Quercetin from Shallots (Allium cepa L. var. aggregatum) Is More Bioavailable Than Its Glucosides

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Abstract

The lipophilic character of quercetin suggests that it can cross enterocyte membranes via simple diffusion. Therefore, it should be more bioavailable than its glucosides, which require preliminary hydrolysis or active transport for absorption. However, the published human studies show that quercetin is less bioavailable than its glucosides. Assuming that low bioavailability of quercetin aglycone provided to humans as a pure substance is the result of its low solubility in the digestive tract, we studied its bioavailability from dietary sources in which quercetin was dispersed in the food matrix. In a randomized crossover study, 9 volunteers took a single dose of either shallot flesh (99.2% quercetin glucosides and 0.8% quercetin aglycone) or dry shallot skin (83.3% quercetin aglycone and 16.7% quercetin glucosides), providing 1.4 mg quercetin per kg of body weight. Blood samples were collected before and after consumption of shallot preparations. Plasma quercetin was measured on HPLC with electrochemical detection after plasma enzymatic treatment. The maximum plasma quercetin concentration of 1.02 ± 0.13 μmol/L was reached at 2.33 ± 0.50 h after shallot flesh consumption compared with 3.95 ± 0.62 μmol/L at 2.78 ± 0.15 h after dry skin consumption. The area under the concentration-time curve after dry skin consumption was 47.23 ± 7.53 μmol·h⁻¹·L⁻¹ and was significantly higher than that after shallot flesh intake (22.23 ± 2.32 μmol·h⁻¹·L⁻¹). When provided along with dietary sources, quercetin aglycone is more bioavailable than its glucosides in humans. Results point to the food matrix as a key factor. J. Nutr. 138: 885–888, 2008.

Introduction

The result of the first investigation on flavonoid bioavailability in animals indicated that most flavonoids present in food were not absorbed in the small intestine as they occur in glycosidic form (1). The author postulated that only flavonoids occurring in a free form may cross the barrier of the small intestine biological membranes. Over 100 reports on bioavailability of flavonoids in humans have been published (2,3) and it is clear now that flavonoids are absorbed into the blood circulation system and are present there in the native and conjugated forms (4–6).

Quercetin is widespread in plant food products and is consequently a commonly consumed flavonoid. It is present in tea, wine, onions, lettuce, cabbage, broccoli, beans, apples, peaches, and buckwheat, among others. A considerable number of current studies have dealt with determining quercetin bioavailability (3) and most of them have shown that onion is an excellent source of quercetin. Quercetin aglycone and its glucosides are absorbed better than quercetin administered in nonglucosidic forms. The bioavailability of 2 quercetin glucosides, 3-glucoside and 4'-glucoside, do not differ (7). However, it is still not certain which form of quercetin, its aglycone or glucosides, is better absorbed in humans. In a study with ileostomy patients, Hollman et al. (8) showed that quercetin absorption was 2 times higher when it was consumed as quercetin glucosides originating from onion compared with quercetin aglycone. However, in the latter case, quercetin was administered in capsules as a pure substance.

One of the most important factors, still not sufficiently explored, that may affect flavonoid absorption from dietary sources is the food matrix. Therefore, one of the questions still unanswered is whether there are any differences in bioavailability of quercetin and its glucosides when both forms are provided from a dietary source. In this study, human bioavailability of quercetin and quercetin glucosides from dry shallot skin and shallot flesh was compared.

Materials and Methods

Material and chemicals. Dry skin and flesh were obtained from shallots (Allium cepa L. var. aggregatum G.Don.: Szalotka 1786) kindly provided by the Research Institute of Vegetable Crops in Skiernicewie and used as a quercetin source. Methanol, acetonitril, and formic acid were purchased from Merck KGaA. Lithium acetate dihydrate, Sulfatase
type H-5 (from *Helix pomatia*) with β-glucuronidase (400–600 unit/mg solid) and sulfatase (15–40 unit/mg solid) and Sulfatase type VI (from *Aerobacter aerogenes*) with sulfatase (10–20 unit/mL) were obtained from Sigma Chemical. Quercetin, quercetin 3-0-β-glucosides, isorhamnetin (Extrasyntese), quercetin 4',3-0-bis-β-glucosides, and quercetin 4'-O-β-glucosides, kindly provided by Dr. T. Tsushida, National Food Research Institute, Tsukuba, Japan, were used for identification and calculation.

