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

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ABSTRACT

This study reports a comprehensive investigation on the presence of flavonoids, limonoids and dietary fiber determined by HPLC in all the by-products of the industrial transformation of orange. Seeds were the richest source of bioactive molecules, with flavanones being the most abundant, followed by phenolic acids (238 mg/kg). However, p-hydroxybenzoic acid and caffeic acid were highly represented also in the exhausted peels and pulps (560 mg/kg). Limonoids were determined exclusively in seeds. Among all the by-products it was found that waste water is extremely rich of hesperidin (19,500 mg/kg). For this reason an only eco-friendly preparative HPLC method for the recovery and purification of hesperidin from waste water was developed. Dietary fiber was determined in exhausted peels and pulps which resulted to be rich sources of insoluble dietary fiber. The results show that the by-products here investigated represent important sources of nutraceuticals as ingredients useful for functional food preparations.

1. Introduction

As reported by the United States Department of Agriculture Foreign Agricultural Service (2014) the global orange production is about 51 million metric tons. The global amount of transformed orange is 20 million metric tons most of which is transformed in China, Brazil and Europe (United States Department of Agriculture Foreign Agricultural Service, 2014). In Italy the citrus production, where orange is the main crop, is mainly located in the southern regions, Sicily and Calabria, being the main producer ones (ISTAT, 2012). The waste products, derived from the local industrial plants in Sicily, represent a growing problem, while the cost to send this material for disposal greatly compromise the commercial trade, with a strong impact on the final cost of the primary product (orange juice). In order to minimize waste and to re-use the by-products it is necessary to deeply characterize their composition, with particular regards to bioactive molecules. For obvious reasons this approach would greatly affect the global citrus industry

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Tamer and Copur, 2014. As reported recently (Russo et al., 2014) our research group is engaged in a research project aimed to re-evaluate the waste generated from the food industry in order to reduce the amounts sent for disposal through the identification of new sources of possible nutraceuticals. For this purpose we are evaluating all the by-products originated from the transformation line of Citrus industry, in order to determine the content of biologically active compounds. Nassar et al. (2008) have reported possible applications of lemon albedo and orange dietary fiber as ingredients for the preparation of foodstuff with improved nutritional values. In a recent study it is reported that the orange fruit is a rich source of molecules with high antioxidant activity (Escobedo-Avellaneda, Gutierrez-Uribe, Valdez-Fragoso, Torres, & Welti-Chanes, 2014). Among these compounds, flavanones in their glycosylated form and as rutinosides, are typical of citrus fruits (Gattuso, Barreca, Gargiulli, Leuzzi, & Caristi, 2007). The most abundant favanone glycosyde in sweet orange is hesperidin (Peterson et al., 2006). In addition to favanones, orange fruits are characterized by the presence of flavones (Bonaccorsi, McNair, Brunner, Dugo, & Dugo, 1999; Russo, Cacciola, Bonaccorsi, Dugo, & Mondello, 2011; Donato, Bonaccorsi, Russo, & Dugo, 2014). In general flavanones and flavones 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 hypocholesterolemic effects (Borrelli & Izzo, 2000; Di Donna et al., 2014; Middleton & Kandaswami, 1992; Tijburg, Mattern, Folts, Weisgerber, & Katan, 1997; Wightman, 2004). Recently it has also been proven that C- and O-glycosyl flavone possess high scavenging properties (Barreca, Bellocco, Leuzzi, & Gattuso, 2014) and that they act as protecting agents against oxidative stress (Bernabé et al., 2013). Moreover the glycosyl flavanones can be hydrolyzed in the gut by microbial β-glycosidases, thus increasing their bioavailability and functional properties (Di Gioia et al., 2014; Jou, Tsai, Tu, & Wu, 2013).

