Enhancing Effect of Lipids and Emulsifiers on the Accumulation of Quercetin Metabolites in Blood Plasma after the Short-term Ingestion of Onion by Rats

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The effects of co-ingested lipids and emulsifiers on the accumulation of quercetin metabolites in blood plasma after the short-term ingestion of onion by rats were investigated. Plasma extracts of rats that had been fed onion-containing diets for one and two weeks were analyzed by HPLC with electrochemical detection after a treatment with sulfatase/β-glucuronidase. Almost all of the quercetin metabolites in the plasma were sulfate/ glucuronide conjugates of quercetin and isorhamnetin. More than 4.6% (w/w) of soybean oil in the diets significantly enhanced the accumulation of quercetin metabolites in the plasma. Fish oil and beef tallow increased this to an extent similar to that with soybean oil, and lecithin was more effective than the other three lipids. Two emulsifiers, sodium caseinate and sucrose fatty acid ester, also showed an enhancing effect on the accumulation of quercetin metabolites. These results indicate that co-ingested lipids and emulsifiers could enhance the bioavailability of quercetin glucosides in onion.

Key words: onion; quercetin metabolite; plasma; lipid; emulsifier

Vegetables and fruits contain a variety of antioxidants such as flavonoids, phenolic acids, and ascorbic acid. Flavonoids are polyphenolic compounds which are widely distributed in foods of plant origin. In recent years, flavonoids have attracted strong attention for their potential beneficial effects on disease prevention. Considerable in vitro studies have demonstrated that flavonoids can prevent oxidative modification of plasma low-density lipoprotein, which has been suggested to participate in the initial process of atherosclerosis leading to coronary heart disease.1–4) Indeed, epidemiological studies have indicated that flavonoid intake was associated with a reduced incidence of coronary heart disease.5–8)

Flavonoids are categorized into flavones, flavanones, flavanols, isoflavones, chalcones, and anthocyanidins. Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is one of the abundant flavonol-type flavonoids and is ubiquitously present in fruits and vegetables, mostly as its glycoside form.9,10) The results of our previous study have demonstrated that Corchorus olitorius, called “morohieya” in Japanese, was rich in quercetin glycosides.11) Quercetin is known to exhibit high antioxidative activity.12–15)

Numerous studies have focused on the absorption and metabolic conversion of flavonoids, quercetin being one of the most studied flavonoids. Although the intestinal absorption and metabolism of ingested quercetin glycosides has been uncertain, substantial previous evidence has suggested that the main pathway is their conversion to conjugate metabolites through aglycone by deglycosylation activity and subsequent conjugation activity.16–27) Moreover, conjugated metabolites of quercetin have recently been found to exhibit antioxidative properties toward lipid peroxidation in vivo and in vitro.18,19,22,24,26,28) It has been shown that the efficiency of intestinal absorption of quercetin is affected by its solubility in the vehicle used for administration.29) We have found that a combination of lipids and emulsifiers enhanced the absorption of orally administered quercetin in rats, and suggested that quercetin’s solubility in lipid micelles was an important factor for its higher absorption from the alimentary tract.30) However, it is still not clear how co-existing food constituents affect the intestinal absorption of quercetin after the ingestion of foods rich in quercetin glycosides.

Onion is one of the most important dietary sources of quercetin because of its high content of quercetin and its regular consumption worldwide. Recent studies have shown the in vivo antioxidative effects of onion oil and onion meal.31–33) It has also been reported that onion had hypoglycemic and hypocholesterol-
leemic effects and a beneficial influence on renal lesions in diabetes.34–36)

This study aims to clarify whether the co-ingestion of lipids and emulsifiers would affect the accumulation of quercetin metabolites in the plasma after short-term ingestion of onion by rats. We investigated their accumulation in rats fed on diets containing onion and different contents and types of lipids for one and two weeks, with or without emulsifier-supplemented drinking water. Quercetin metabolites in the plasma were deconjugated and quantitatively determined by HPLC with electrochemical detection.

