Journal of Functional Foods 40 (2018) 68–75

Contents lists available at ScienceDirect

Journal of Functional Foods

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

Antioxidant and anti-inflammatory activities of quercetin and its derivatives

Marija Lesjak*, Ivana Beara, Nataša Simin, Diandra Pintać, Tatjana Majkić, Kristina Bekvalac, Dejan Orčić, Neda Mimica-Dukić

Department of Chemistry, Biochemistry and Environmental Protection, Faculty of Sciences, University of Novi Sad, Trg Dositeja Obradovića 3, 21000 Novi Sad, Serbia

ARTICLE INFO

Keywords:
Quercetin
Quercetin derivatives
Quercetin glycosides
Antioxidant activity
Anti-inflammatory activity

ABSTRACT

Quercetin is hardly bioavailable and largely transformed to different metabolites. Although little is known about their biological activities, these metabolites are crucial for explanation of health benefits associated with quercetin dietary intake. In this study, the antioxidant and anti-inflammatory activities of six quercetin derivatives (quercetin-3-*O*-glucuronide, tamarixetin, isorhamnetin, isorhamnetin-3-*O*-glucoside, quercetin-3,4′-di-*O*-glucoside, quercetin-3,5,7,3′,4′-pentamethylether) were compared with the activity of common onion extract as the main source of dietary quercetin and standards (butylated hydroxytoluene and aspirin). The quercetin derivatives demonstrated notable bioactivities, similar to standards and onion. Derivatization of quercetin hydroxyl groups resulted in decrease of antioxidant potency. However, the number of quercetin free hydroxyl groups was not in direct correlation with its potential to inhibit inflammatory mediators production. To conclude, quercetin derivatives present in systemic circulation after consumption of quercetin may act as potent antioxidant and anti-inflammatory agents and can contribute to overall biological activity of quercetin-rich diet.

1. Introduction

Quercetin is a natural flavonoid found abundantly in almost all edible vegetables and fruits. The daily intake of quercetin in the Western diet is high, being approximately 15 mg. As an example, it is estimated that red onion, common onion, cranberry, blueberry and fig have 39, 20, 15, 8 and 5 mg of quercetin aglycone per 100 g of fresh weight of edible portion, respectively (Bhagwat, Haytowitz, & Holden, 2014).

There is growing body of evidence showing that quercetin has great therapeutic potential in the prevention and treatment of different chronic diseases, including cardiovascular and neurodegenerative diseases, as well as cancer (Boots, Haenen, & Bast, 2008; Dajas, 2012; D'Andrea, 2015; Russo, Spagnuolo, Tedesco, Bilotto, & Russo, 2012; Serban et al., 2016). It has been shown that quercetin exerts health beneficial effects in a number of cellular and animal models, as well as in humans, through modulating the signaling pathways and gene expression involved in these processes (Wang et al., 2016). Consequently, intake of a quercetin-rich diet is supported and is positively correlated with health promotion (D'Andrea, 2015). Quercetin can also be taken as a dietary supplement with daily recommended doses of 200–1200 mg, as well as a nutraceutical through functional foods with a concentration range of 10–125 mg per serving. Dietary supplementation with quercetin and its addition into food is highly supported by data on its safety (Harwood et al., 2007; Okamoto, 2005). Bioavailability of quercetin, defined as the portion of an initially administered dose that reaches the systemic circulation unchanged (Jackson, 1997), is very low, mostly due to its extensive metabolism. Namely, quercetin is present in plants mainly in its highly hydrophilic glycosylated forms, primarily as β-glycosides of various sugars (Lee & Mitchell, 2012). Prior to absorption in the gut, flavonoids first need to be freed from plant tissue by chewing in the oral cavity and then processed by digestive juices in the intestine or by microorganisms in the colon. There are two main routes of quercetin glycosides absorption by the enterocytes. Firstly, absorption goes via a transporter, followed by deglycosylation within the enterocyte by cytosolic glycosidase. Secondly, deglycosylation can occur by luminal hydrolases, followed by transport of the aglycone into enterocyte by passive diffusion or via different transporters (Day, Gee, DuPont, Johnson, & Williamson, 2003; Walle, Otake, Walle, & Wilson, 2000; Wolffram, Blöck, & Ader, 2002; Ziberna, Fornasaro, Čvorović, Tramer, & Passamonti, 2014).

