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Quercetin and related flavonoids conserve their antioxidant properties despite undergoing chemical or enzymatic oxidation

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ABSTRACT

Oxidation of a phenolic group in quercetin is assumed to compromise its antioxidant properties. To address this assumption, the ROS-scavenging, Folin-Ciocalteau- and Fe-reducing capacities of quercetin and thirteen structurally related flavonoids were assessed and compared with those of mixtures of metabolites resulting from their chemical and enzymatic oxidation. Regardless of the oxidation mode, the metabolites mixtures largely conserved the antioxidant properties of the parent molecules. For quercetin, 95% of its ROS-scavenging and over 77% of its Folin-Ciocalteau- and Fe-reducing capacities were retained. The susceptibility of flavonoids to oxidative disappearance (monitored by HPLC-DAD) and that of the mixtures to retain their antioxidant capacity was favourably influenced by the presence of a catechol (ring-B) and enol (ring C) function. This is the first study to report that mixtures resulting from the oxidation of quercetin and its analogues largely conserve their antioxidant properties.

1. Introduction

Quercetin, the most abundant flavonoid in edible plant foods, has been subject to many studies due to its antioxidant properties (Brown, Khodr, Hider, & Rice-Evans, 1998; Deng, Fang, & Wu, 1997; Rice-Evans, Miller, & Paganga, 1996; Thompson & Williams, 1976; Yoshino & Murakami, 1998). These properties are largely attributed to the capacity of quercetin to reduce the formation of reactive oxygen species (ROS) *via*, for instance, chelation of metals involved in ROS generation (Brown et al., 1998; Thompson & Williams, 1976; Yoshino & Murakami, 1998), inhibition of various ROS-generating enzymes (Chimenti et al., 2006; Leyva-López, Gutierrez-Grijalva, Ambriz-Perez, & Heredia, 2016) and/or down-regulation of expression of relevant enzymes (Leyva-López et al., 2016). The antioxidant actions of quercetin can also arise from its capacity to favour ROS removal *via*, for instance, direct scavenging of such species (Rice-Evans et al., 1996), induction of the synthesis of ROS-removing enzymes (Molina, Sanchez-Reus, Iglesias, & Benedi, 2003; Tang et al., 2012), and/or up-regulation of the expression of genes encoding endogenous antioxidant-synthesizing enzymes (Lay Saw et al., 2014). In addition, quercetin is intensively studied for its increasingly recognized ROS-scavenging-independent actions (D'Andrea, 2015; Khan et al., 2016; Nabavi, Russo, Daglia, & Nabavi, 2015).

During direct ROS-scavenging action, the quercetin molecule is expected to engage in a series of single-electron transfer and/or hydrogen atom-donation reactions that will initially lead to the oxidation of its catecholic group and subsequently to other oxidative changes that could affect its flavonoid skeleton (Zhou & Sadik, 2008). Such changes have been studied by many investigators; some metabolites generated during the oxidation of quercetin are endowed with clear electrophilic and/or pro-oxidant potential (Boots et al., 2007; Galati, Moridani, Chan, & O'Brien, 2001; Li, Jongberg, Andersen, Davies, & Lund, 2016; Sanda Chedea, Choueiri, Jisaka, & Kefalas, 2012). Chemical, enzymatic, electrochemical and ROS-mediated oxidation studies, among others, have been reported (Zhou & Sadik, 2008). Although many such studies have investigated the chemical identity and possible mechanisms underlying the formation of metabolites (Boots et al., 2007; Galati et al., 2001; Li et al., 2016; Zhou & Sadik, 2008), the actual consequences that the oxidation of quercetin could have on its antioxidant properties have not been evaluated yet. In the present work, we assessed the ROS-scavenging (ORAC), Folin-Ciocalteau (FC)- and Fe-reducing capacities of a mixture of metabolites that resulted from chemical (alkaline dissolution-auto-induced) (Jurasekova, Torreggiani, Tamba, Sanchez-Cortez, & Garcia-Ramos, 2009) and

Abbreviations: ACN, acetonitrile; FRAP, Fe-reducing capacity; ORAC, oxygen radical absorbance capacity; PPO, mushroom tyrosinase; TP, total phenolics.

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enzymatic (mushroom tyrosinase-mediated) oxidation of quercetin (Kubo, Nihei, & Shimizu, 2004). For comparison, and to define possible structural determinants of the susceptibility of quercetin to oxidation, we extended our study to thirteen structurally related flavonoids. The present study reveals that the mixtures of metabolites that result from the oxidation of quercetin or related flavonoids, rather than losing the antioxidant properties of the parent molecules, tend to largely conserve them. Among flavonoids, differences in susceptibility to oxidation and/or retention of their original antioxidant properties were found to primarily depend on structural features and only marginally on the mode of oxidation. Despite undergoing oxidation, quercetin and many other flavonoids conserve a substantial portion of their original antioxidant properties; the potential biological and/or methodological implications are discussed.

2. Materials and methods

2.1. Materials

Mushroom tyrosinase (2684 units/mg of solid, EC 1.14.18.1) and all flavonoids used in this study were purchased from Sigma–Aldrich (St. Louis, MO, USA), and their purity was as follows: apigenin (≥97%), (+)-catechin (≥98%), dideoxyquercetin (≥98%), (-)-epicatechin (≥98%), (±)-eriodictyol (≥95%), fisetin (≥98%), galangin (≥95%), isorhamnetin (≥95%), kaempferol (≥97%), luteolin (≥98%), morin (≥97%), myricetin (≥96%), quercetin (≥95%) and (+)-taxifolin(≥90%). Acetonitrile (ACN) and ethanol were HPLC grade. Formic acid, sodium hydroxide, sodium dihydrogen phosphate and di-sodium hydrogen phosphate were analytical grade and purchased from Merck (Darmstadt, Germany).