Materials and Methods

Chemicals. Quercetin, soybean oil, soybean lecithin, beef tallow, and sodium caseinate were purchased from Wako Pure Chemical Industries (Osaka, Japan). Quercetin 3-glucoside (Q3G) andisorhamnetin were obtained from Extrasyntese (Geney, France). Quercetin 4′-glucoside (Q4′G) and quercetin 3,4′-diglucoside (Q3,4′G) were kindly supplied by Dr. T. Tsushida of the National Food Research Institute (Japan). Sulfatase type H-5 (from Helix pomatia) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Cornstarch, sucrose, casein, cellulose powder, AIN-76 mineral mix, and AIN-76 vitamin mix supplemented with choline caseinate or 0.5% (w/w) lecithin diet were purchased from Oriental Yeast Co. (Tokyo, Japan). Fish oil (EPA-28G, containing 28% of eicosapentaenoic acid and 13% of docosahexaenoic acid) was obtained from Nippon Chemical Feed Co. (Hakodate, Japan). The hydrophilic sucrose fatty acid ester, “Ryoto sugar ester S1670” was kindly supplied by Mitsubishi-Kagaku Foods Corp. (Tokyo, Japan). All other chemicals were of analytical or HPLC grade.

Onion sample. One cultivated variety of yellow-type onion (Allium cepa L.), “Kamui”, was harvested in Hokkaido and kindly supplied by Takii Seed Co., (Osaka, Japan) to use for the experiment.

Measurement of quercetin glucosides in onion. Quercetin glucosides were extracted by suspending 200 mg of onion powder in 4 ml of 70% methanol for 24 h in darkness at room temperature with occasional vigorous shaking. After centrifuging at 3,000 g for 5 min, the extraction procedure was repeated one more time. The supernatants were combined and made up to 10 ml with 70% methanol. The extract was passed through a 0.20-µm filter and analyzed by HPLC under the following conditions: column, TSK gel ODS-80Ts (5 µm, 150 mm × 4.6 mm I.D.; Tosoh Co., Tokyo, Japan); mobile phase, water-methanol-acetic acid (30:68:2, v/v/v); flow rate, 0.9 ml/min; column temperature, 35°C. The quercetin glucosides eluted from the column were monitored at 254 nm. They were identified from their retention times against those of respective standard compounds, and their concentrations were calculated by using standard curves for each quercetin glucoside.

Animals and diets. Nine-week-old male Wistar rats weighing 190–200 g were supplied by Japan SLC (Hamamatsu, Japan). The animals were kept in an environmentally controlled animal facility operated on a 12 h dark/light cycle at 23±1°C and 55% humidity for 5–6 days before the experiments, with free access to tap water and the standard MF diet (Oriental Yeast Co., Tokyo, Japan). Fifty rats were divided into ten groups of five rats each (Table 1). Each group received one of eight semipurified diets which had been formulated to be isocaloric and to contain 0–18.8% (w/w) of lipids (Table 2) for two weeks. Each diet per day per rat contained 1.0 g of onion powder (with about 3.9 mg of quercetin aglycone equivalent). Groups 9 and 10 had free access to drinking water supplemented with 5% (w/v) of sodium caseinate or 0.5% (w/v) of sucrose fatty acid ester. The rats were fasted for 13 h after feeding for one and two weeks, and blood samples were collected from the tail vein. Blood plasma was immediately prepared by centrifugation for 15 min at 4°C and 1,000 g. These animal experiments were performed under the guidelines for animal experiments according to Notification No. 6 of the Japanese government.

