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Underestimated sources of flavonoids, limonoids and dietary fibre: Availability in lemon's by-products

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A B S T R A C T

The distribution of bioactive molecules has been determined in all by-products (seeds, exhausted peels and pulps, waste waters) generated by the industrial processing of lemon fruits. These are usually sent for disposal, despite being rich sources of bioactive molecules, in particular flavones, flavanones, flavanols, phenolic acids, limonoids, carotenoids, coumarins, furocoumarins, polymethoxyflavones, and dietary fibre, among others. All samples were treated with solvents (methanol, ethanol, ethyl acetate, petroleum ether) to extract flavonoids, carotenoids, limonoids and oxygen heterocyclic compounds. Analyses were performed by HPLC/PDA/MS. The major classes of compounds determined were flavanones, flavones and phenolic acids. Carotenoids were not detected due to probable chemical degradation. The peel and pulp contained dietary fibre (soluble and insoluble) with an optimal percentage of the soluble fraction of about 35% as recommended by official dietary guidelines. The information here reported may stimulate operators to re-evaluate their by-products, with the possibility to recover significant amounts of nutraceuticals and produce functional foods.

1. Introduction

Citrus industry generates a considerable amount of by-products (or waste) with high amounts of valuable bioactive components. In fact, the annual *Citrus* world production is over 100 million metric tonnes, and lemon is one of the main *Citrus* crops, with a world annual production of over 6.0 million tonnes (United States Department of Agriculture – Foreign Agricultural Service, 2014). The Italian annual production of *Citrus* fruits is about 37 thousand tonnes. Lemon fruits constitute a considerable amount (about 5 thousand tonnes a year) of the Italian market (ISTAT, 2013) and more than 30% of the total production is industrially processed. Essential oil and juice are the main products obtained, and the amount of by-products generated from the industrial processes is consistent. The by-products obtained from lemon transformation are represented by peels, pulps, seeds and waste water. These are known to be rich in bioactive molecules (Atrooz, 2009; Bocco, Cuvelier, Richard, & Berset, 1998; Barreca, Bellocco, Caristi, Lezzi, & Gattuso, 2011; Yang et al., 2011), such as flavonoids, limonoids, phenolic acids, coumarins, furocoumarins,

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polymethoxyflavones, and carotenoids. *Citrus* peel and pulp also represent an important source of dietary fibre since they provide a well balanced amount of water soluble fraction (Gorinstein et al., 2001; Griguelmo-Miguel & Martin-Belloso, 1999; Prosky, Asp, Schweizer, DeVries, & Furda, 1992). Recently Chinapongtitiwat, Jongaroontaprangsee, Chewchan, and Devahastin (2013) reported the amount of soluble dietary fibre (SDF) determined in different *Citrus* ranging between 34.2% and 46.6% of the total dietary fibre amount (TDF). These ratios are ideal, considering that the optimal intake of SDF should be above 30% of the TDF. All these compounds can be recovered from the wastes and recycled for different uses, thus reducing the costs of waste disposal as well as the amount of total waste.

Recent literature data report on the utilization of different food industry by-products, such as proteolytic enzymes recovery from sardine by-products (Castro-Cesena, Del Pilar Sanchez-Saavedra, & Marquez-Rocha, 2012); natural dyes for textile dyeing from olive mill wastewater (Meksi, Haddar, Hammami, & Mhenni, 2012), functional proteins from canned and fresh tuna by-products (Sanmartin et al., 2012), adsorbent for removal of Pb(II) and Ni(II) from water from pigeon peas hulls waste (Ramana, Reddy, Yu, & Seshaiah, 2012), herpes Simplex virus inhibitors from apple pomace (Alvarez et al., 2012), phenolics compounds recovery from tomato leaves (Taveira et al., 2012), tomato waste (Cetkovic et al., 2012) and from olive oil by-products (Herrero et al., 2011); flavonoids (Sommella et al., 2014; Yang et al., 2011) and dietary fibre (Marin, Soler-Rivas, Benavente-Garcia, Castillo, & Pérez-Alvarez, 2007; Tanaka, Takamizu, Hoshino, Sasaki, & Goto, 2012) recovered from *Citrus*-processing by-products.

Numerous articles are found in the literature reporting on the distribution of flavonoids, limonoids, phenolic acids, coumarins, furocoumarins, polymethoxyflavones, and carotenoids, in *Citrus* fruits. These are mainly analyzed by HPLC, as demonstrated by many different publications dealing with the determination of flavonoids (Chinapongtitiwat et al., 2013; Dugo et al., 2005; Gattuso, Barreca, Gargiulli, Leuzzi, & Caristi, 2007; Russo, Cacciola, Bonaccorsi, Dugo, & Mondello, 2011), carotenoids (Agocs et al., 2007; Bonaccorsi et al., 2003; Giuffrida, La Torre, Stelitano, Pellicanò, & Dugo, 2006; Rouseff, Raley, & Hofsommer, 1996), coumarins and furocoumarins (Bonaccorsi, McNair, Brunner, Dugo, & Dugo, 1999; Dugo et al., 2009; Russo et al., 2012), and limonoids (Chinapongtitiwat et al., 2013; Manners, 2007).

