JOURNAL OF FUNCTIONAL FOODS 11 (2014) 121–129

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Metabolites of dietary quercetin: Profile, isolation, identification, and antioxidant capacity

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ARTICLE INFO

Article history:
Received 6 June 2014
Received in revised form 18 September 2014
Accepted 24 September 2014
Available online

Keywords:
Onion
Quercetin
Pig quercetin metabolites
Isolation
HPLC-MS/MS
Antioxidant activity

ABSTRACT

The study of the profile, isolation and antioxidant capacity of pig quercetin metabolites after onion dry skin intake was performed. Onion dry skin contain very high content of quercetin, which occurred mainly in aglycone form (87%). Urine was collected from seventeen pigs before and within 2-6 hours after intake of onion dry skin providing quercetin. Quercetin metabolites profile was analyzed by HPLC-MS/MS. Upon quercetin intake, quercetin was absorbed and occurred in pig urine in methylated, glucuronided, sulfated and combined derivatives. Among 12 quercetin metabolites identified, quercetin monoglucuronides were predominant. Quercetin metabolites were isolated and purified by Amberlite XAD-16 column chromatography and on HPLC C18 semi-preparative column. Quercetin metabolites mixture showed higher radical-scavenging activities compared to native quercetin derivatives of onion skin. Results indicated that the profile of quercetin metabolites in pig reflects that in a humans. Moreover, quercetin metabolites present in systemic circulation after quercetin intake still acted as antioxidants.

1. Introduction

In order to understand the biological activity of dietary quercetin, it is important to explore its fate in the organism. Since the bioavailability studies allow the determination of real exposure of the organism to quercetin, their results are an essential topic for other experiments aiming at the explanation of quercetin physiological functions (Ting, Jiang, Ho, & Huang, 2014).

There have been a number of publications on the bioavailability of quercetin (Manach, Williamson, Morand, Scalbert, & Remesy, 2005). So far, it has been shown that quercetin is absorbed and occurs in animal and human blood plasma and urine in the form of conjugated derivatives (Terao, Murota, & Kawai, 2011). The extent of absorption, bioavailability and metabolism is highly dependent on the nature of quercetin derivatives consumed as well as on the food matrix used (Wiczkowski et al., 2008). In this respect, the pathways of quercetin fate after intake are not fully characterized and understood yet; however, a number of phenomena related to quercetin absorption, bioavailability and metabolism have already been described (Manach et al., 2005). Methylated, glucuronidated, sulfated, and combined conjugates of quercetin after the intake

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http://dx.doi.org/10.1016/j.jff.2014.09.013
1756-4646/

of food rich in quercetin glucosides and aglycone have been found (Terao et al., 2011).

There have also been numerous studies on the biological activity of quercetin, where it is implied that quercetin present in food products has a protective potential against chronic degenerative diseases. Nonetheless, the mechanisms of this action are not completely explained. It is noteworthy that in most of the studies only quercetin 3-glucuronide was used (Bansal et al., 2012; Terao et al., 2011; Williamson, Barron, Shimoi, & Terao, 2005).

To the best of our knowledge, in the previous studies related to the biological properties of quercetin metabolites, the strategy with dietary-relevant profile of quercetin metabolites has never been used. Although, as mentioned above, earlier studies indicated that after the intake of quercetin, the profile of quercetin metabolites in biological fluids was noted to consist of several compounds with different concentration (Graf et al., 2005; Mullen, Boitier, Stewart, & Crozier, 2004). Therefore, the main problem identified is connected with obtaining the mixture of quercetin metabolites, reflecting the profile of quercetin compounds in humans after quercetin consumption, as well as measuring its biological activities.

Taking the above into account, determination of quercetin metabolites with their profile being close to that in human tissues, and subsequent isolation of these compounds are essential for further investigations explaining the physiological function of quercetin metabolites. Since previous studies indicated that onion is one of the best sources of bioavailable quercetin (de Vries, Hollman, Amersfoort, Olthof, & Katan, 2001; Hollman et al., 1997) and that aglycone of quercetin is better bioavailable than quercetin glucosides when consumed in the form of food matrix (Wiczkowski et al., 2008), in this study, dry skin of onion was used as a source of dietary quercetin. Because the digestive tract of a pig is similar to that of a human, this animal was chosen as the investigation model for production of quercetin metabolites. Four different assays were used for determination of antioxidant capacity of quercetin metabolites mixture in comparison to native quercetin derivatives found in onion skin.