Further biotransformation of quercetin aglycone involves

Abbreviations: 12-HETE, 12(*S*)-hydroxy-(5*Z*,8*Z*,10*E*,14*Z*)-eicosatetraenoic acid; 12-HHT, 12(*S*)-hydroxy(5*Z*,8*E*,10*E*)-heptadecatrienoic acid; 12-LOX, 12-lipoxygenase; AA, arachidonic acid; BHT, butylated hydroxytoluene; COX-1, cyclooxygenase-1; DMSO, dimethyl sulfoxide; DPPH, 1,1-diphenyl-2-picryl-hydrazyl; FRAP, ferric reducing ability of plasma; iso-glucoside, isorhamnetin-3-*O*-glucoside; LP, lipid peroxidation; PGE_2, prostaglandin E_2; q-5methyl, quercetin-3,5,7,3′,4′-pentamethylether; q-diglucoside, quercetin-3,4′-di-*O*-glucoside; q-glucuronide, quercetin-3-*O*-glucuronide; SD, standard deviation; TXB_2, thromboxane B_2

* Corresponding author.
E-mail address: marija.lesjak@dh.uns.ac.rs (M. Lesjak).

http://dx.doi.org/10.1016/j.jff.2017.10.047
Received 29 November 2016; Received in revised form 8 October 2017; Accepted 30 October 2017

glucuronidation, sulfation and methylation of hydroxyl groups, which primarily occurs in enterocytes and hepatocytes. Consequently, after the intake of food rich in quercetin glucosides and aglycone, methylated, glucuronidated, sulfated and combined derivatives of quercetin, such as isorhamnetin-3-glucuronide, quercetin diglucuronide, quercetin glucuronide sulphate, methylquercetin diglucuronide, etc., have been found in the human plasma (Terao, Murota, & Kawai, 2011). It is assumed that they are produced in the small intestine, transported into the portal vein and are further converted into other metabolites in the liver within phase II metabolism. After returning to the bloodstream they are excreted in urine via kidneys. Additionally, a portion of quercetin is converted to low molecular weight phenolic acids, such as 3-hydroxyphenylpropionic acid, 3,4-dihydroxyphenylpropionic acid and 3-methoxy-4-hydroxyphenylpropionic acid (Mullen, Edwards, & Crozier, 2006; Olthof, Hollman, Buijsman, Amelsvoort, & Katan, 2003).

In light of the efficient metabolism of quercetin, some authors consider that evidence on biological effects and mechanisms of quercetin are insufficient, as they are mainly based on *in vitro* studies with quercetin aglycone, which is hardly present in human plasma (Kroon et al., 2004; Mullen et al., 2006). In addition, some research on quercetin activities in humans have so far shown inconclusive and even conflicting results (Egert et al., 2008). On the other side, studies have shown that the biological properties of quercetin aglycone and its metabolites may be different (Amić et al., 2017; Chuang et al., 2016; Kroon et al., 2004; Terao et al., 2011). Taking into account that metabolites are the potential bioactive forms *in vivo*, more studies should be carried out on quercetin derivatives regarding their biological activities.

Bearing in mind that there is insufficient data on the antioxidant and anti-inflammatory effects of quercetin plasma metabolites (Al-Shalmani et al., 2011; Boesch-Saadatmandi et al., 2011; Cho et al., 2012; Derlindati et al., 2012; Dueñas, Surco-Laos, González-Manzano, González-Paramás, & Santos-Buelga, 2011; Justino et al., 2004; Messer, Hopkins, & Kipp, 2015; Morand et al., 1998; Santos et al., 2008; Wang et al., 2016; Wiczkowski et al., 2014), the overall goal of this study was to address these gaps in knowledge. Thus, this study was done to evaluate structure-antioxidant and anti-inflammatory activity relationships of glucuronidated and methylated quercetin metabolites commonly found in human plasma (Cialdella-Kam et al., 2013; Day et al., 2001; Wittig, Herderich, Graefe, & Veit, 2001), such as quercetin-3-*O*-glucuronide (q-glucuronide), 4′-*O*-methylquercetin (tamarixetin) and 3′-*O*-methylquercetin (isorhamnetin), as well as two forms of quercetin found in edible plants – quercetin-3,4′-di-*O*-glucoside (q-diglucoside) and isorhamnetin-3-*O*-glucoside (iso-glucoside). Besides, quercetin-3,5,7,3′,4′-pentamethylether (q-5methyl) was included in order to evaluate the importance of free hydroxyl groups in quercetin biopotential (Fig. 1). Since common onion (*Allium cepa* L.) is one of the best known sources of bioavailable quercetin (Wiczkowski et al., 2014), the extract of this plant was also examined in order to compare biopotential of dietary quercetin with biopotential of quercetin metabolites as pure compounds. In general, this research should contribute to a better understanding of factors regulating nutraceutical potency of quercetin and its role in managing different diseases.