The solid residues originated from the extraction of orange juice and essential oils (exhausted peels and pulps) have been also analyzed to determine the amount of dietary fiber (DF). Fiber is often classified as soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) (Gorinstein et al., 2001). The SDF/IDF ratio is important for both dietary and functional properties. It is generally accepted that optimal DF for the best nutritional properties should have an SDF/IDF ratio of at least 1:2 (Jaime et al., 2002). Literature reports that citrus fruits contain a ratio of SDF/IDF of about 1:5 (Figuerola, Hurtado, Estevez, Chiffelle, & Asenjo, 2005). This fraction has been proven to exert different beneficial effects on risk factors for developing chronic diseases (Fernandez-Lopez et al., 2004; Guillon & Champ, 2000; Harris & Ferguson, 1999; Lipkin, Reddy, Newmark, & Lamprecht, 1999). DF value is not only related to its nutritional properties, but also for its functional and technological properties so as to be a desirable ingredient in functional food preparations as gluten free ones (Figuerola et al., 2005; Ocen & Xu, 2013; O'Shea, Roßle, Arendt, & Gallagher, 2014).

The present study reports on the results obtained by analyzing samples of juice, seeds, solid residues (exhausted peels and pulps), and waste water, all derived from the transformation of Sicilian oranges. Based on the fact that phenolic acids, flavonoids and limonoids are mainly analyzed by HPLC (Chinapongtitiwat, Jongaroontaprangsee, Chewchan, & Devahastin, 2013; Dugo et al., 2005; Gattuso et al., 2007; Manners, 2007; Russo et al., 2011; Sommella et al., 2014) our analytical approach consisted of the separation by RP-HPLC and the quantitative determination of single components of different chemical classes and of the determination of the dietary fiber as previously reported (Russo et al., 2014) on a similar study performed on lemon by-products.

2. Materials and methods

2.1. Materials and samples

This research was carried out on an orange juice, and on the by-products resulting from the entire industrial process: waste waters, solid residue (peels and pulps) and seeds. The samples were collected at a local *Citrus* plant located in Barcellona Pozzo di Gotto (Messina, Italy). For the determination of bioactive molecules the samples of waste water and orange juice were analyzed without any pre-treatment, while all the seed and solid residues were subjected to solvent extraction before HPLC analysis. Waste water was diluted 1:4 while orange juice required a dilution 1:5 with distilled water, then they were filtered on Acrodisc filter 0.45 μm (Sigma-Aldrich, Milan, Italy) and injected into HPLC. Each sample was analyzed in triplicate.

The standard compounds: p-hydroxybenzoic acid, caffeic acid, eriocitrin, narirutin and hesperidin were obtained from Extrasynthese (Genay Cedex, France). Apigenin 6,8-di-C-glucoside was previously isolated in our laboratory (Russo et al., 2014) by preparative LC–LC/PDA/MS separation from a concentrated bergamot juice.

Methanol and *n*-hexane employed for the extraction procedure were obtained from VWR (Milan, Italy). Formic acid was purchased from Riedel-de Haën (Germany). For LC and preparative LC–LC analyses, water, acetonitrile and ethanol were obtained from Sigma-Aldrich. Enzymes α-amylase, protease and amyloglucosidase used for the determination of DF were obtained from Sigma-Aldrich.

2.2. Instrumentation and software

LC analyses were carried out using a Shimadzu Prominence LC-20A system (Shimadzu, Milan, Italy), including a CBM-20A controller, two LC-20 AD dual-plunger parallel-flow pumps, a DGU-20A_3 on-line degasser, and a CTO-20A column oven. As detectors, an SPD-M20A UV detector and an LCMS-2020, through both ESI and APCI interfaces (Shimadzu), were respectively employed for quantification and characterization of the bioactive molecules. MS data acquisition was performed by the LCMS solution Ver. 3.30 software (Shimadzu).

Preparative LC–LC analyses were carried out using a Shimadzu Prominence LC-20A system (Shimadzu), including a CBM-20A controller, one LC-20 AD dual-plunger parallel-flow pump, three LC-20 AP preparative pumps, a DGU-20A3R on-line degasser, one FCV-20AH2 valve, two FCV-14AH valves and an APV Split. As detectors, an SPD-20A UV detector with a preparative cell, an SPD-M20A UV detector, and an LCMS-2020, through ESI interface (Shimadzu), were employed. As