**Determination of quercetin in shallot and plasma.** Extraction and analysis of quercetin derivatives in shallot were carried out as described previously by Wiczkowski et al. (9). Briefly, freeze-dried and pulverized shallot tissues were extracted 5 times with 80% methanol by sonication and vortexing. We proved that high extraction repetition rate and 10:1 (v:w) samplesolvent ratio ensured a minimum 98% recovery of both quercetin aglycone and its glucosides. Chromatographic determinations of the extracts were performed on HPLC with UV detection at 360 nm (Shimadzu) at 35°C with the flow rate of 0.2 mL/min on a C18(2) Luna 3-μm column, 150 × 2 mm (Phenomenex). The flavonones were eluted in a gradient system composed of water:formic acid (99.5:0.5, v/v) and acetonitrile. Confirmation of the compound identity was conducted on a mass spectrometer (QP8000x, Shimadzu). Analytical parameters are listed in [Supplemental Table 1](#).

Plasma quercetin was determined according to Piskula and Terao (10). Analysis of quercetin was performed on HPLC after enzymatic hydrolysis and extraction from blood plasma. Briefly, 50 μL plasma was mixed and incubated at 37°C for 75 min with 50 μL of 0.2 mmol/L acetate buffer pH 5.5 containing 750 units of β-glucuronidase and 30 units of sulfatase from Sulfatase type H-3 and 0.5 units of sulfatase from Sulfatase VI. The latter was added to ensure complete hydrolysis of dominant Q-3-sulfate metabolite (11). Released quercetin aglycone was extracted with 0.9 mL of methanol:acetic acid (95:5, v/v) solution by triplicate 30-s sonication and vortexing. We proved that high extraction repetition rate and 10:1 (v:wt) sample:solvent ratio ensured a minimum 98% recovery of both quercetin aglycone and its glucosides. Chromatographic determinations of the extracts were performed on HPLC with UV detection at 360 nm (Shimadzu) at 35°C with the flow rate of 0.2 mL/min on a C18(2) Luna 3-μm column, 150 × 2 mm (Phenomenex). The flavonones were eluted in a gradient system composed of water:formic acid (99.5:0.5, v/v) and acetonitrile. Confirmation of the compound identity was conducted on a mass spectrometer (QP8000x, Shimadzu). Analytical parameters are listed in [Supplemental Table 1](#).

**Subjects, diets, and study design.** The study was conducted in accordance with the ethical principles of the Helsinki Declaration and the study protocol was approved by the Bioethical Committee of the Warmia and Mazuria Regional Medical Chamber in Olsztyn. All subjects were fully informed about the study and signed an informed consent form.

Nine volunteers (5 women and 4 men), with a mean age of 29 yrs and a body weight of 63 kg, were randomly assigned to 2 groups. Based on a medical questionnaire, they were considered to be healthy and passed the exclusion criteria: smoking (>15 cigarettes per day), drinking coffee (>5 cups per day; 150 mL/cup), intolerance of and hypersensitivity to onion, garlic, hay fever, and/or other allergies. All were included in the study protocol were approved by the Bioethical Committee of the Warmia and Mazuria Regional Medical Chamber in Olsztyn. All subjects were fully informed about the study and signed an informed consent form.

The subjects were not allowed to drink alcohol for 2 wk before and during the study or to take any medicine during the study, except for oral contraceptives. They followed a quercetin-free diet for 3 d prior to and throughout the experiment. For this purpose, the list of authorized foods was given to the participants (see [Supplemental Table 2](#)). Moreover, during the first 4 h after the trial, they were allowed to drink water and then a light lunch (cheese dumplings) was provided. After that period for the next 68 h, the volunteers were allowed to resume their daily diet with restriction to non-quercetin foods.