2. Material and methods

2.1. Chemicals and reagents

Quercetin, q-glucuronide, tamarixetin, isorhamnetin, iso-glucoside, q-diglucoside and q-5methyl were purchased from Extrasynthese (Genay, France). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Extract preparation

Samples of cultivated common onion (*A. cepa L. var. cepa*) were collected in July 2009 in the village of Neradin, the Fruška Gora Mountain, Serbia. The voucher specimen No. 2-1762 was prepared, identified and deposited at the Herbarium of the Department of Biology and Ecology (BUNS Herbarium), University of Novi Sad Faculty of Sciences, Serbia. 30 g of fresh plant material (whole plants) was grounded and macerated with 70% aqueous methanol (8 mL per 1 g of plant material) during 72 h at 30 °C. After filtration, the solvent was evaporated to dryness under vacuum at 45 °C and dry residues were redissolved in 70% aqueous methanol to the final concentration of 200 mg/mL and used for determination of antioxidant and anti–inflammatory activities. The extract was made in triplicate.

2.3. Estimation of antioxidant potential

Antioxidant potential of quercetin, its metabolites and glycosides, as well as *A. cepa* extract, was determined using previously adapted assays for 96-well microplates related to free radical scavenging ability towards 1,1-diphenyl-2-picrylhydrazyl radical ($DPPH^{\cdot}$), ferric reducing ability of plasma (FRAP) and inhibitory potential towards lipid peroxidation (LP; Lesjak et al., 2013, 2014). The results were compared with the synthetic antioxidant butylated hydroxytoluene (BHT). Additionally, total flavonoid content was determined in *A. cepa* extract (Lesjak et al., 2013). All compounds were dissolved in dimethyl sulfoxide (DMSO) to obtain 3.33 mg/mL stock solutions and afterwards used for estimation of antioxidant potential. Each experimental procedure is briefly explained in the text below.

2.3.1. $DPPH^{\cdot}$ scavenging ability

Ten microliters of each sample, quercetin and its derivatives dissolved in DMSO and *A. cepa* extract dissolved in 70% aqueous methanol in series of concentrations, were added to 100 µL of 90 µmol/L DPPH solution in methanol, and the mixture was diluted with additional 190 µL of methanol. In controls, the 10 µL of sample was substituted with DMSO (for quercetin derivatives) or 70% aqueous methanol (for *A. cepa* extract). In blank probes, only methanol (290 µL) and each sample (10 µL) were mixed, while in blank probe for controls, only 300 µL of methanol were added. Measurements of absorbance were read at 515 nm after 1 h. All samples and the control were made in triplicate. The percentage of inhibition achieved by different concentrations of samples in the antioxidant assays performed was calculated by using the following equation: $I\ (\%) = (A_0 - A)/A_0 \times 100$, where A_0 was the absorbance of the control reaction and A was the absorbance of the examined samples, both corrected for the value of the corresponding blank probes. Corresponding inhibition-concentration curves were drawn using Origin software, version 8.0 and IC_{50} values (concentration of extract that inhibited $DPPH^{\cdot}$ formation by 50%) were determined. For each assay final result was expressed as mean ± standard deviation (SD) of three measurements.

2.3.2. FRAP

Examined samples were tested in series of different concentrations and ascorbic acid (1.25–160 µg/mL) was used for creating a standard curve. FRAP reagent was prepared by mixing 10 mmol/L 2,4,6-tripyridyl-*S*-triazine in 40 mmol/L HCl, 0.02 mol/L $FeCl_3$, and acetate buffer (22.78 mmol/L CH_3COONa, 0.28 mol/L CH_3COOH, pH 3.6) at ratio of 1:1:10 (v/v/v), respectively. After the addition of the sample or ascorbic acid (10 µL, substituted with DMSO or 70% aqueous methanol in the control) to 290 µL of FRAP reagent and 6 min of incubation at room temperature, absorbance was read at 593 nm. In blank probes samples (10 µL) were mixed with 290 µL of distilled water. All samples and blank probes were made in triplicate and mean values of reducing power were expressed as µg of ascorbic acid equivalents per µg of dry extract or pure compound, calculated according to the standard calibration curve.