Table 1. Rat Groups

<table>
<thead>
<tr>
<th>No.</th>
<th>Diet</th>
<th>Lipid Content (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>non-oil diet</td>
<td>0%</td>
</tr>
<tr>
<td>2</td>
<td>1.8% soybean oil diet</td>
<td>1.0%</td>
</tr>
<tr>
<td>3</td>
<td>4.6% soybean oil diet</td>
<td>4.0%</td>
</tr>
<tr>
<td>4</td>
<td>9.5% soybean oil diet</td>
<td>9.0%</td>
</tr>
<tr>
<td>5</td>
<td>18.8% soybean oil diet</td>
<td>18.0%</td>
</tr>
<tr>
<td>6</td>
<td>9.5% fish oil diet</td>
<td>9.0%</td>
</tr>
<tr>
<td>7</td>
<td>9.5% beef tallow diet</td>
<td>9.0%</td>
</tr>
<tr>
<td>8</td>
<td>9.5% lecithin diet</td>
<td>9.0%</td>
</tr>
<tr>
<td>9</td>
<td>4.6% soybean oil diet,</td>
<td>18.8%</td>
</tr>
<tr>
<td></td>
<td>drinking water added with 5%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sodium caseinate</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>4.6% soybean oil diet,</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>drinking water added with 0.5%</td>
<td></td>
</tr>
</tbody>
</table>

Determination of the quercetin metabolites in rat plasma. The quercetin metabolites in rat plasma were quantitatively determined by HPLC according to the method previously described.30) Plasma (50 µl) was mixed with 50 µl of a sulfatase type H-5 (25 units of sulfatase and 500 units of β-glucuronidase) solution in a 0.1 mM sodium acetate buffer at pH 5.0. The mixture was incubated at 37°C for 50 min. The released compounds were extracted by adding 900 µl
Table 2. Composition of the Diets

<table>
<thead>
<tr>
<th>Component</th>
<th>1</th>
<th>2</th>
<th>3, 9<em>1, 10</em>2</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornstarch</td>
<td>9.00</td>
<td>8.32</td>
<td>7.31</td>
<td>5.75</td>
<td>3.15</td>
<td>7.31</td>
<td>7.31</td>
<td>7.31</td>
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<tr>
<td>Sucrose</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
<td>1.50</td>
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<tr>
<td>Casein</td>
<td>3.75</td>
<td>3.75</td>
<td>3.75</td>
<td>3.75</td>
<td>3.75</td>
<td>3.75</td>
<td>3.75</td>
<td>3.75</td>
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<tr>
<td>Cellulose powder</td>
<td>1.20</td>
<td>1.20</td>
<td>1.20</td>
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<td>1.20</td>
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<td>Mineral mix*3</td>
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<td>0.52</td>
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<td>0.52</td>
<td>0.52</td>
<td>0.52</td>
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<tr>
<td>Vitamin mix*4</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
<td>0.15</td>
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<tr>
<td>Soybean oil</td>
<td>—</td>
<td>0.30</td>
<td>0.75</td>
<td>1.45</td>
<td>2.60</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Fish oil</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.75</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Beef tallow</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.75</td>
<td>—</td>
</tr>
<tr>
<td>Lecithin</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.75</td>
</tr>
<tr>
<td>Onion powder</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Lipid content (%)  
0 1.8 4.6 9.5 18.8 9.5 9.5 9.5

The caloric value of each diet was the same.
*1 Drinking water was added with 5% (w/v) sodium caseinate.
*2 Drinking water was added with 0.5% (w/v) sucrose fatty acid ester.
*3 AIN-76 mineral mix (Oriental Yeast Co.).
*4 AIN-76 vitamin mix supplemented with choline hydrogen tartrate (Oriental Yeast Co.).

Table 3. Contents of Quercetin Glucosides in Freeze-dried Onion, "Kamui"

<table>
<thead>
<tr>
<th>Compound</th>
<th>Content (mg of quercetin aglycone equivalent/g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin 3,4'-diglucoside</td>
<td>1.52±0.12</td>
</tr>
<tr>
<td>Quercetin 3-glucoside</td>
<td>0.07±0.01</td>
</tr>
<tr>
<td>Quercetin 4'-glucoside</td>
<td>2.35±0.20</td>
</tr>
<tr>
<td>Total</td>
<td>3.94±0.33</td>
</tr>
</tbody>
</table>

Each value is the mean±SD of three replications.

Data analysis. Each reported value is presented as the mean±SD. A statistical analysis was conducted by the Dunnett post-hoc multiple-comparison test to identify significantly different means, using StatView for Windows Ver. 5.0 (SAS Institute, Cary, NC, USA). The level of significance was set at \( P < 0.05 \).