To equilibrate the quercetin content in the doses, a quantity of shallots was peeled, quartered, and mixed and the dry skins were mixed and pulverized. The study was designed as 2 3-d crossover experiments separated by 7-d quercetin wash-out intervals. At 0800 on d 1 of each experiment, after an overnight fast, shallot preparations providing 1.4

| TABLE 1 Quercetin and quercetin glucoside concentrations in shallot dry skin and flesh 1 |
|------------------------|------------------------|------------------------|
|                        | Flesh                  | Dry skin               |
| Total quercetin, μmol/g| 4.93 ± 0.03            | 111.50 ± 0.03          |
| Quercetin aglycone, %  | 0.8                    | 83.3                   |
| Quercetin glucosides, %| 99.2                   | 16.7                   |
| Quercetin 4'-O-β-glucosides | 49.2                  | 13.2                   |
| Quercetin 4',3-O-bis-β-glucosides | 49.2               | 3.2                    |
| Quercetin 3-0-β-glucosides | 0.3                   | 0.2                    |
| Quercetin 3'-O-methyl 4'-O-β-glucosides | 0.5               | 0.1                    |

1 Values are means ± SEM, n = 3 or 4.

mg (4.63 μmol) quercetin per kg of body weight (shallot flesh or dry skin) with 200 mL water were served to the subjects. Immediately before consumption, the doses were homogenized in part of the water offered. To control the possible influence of homogenization on the quercetin derivative composition, 2 portions of shallot preparations were stored at −80°C until analyzed. Before (0 h, control) and after shallot consumption (0.25, 0.5, 1, 2, 3, 6, 8, 12, 24, 36, 48, and 72 h), elbow or forearm vein blood samples were drawn into heparinized BD vacutainers. After each sampling, blood was centrifuged at 4°C in 2 stages (500 × g; 10 min and 1000 × g; 5 min) and plasma was then divided into 50-μL portions, frozen, and stored at −80°C until analysis.

**Pharmacokinetic methods and statistical analysis.** The data are presented as means ± SEM. The maximum quercetin concentration (C_{max})5, time to reach C_{max} (t_{max}), elimination constant (k), elimination half-life (t_{1/2}), area under the concentration-time curve (AUC_{0-24h}), and elimination half-life (t_{1/2}, AUC_{0-24h}) were determined using Kinetica v. 4.3 (InnaPhase) software. The mean values of pharmacokinetic parameters (t_{max}, C_{max}, k, t_{1/2}, AUC_{0-24h}) for treatment groups were compared using 1-way ANOVA with a Bonferroni post hoc test. To measure the differences between means of measurement points between treatment groups, we applied a repeated ANOVA with Tukey’s post hoc test. P < 0.05 was considered significant. The statistical analysis was performed using Statistica v. 6 (Stat Soft).

**Results** We found 5 quercetin derivatives: quercetin 3,4'-O-bis-β-glucoside, quercetin 3-0-β-glucoside, quercetin 4'-O-β-glucoside, isorhamnetin 4'-O-β-glucoside, and quercetin aglycone (Table 1). Quercetin content in dry shallot skin was over 20 times higher than in shallot flesh. In dry skin, 83% of total quercetin was in a free form, whereas in shallot flesh it was <1%. This means that >99% of quercetin in shallot flesh was formed by its glucosides, mainly by quercetin 3,4'-O-bis-β-glucoside and quercetin 4'-O-β-glucoside.

The plasma quercetin concentration was consistently higher after dry skin consumption compared with flesh consumption and it was significantly higher between 0.5 h and 8 h (Fig. 1). After the intake of dry skin, the C_{max} was ~3 times higher than after shallot flesh intake (P < 0.05) (Table 2). In both cases, t_{max} occurred more than 2 h after intake. The individual maximum concentration after the intake of shallot flesh was measured in most subjects in blood sampled after 2 h and it ranged between 0.36 μmol/L and 1.90 μmol/L, whereas after dry skin intake after 3 h it ranged from 2.16 μmol/L to 8.21 μmol/L. There was a very high inter-individual variation in plasma quercetin concentration among the subjects. The subjects who were the highest and the lowest quercetin absorbers after shallot flesh consumption were also the highest and the lowest absorbers after the shallot dry skin consumption.