2. Material and methods

2.1. Reagents

Reagents including acetonitrile, methanol, formic acid, ethyl acetate, sodium carbonate were purchased from Merck KGaA (Darmstadt, Germany). 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and Sulfatase type H-5 were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Hydrophilic condition (ACW) and lipophilic condition (ACL) kits (model no. 400.801) for the photochemiluminescence (PCL) assay were received from Analytik Jena AG (Jena, Germany). Water was purified with a Mili-Q system (Millipore, Bedford, MA, USA). Quercetin (Q), quercetin 3-glucoside (Q3G), isorhamnetin (iR), isorhamnetin 3-glucoside (iR3G) (Extrasynthese, Genay, France) and quercetin 4′,3-diglucoside (Q3,4′G) and quercetin 4′-glucoside kindly provided (Q4′G) by Dr. T. Tsushida Tsukuba (Japan) were used for identification and calculation.

2.2. Plant material

Dry skin was obtained from yellow common onion (*Allium cepa* L., Hercules variety) kindly provided by Yara Poland (Szczecin, Poland) and was used as quercetin source. The bulbs of onion were peeled off from the dried outer leaves. Next, dry skin (approximately 5 kg) was dried in the laboratory oven at a temperature of 40 °C. Upon drying, the samples obtained were pulverized and stored at –20 °C until analysis.

2.3. Determination of quercetin in onion dry skin

Extraction and analysis of Q derivatives in onion were carried out as described previously by Wiczkowski et al. (2008). About 0.05 g of freeze-dried and pulverized onion dry skin was extracted by using 30 s sonication (VC 750, Sonics & Materials, Newtown, CT, USA) with 1 mL of 80% methanol. Subsequently, the mixture was vortexed for 30 s, again sonicated and vortexed, and centrifuged (Centrifuge 5415R, Eppendorf, Germany) for 10 min (13,200 × *g* at 4 °C). Supernatant was collected in a 5 mL flask. This step was repeated five times. Finally, before the analysis, the extract was centrifuged (20 min, 13,000 × *g*, 4 °C) and 5 μL of the extract was submitted to HPLC-DAD system (Shimadzu, Kyoto, Japan) equipped with a 150 × 2.1 mm i.d. XBridge C18 3.5 μm column (Waters, Milford, MA, USA). The HPLC system consisted of two pumps (LC-10 AD_{VP}), DAD detector (SPD-M10 A_{VP}) set at 360 nm, autosampler (SIL-10 AD_{VP}), column oven (CTO-10 AS_{VP}) and system controller (SCL-10 A_{VP}). All chromatographic determinations were performed at 45 °C with the flow rate of 0.23 mL/min. Q derivatives were eluted in gradient system composed of water/formic acid (99:1, v/v, phase A) and acetonitrile/formic acid (99:1, v/v, phase B). Gradient was composed of: 10–20% B (0–14 min), 20–80% B (14–25 min), 80–10% B (25–26 min), and 10% B (26–45 min). Compounds were identified by comparison of their retention times and UV-visible spectrum with standards and their quantity was calculated from HPLC-DAD peak area at 360 nm against appropriate external standard. The calibration curve (the range of 0.3–45 μM) was linear with a correlation coefficient of 0.98.

2.4. Animals, diets and study design

The study was conducted in accordance with the study protocol approved by the Ethical Committee of the University of Warmia and Mazury in Olsztyn (no 73/2010/N). Seventeen 6-month-old female pigs of Large White Landrace, each weighing approximately 100 kg, were used. Animals were kept in a light and temperature-controlled animal room of the Pig Breeding Farm in Dobrzejowice (Poland) in separate cages with a free access to tap water. Pigs followed a quercetin-free diet for 1 day prior to the experiment. After overnight fast, onion dry skin preparations providing 15 mg of Q (calculated as Q aglycone) per kg of body weight were mixed with pigs' fodder and fed to each animal. Consumption of that mix was approximately 95%. Collection of urine was carried out before and within the period of 2–6 h after the Q intake. Finally, 7 L and 12 L of urine