Results and Discussion

Contents of quercetin glucosides in onion

The contents of quercetin glucosides in the freeze-dried onion used here were measured by HPLC (Table 3). Q3,4’G and Q4’G were contained as the major quercetin glucosides and Q3G as a minor glucoside, and their composition agreed with the results previously reported.\(^{23,37,38}\) The total content of quercetin glucosides in this onion was 3.94±0.33 mg of quercetin aglycone equivalent per gram of dry weight. The 10.0% of water content of this onion enabled its total quercetin content per 100 g fresh weight to be calculated as 39.4 mg. Seven other varieties of yellow onion cultivated in Japan have been reported to contain 30.3–43.1 mg of quercetin glucosides (17.2–24.9 mg of quercetin aglycone equivalent) per 100 g fresh weight.\(^{37}\) Patil et al.\(^{39}\) have demonstrated that the total quercetin contents of 55 yellow onion entries in USA were in the range of 5.4–28.6 mg/100 g fresh weight. These data indicate that the onion variety used here is one of the richest in quercetin glucosides among the yellow onion varieties.

Effect of soybean oil content in the diets on the plasma accumulation of quercetin metabolites

Rat plasma after one and two weeks of feeding was analyzed by HPLC. Hydrolysis with sulfatase H-5,
possessing both sulfatase activity and glucuronidase activity, released quercetin and isorhamnetin (3′-methoxyquercetin), as identified by da Silva et al.,19 as shown in Fig. 1. A trace of free, nonconjugated quercetin and no nonconjugated isorhamnetin were detected in the plasma of every rat group.

The effect of the soybean oil content in the diets on the accumulation of quercetin metabolites in the plasma was investigated in the range of 0–18.8% (w/w). The calorie intake in each group was adjusted to be equal by reducing the amount of cornstarch in the diet to match the increase in soybean oil content. We had already confirmed that the cornstarch content in each diet did not affect the accumulation of quercetin metabolites in the plasma (preliminary results).

Figure 2 shows the plasma concentrations of the total conjugates of quercetin and isorhamnetin in the rat groups fed on the diets containing 0–18.8% (w/w) of soybean oil (rat group nos. 1–6). Although there were no significant differences in their plasma concentrations between the ingestion periods of one and two weeks, the concentrations tended to increase with lengthening ingestion period. In the rat groups maintained on the diets containing more than 4.6% of soybean oil, both the quercetin conjugates and isorhamnetin conjugates accumulated at significantly higher concentrations than in the groups fed on the diets containing 0 or 1.8% soybean oil.

The results of our previous study have shown that the co-administration of such lipids as soybean oil and lecithin had no statistically significant effect on the absorption of orally administered quercetin in rats.30 On the other hand, the results of the present study reveal that the plasma accumulation of quercetin metabolites after onion ingestion was significantly enhanced by the co-ingestion of soybean oil.

Piskula and Terao29 have demonstrated that the solubilization of quercetin enhanced its intestinal absorption. We have shown that quercetin’s solubility in the vehicles used for its administration was increased by the addition of lipids and/or emulsifiers, and this supports the importance of solubilization for the efficient intestinal absorption of quercetin.30 It is
Fig. 3. Effect of the Type of Co-ingested Lipid on the Plasma Concentration of Quercetin Metabolites after Onion Ingestion.

Rats received one of the onion-containing diet nos. 3 and 6–8 shown in Table 2 for two weeks. Plasma taken after one or two weeks of feeding was analyzed as described in the Materials and Methods section. Shown are the plasma concentrations of conjugated quercetin after one week (1A), conjugated isorhamnetin after one week (1B), conjugated quercetin after two weeks (2A), and conjugated isorhamnetin after two weeks (2B). The types of lipid in the diets were as follows: S, soybean oil; F, fish oil; B, beef tallow; L, lecithin. Each value is the mean ± SD for five rats. Values within the same panel that do not share a common lowercase letter are significantly different at P < 0.05.