5 Abbreviations used: AUC, area under the concentration-time curve; C_{max}, maximum quercetin concentration; k, elimination constant; t_{max} time to reach C_{max}.
glucoside) in the diet.

differences we found resulted from the quercetin form (aglycone or

calculated after shallot flesh intake, where quercetin occurred

drug skin and flesh (Table 2), which confirms that the

m 1 Values are means ± SEM, n = 9. *Different from flesh at that
time, P < 0.05.

TABLE 2 Plasma pharmacokinetic parameters of quercetin in
tsuts who consumed 4.63 μmol quercetin per kg body weight

<table>
<thead>
<tr>
<th>Quercetin sources</th>
<th>Flesh</th>
<th>Dry skin</th>
</tr>
</thead>
<tbody>
<tr>
<td>( t_{max} ), μmol/L</td>
<td>( 1.02 ± 0.13 )</td>
<td>( 3.95 ± 0.62^* )</td>
</tr>
<tr>
<td>( t_{max} ), h</td>
<td>( 2.33 ± 0.50 )</td>
<td>( 2.78 ± 0.15 )</td>
</tr>
<tr>
<td>( k ), h⁻¹</td>
<td>( 0.02 ± 0.003 )</td>
<td>( 0.02 ± 0.003 )</td>
</tr>
<tr>
<td>( t_{1/2} ), h</td>
<td>( 45.68 ± 9.09 )</td>
<td>( 32.73 ± 4.11 )</td>
</tr>
<tr>
<td>AUC, μmol·h·L⁻¹</td>
<td>( 22.23 ± 2.32 )</td>
<td>( 47.32 ± 7.53^* )</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 9. *Different from flesh, P < 0.05.

Discussion

The low hydrophilicity of quercetin aglycone allows it to enter intestinal enterocytes as a result of passive transport (12,13). The presence of polar glucose substitution in the quercetin molecule increases hydrophilicity and limits its absorption via this route. Therefore, 2 mechanisms enabling quercetin glucoside absorption are postulated. According to the first one, prior to absorption by an enterocyte, quercetin glucosides must be hydrolyzed in the small intestine by phlorizin lactase (EC 3.2.1.23 and 62), which is capable of hydrolyzing flavonoid glucosides and thus released free quercetin may be absorbed (14,15). Hydrolysis by phlorizin lactase was the main event in the absorption of quercetin-3-glucoside present in onion (13,16,17). Another possibility is the active transfer of quercetin glucosides through the small intestine mucosa cell wall via the Na⁺/glucose transporter and subsequent glucoside hydrolysis by cytosolic β-glucosidas.

In humans, Hollman et al. (8) demonstrated better bioavailability of quercetin from its β-glucosides originating from fried onion than from capsules containing quercetin aglycone. Morand et al. (20) showed better absorption of quercetin-3-glucoside in rats than of quercetin aglycone. This is rather inconsistent with the above-described mechanisms of quercetin absorption. It could be expected that quercetin entering enterocytes via passive transport should be better absorbed than its glucosides, which, to be absorbed, require the action of hydrolytic enzymes or transporters. We hypothesized that the reason for this discrepancy is the form in which quercetin aglycone was offered. In both the above-mentioned studies, quercetin was given as a pure substance poorly soluble in the digestive tract environment.