The antioxidant activity of these bioactive molecules is well assessed (Atrooz, 2009; Bocco et al., 1998; Chen, Yuan, & Liu, 2010; Cook & Samman, 1996; Manners, 2007; Patil, Jayaprakasha, Murthy, & Vikram, 2009; Pernice et al., 2009). Carotenoids and flavonoids have been implicated in the protection against cancer, prevention of serious human health disorders such as heart and cardiovascular diseases, macular degeneration, cataracts, osteoporosis, hypertension and hyperlipidaemia (Gattuso et al., 2007; Rao & Rao, 2007; Srinivasan & Pani, 2013). Coumarins have also been demonstrated to exert many pharmacological and toxicological activities (Kleiner et al., 2008; Middleton, Kandaswami, & Theoharides, 2000; Murray, Mendey, & Brown, 1982; Row, Brown, Stachulski, & Lennard, 2006), possessing antibacterial (Kayser & Kolodziej, 1999), antiplatelet aggregation, anti-HIV (Wu et al., 2001) and intestinal anti-inflammatory (Luchini et al., 2008) properties. Moreover, flavanones and flavons exhibit beneficial effects on capillary fragility, anti-inflammatory, antimicrobial and antiviral activities, and possess the capability to inhibit human platelet aggregation, antiallergenic, and antiulcer properties and hypocholesterolaemic effects (Borrelli & Izzo, 2000; Di Donna et al., in press; Middleton & Kandaswami, 1992; Tijburg, Mattern, Folts, Weisgerber, & Katan, 1997; Wightman, 2004).

Dietary fibre has been proven to exert different beneficial effects, and its intake has beneficial effects on risk factors for developing chronic diseases. In fact, dietary reference intakes (DRI, 2002/2005) recommend consumption of 25 g of fibre for adult women and 38 g for adult men, based on epidemiologic studies showing protection against cardiovascular disease (Fernandez-Lopez et al., 2004; Guillon & Champ, 2000; Harris & Ferguson, 1999; Lipkin, Reddy, Newmark, & Lamprecht, 1999). Moreover, in addition to its nutritional role, dietary fibre is also desirable for its functional and technological properties (Marin et al., 2007).

This study, which is a part of a more extended project aimed to endorse the use of the waste produced by Italian agro-food industries, is focused on the evaluation of different lemon-processing by-products as potential sources of bioactive molecules and dietary fibre. The samples (juice, waste water, peels, pulps and seeds) provided by the local *Citrus* industries were thus analyzed by RP-HPLC/PDA/MS.

2. Experimental

2.1. *Materials and samples*

This research was carried out on a 48°Brix concentrated lemon juice and on the by-products resulting from the entire industrial process, namely waste water, peels, pulp and seeds. The whole samples were collected at local *Citrus* plants located in Barcellona Pozzo di Gotto (Messina, Italy). For the determination of polyphenols the samples of waste water and of concentrated lemon juice were analyzed without any pre-treatment, while all the other samples were subjected to solvent extraction before HPLC analysis. Waste water was diluted 1:4 (v/v) while concentrated lemon juice required a dilution 1:40 (v/v) with distilled water, then they were filtered using Acrodisc filter 0.45 μm (Sigma-Aldrich, Milan, Italy) and injected into HPLC. Each sample was analyzed in triplicate.

The standard compounds, namely gallic acid, protocatecuic acid, p-hydroxybenzoic acid, caffeic acid, p-coumaric acid, ferulic acid, synapic acid, eriocitrin, rutin, narirutin, hesperidin, neoesperidin and β-carotene were obtained from Extrasynthese (Genay Cedex, France). Apigenin 6,8-di-C-glucoside and diosmetin 6,8-di-C-glucoside were obtained by preparative HPLC-HPLC/PDA/MS separation from concentrated bergamot juice. Coumarin and phloroglucinol were purchased from Fluka (Milan, Italy); 8-geranyloxy-psoralen was procured from Herboreal Ltd (Edinburgh, UK); bergapten and 8-methoxy-psoralen were purchased from Sigma-Aldrich. Bergamottin, 5-isopentenyloxy-7-methoxy-coumarin and 5-geranyloxy-7-methoxy-coumarin were previously isolated in our laboratory (Dugo et al., 2009; Russo et al., 2012). Imperatorin, isoimperatorin and citropten were previously synthesized in