Effect of the type of lipids co-ingested on the plasma accumulation of quercetin metabolites

Figure 3 presents the plasma concentrations of the quercetin and isorhamnetin conjugates in the rat groups fed on diets containing 4.6% of soybean oil, fish oil, beef tallow, or lecithin (rat group nos. 3 and 6–8). Fish oil and beef tallow significantly enhanced the accumulation of these quercetin metabolites in the plasma to almost the same extent as that of soybean oil. Lecithin was significantly more effective for enhancing their accumulation than the other three types of lipid. It can be expected that these lipids could raise the solubility of quercetin glucosides in the intestines independently of the type of lipid, leading to enhanced accumulation of quercetin metabolites in the plasma. The additional effect of lecithin may probably have been due to its emulsifying action, this being responsible for further increasing the solubility of quercetin glucosides.

Effect of co-ingested emulsifiers on the plasma accumulation of quercetin metabolites

Figure 4 shows the plasma concentrations of the quercetin and isorhamnetin conjugates in the rat groups adapted to drinking water with no added emulsifier, 5% sodium caseinate, or 0.5% sucrose fatty acid ester, together with the diet containing 4.6% soybean oil (rat group nos. 3, 9, and 10). Since the sucrose fatty acid ester had low solubility in water, the low concentration of 0.5% was used. Both emulsifiers significantly increased the plasma concentrations of quercetin metabolites, with the exception of the sucrose fatty acid ester having no significant effect after one week of feeding. We have reported that the combination of emulsifiers including this sucrose fatty acid ester with lipids enhanced the absorption of orally administered quercetin in rats. In the present trial, this sucrose fatty acid ester was also effective for enhancing the plasma accumulation of quercetin metabolites after onion intake. Sodium
Fig. 4. Effect of Co-ingested Emulsifiers on the Plasma Concentration of Quercetin Metabolites after Onion Ingestion.

Rats were kept on an onion-containing diet and drinking water with no added emulsifier, sodium caseinate, or sucrose fatty acid ester (nos. 3, 9, and 10 in Table 2) for two weeks. Plasma taken after one or two weeks of feeding was analyzed as described in the Materials and Methods section. Shown are the plasma concentrations of conjugated quercetin after one week (1A), conjugated isorhamnetin after one week (1B), conjugated quercetin after two weeks (2A), and conjugated isorhamnetin after two weeks (2B). Each value is the mean ± SD for five rats. Symbols: N, no emulsifier; SC, 5% sodium caseinate; SF, 0.5% sucrose fatty acid ester. Values within the same panel that do not share a common lowercase letter are significantly different at P < 0.05.

caseinate, which was used at a concentration as high as 5%, is likely to have shown stronger effects than 0.5% of the sucrose fatty acid ester. It seems that the accumulation-enhancing function of these emulsifiers is attributable to the increased solubility of quercetin glucosides in lipid micelles.

The results of this study strongly suggest that the co-ingestion of lipid foods and emulsified foods could enhance the accumulation of quercetin metabolites in the plasma after the short-term ingestion of onion by humans. Ioku et al. have reported that cooking onion by frying had little effect on the quercetin content, judging from the results that its content was not significantly altered after 40 min of frying with oil or butter. Thus, frying onion with fat or oil could also increase the bioavailability of dietary quercetin. In addition, it is suggested that the daily intake of quercetin-rich foods by humans is important for increased accumulation of the quercetin metabolites in the plasma. There is some evidence that the conjugated quercetin metabolites present in blood plasma retained the inhibitory effect on lipid peroxidation in rat plasma and human LDL. Therefore, higher plasma levels of the quercetin metabolites can be expected to produce a more effective antioxidative defence in the circulation system. The relationship between the intake of quercetin-rich foods and in vivo oxidation resistance should be clarified to understand the potentially beneficial impact of dietary quercetin on human health.

Acknowledgments

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References


29) Piskula, M., and Terao, J., Quercetin's solubility affects its accumulation in rat plasma after oral...
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