When comparing their bioavailability, it seems crucial to consider the differences in the solubility of crystalline quercetin aglycone and its glucosides. There are studies that indicate that the efficiency of intestinal absorption of flavonoids is strongly affected by their solubility in the vehicle and the diet composition. In our previous research (10), we observed that absorption of quercetin, which was administered to rats in a combination of propylene glycol and water, improved along with better quercetin solubility in the vehicle. In another animal study, the absorption of onion quercetin was enhanced when the diets were enriched with lipids (21–23). The bioavailability of isolated quercetin glucosides was also enhanced when offered with 5% ethanol and sodium chloride dissolved in water (7,24). However, Azuma et al. (23) indicated that ethanol may serve as a quercetin bioavailability enhancer only in concentrations ≥30%. It must be noted here that according to Graefe et al. (25), the bioavailability of quercetin from onion, in which a variety of quercetin glucosides is present, is comparable to the bioavailability of isolated quercetin 4'-glucoside.

We aimed to compare the bioavailability of quercetin aglycone and its β-glucosides in humans from dietary sources. For that purpose, dry shallot skin, where most quercetin was present as aglycone, and shallot flesh, with almost exclusive content of quercetin glucosides, were served to volunteers in the amounts corresponding to the consumption of a middle-sized onion bulb (90–120 g) (26). Onion can be consumed in many ways: raw, cooked in water, or fried in oil. Considering the enhancing effect of lipids on quercetin absorption, we decided to provide shallot preparations to the subjects in water, the less favorable environment for quercetin aglycone solubility and thus for its absorption.

Ng et al. (27) showed that dry skin was more resistant to mechanical damage than other onion tissues. To minimize the differences in the mechanical properties of the offered preparations, shallot flesh and dry skin were homogenized in water using a kitchen food processor immediately before the intake. With this treatment, we tried to unify the accessibility of the digesta to the plant tissues after consumption. Because onion tissues contain β-glucosidase able to hydrolyze quercetin glucosides (28), the stability of quercetin derivatives was controlled after homogenization. The results showed that within 1 h after the treatment, the differences in composition of quercetin derivatives were <5%.

Quercetin was found in volunteers’ blood plasma only in the conjugated forms. It appeared as early as 15 min after the consumption of shallot flesh (0.20 ± 0.02 μmol/L) and dry skin (0.71 ± 0.15 μmol/L). This indicates that absorption occurred in the upper part of the digestive tract (8,21,29), irrespective of quercetin source. The rapid increase of quercetin concentration
in plasma was substantially faster after the intake of shallot skin than that of shallot flesh. This phenomenon may at least in part result from absorption of flavonoid aglycone but not of their glucosides from the stomach (30–32). We attribute the slower increase in plasma quercetin concentration after the consumption of shallot flesh rich in quercetin glucosides to the fact that quercetin absorption must be preceded by hydrolysis of its glucosides or it requires active transport.

The main conclusion emerging from this study is that when quercetin and its derivatives are consumed as immanent components of food, the bioavailability of quercetin aglycone in humans is better than that of quercetin glucosides. We previously reported the same observation in rats, which were also given onion preparations (9). The better bioavailability of quercetin aglycone than of its glucosides observed here is in opposition to other studies based on the same comparison. The basic difference in our approach was the use of plant products rich in quercetin aglycone instead of quercetin in standard purified commercial form. To the best of our knowledge, this is the first report in which quercetin was offered to humans along with the natural source, which allowed us to overcome the problems with crystalline quercetin solubilization. Our contrary results may result from many factors that may considerably contribute to bioavailability of quercetin, among which are the mechanisms of transportation, hydrolysis, and solubility. In dry shallot skin, quercetin aglycone does not appear in crystalline form but it is dispersed in the onion matrix, which makes it very easily accessible. Single molecules of quercetin aglycone and quercetin glucosides are naturally built into the food matrix to some extent.

To conclude, we demonstrated that when quercetin and its derivatives are provided for consumption along with their natural sources in which these compounds are dispersed in the matrix, quercetin aglycone is more bioavailable than its glucosides. This finding suggests that in some cases, bioavailability of isolated food components consumed as food supplements could be less than when they are consumed with the food matrix.

Literature Cited

20. Morand Ch, Manach C, Crespy V, Remyes Ch. Quercetin 3-O-beta-glucoside is better absorbed than other quercetin forms and is not present in rat plasma. Free Radic Res. 2000;33:667–76.