2.2. Methods

2.2.1. Alkali-mediated oxidative disappearance of flavonoids

Each flavonoid was dissolved in 1 M NaOH to a final concentration of 0.1 M (pH = 12) and immediately incubated at 22 °C for a maximum of 300 min. Prior to analysis of the remnant concentration, samples of the incubated solutions were neutralized by the addition of sodium phosphate buffer (75 mM, pH 7.0) and diluted immediately with the mobile phase (see below). Control oxidation experiments were performed in parallel by incubating flavonoids dissolved in pure ethanol instead of alkali. After sampling, the ethanolic solutions were treated identically to the alkali samples.

2.2.2. Enzyme-mediated oxidative disappearance of flavonoids

Stock solutions of each flavonoid were prepared fresh in ethanol to 20 mM; prior to their incubation these were diluted to a final concentration of 100 μM with phosphate buffer (75 mM, pH 6.6). Oxidation was induced by incubating samples of the latter solutions with 42.5 U/mL of mushroom tyrosinase (PPO) at 30 °C for a maximum of 300 min. Prior to the analysis of the remnant concentration of the flavonoids, incubated samples were diluted with an equal volume of a 40:60% v/v mixture of ACN and water acidified with formic acid (at 0.1% v/v) to stop the oxidation reaction. As control oxidation experiments, solutions of each flavonoid (containing 0.5% v/v ethanol) were incubated in parallel in the absence of PPO.

2.2.3. HPLC analysis of the flavonoids

HPLC analysis was performed using an Agilent 1200 series bomb, equipped with an autosampler and a photodiode array detector (Santa Clara, CA, USA). The HPLC system was controlled by Agilent ChemStation (Agilent Technologies 2010). The concentration of each flavonoid was assessed in incubated samples and estimated from the area under the curve of its chromatographic peak using the standard curves prepared for each flavonoid on the day of the experiment. The standard deviation of each determination was always less than 5%. Detection wavelengths were 294 nm for (+)-catechin, epicatechin, eriodictyol and taxifolin; 334 nm for apigenin; 370 nm for dideoxyquercetin, fisetin, galangin, isorhamnetin, kaempferol, luteolin, morin, myricetin and quercetin. Other chromatographic conditions were as follows: flow rate of 0.8 mL/min, column (250 x 4.6 mm i.d., 5 μm, RP-18e Purospher® Star, Merck, Darmstadt, Germany), oven column at 25 °C. All flavonoids were analysed under isocratic conditions using a mobile phase mixture of ACN (A) and water/formic acid (0.1%) (B), whose composition (v/v) varied according to the analysed flavonoid, as follows: catechin and epicatechin, 20% A and 80% B (t_R = 6.4 and 7.6 min, respectively); taxifolin, 25% A and 75% B (t_R = 10.7 min); fisetin and myricetin, 30% A and 70% B (t_R = 8.8 and 9.5 min, respectively); quercetin, eriodictyol and luteolin, 35% A and 65% B (t_R = 11.4, 10.9 and 10.6 min, respectively); apigenin, dideoxyquercetin, isorhamnetin, kaempferol and morin, 40% A and 60% B (t_R = 11.1, 9.7, 13.1, 10.1, 6.7 min, respectively); galangin, 55% A and 45% B (t_R = 12.6 min). For quercetin, in addition to assessing the changes in the area under the curve of the characteristic peak, the appearance of new peaks with progressive oxidative disappearance was investigated (shown in Fig. 1). The disappearance of quercetin and appearance of its metabolites was monitored at 294 nm employing the following HPLC gradient

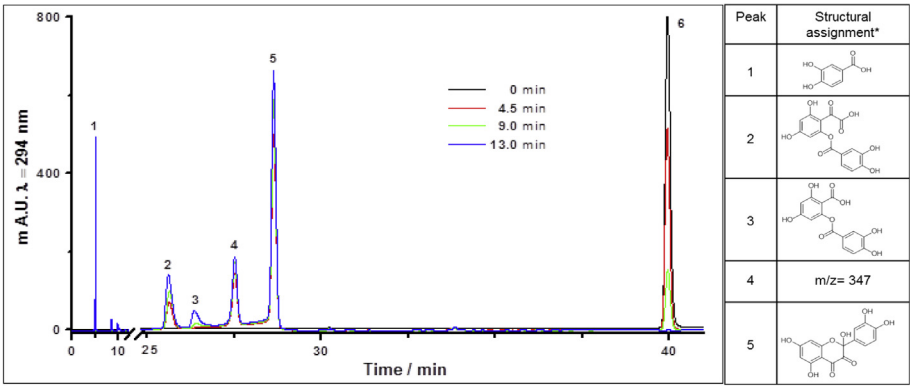

Fig. 1. HPLC chromatogram of the time-dependent disappearance of quercetin, and the appearance of major oxidation products during the exposure of quercetin to alkaline conditions. Under the chromatographic conditions employed in this study, quercetin corresponds to peak 6, with a retention time of 39.9 min. The t_R of peaks 1–5, associated with quercetin oxidation products, were 5.0, 25.6, 26.4, 27.5 and 28.6 min, respectively. Based on ESI-MS/MS analysis, the figure includes the chemical structure assigned to compounds in peaks 1–5.