata × *C. sinensis*	Tangor	Elendale	June	A
			August	B
Citrus aurantium ssp. *bergamia*	Bergamot	–	April	–
Citrus meyeri	Lemon	Meyer	April	A
			May	B
C. reticulata × *C. paradisis*	Tangelo	Mineola	June	1A
			August	1B
		Orlando	August	2
		Ugli	June	3A
			August	3B
Fortunella margarita	Kumquat	Nagami	April	A
			June	B
Citrus mitis	Calamondin	–	June	A
			August	B
Citrus grandis	Pamplemousses	Rainking	May	1A
			August	1B
		Kaopan	May	2A
			August	2B
		Pink	May	3A
			August	3B
		Chandler	August	4