Structural Changes and Digestibility of Onion Quercetin and Grape Resveratrol during In Vitro Human Digestion

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Running title: Structural changes of quercetin and resveratrol

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Abstract: This study was conducted to investigate the effects of in vitro human digestion on digestibility and structural changes of onion quercetin and grape resveratrol that simulates the composition of saliva, gastric, duodenal, and bile juice. We observed that the change the content of resveratrol and quercetin in grapes and onion were 52.45 ± 0.32, 46.48 ± 0.32% in small intestine of in vitro human digestion system. In LC-MS analysis, we found that the structure of grape resveratrol was influenced in vitro human digestion, whereas onion quercetin was less influenced. Before and after in vitro human digestion, the DPPH radical scavenging activities of homogenized, water and ethanolic extracts from grapes were higher than those of onion extracts, with 72.96 ± 0.32, 66.60 ± 0.16 and 67.36 ± 0.12 at mg/ml, respectively. The DPPH radical scavenging activities was decreased by in vitro human digestion in both of grape and onion extracts. These results will improve the understanding how in vitro human digestion influenced the structural changes and free radical scavenging activity of onion quercetin and grape resveratrol during digestion.
1. Introduction

Among natural products, resveratrol, a natural polyphenol that can be extract or isolated from grapes skin and by-products of the wine [1]. These resveratrol is widely demonstrated biological and pharmacological activities such as, protective effect of liver disease [2], ethanol toxicity [3], high fat diet [4], anti-cancer [5], anti-inflammatory effects [6] and reduction of hypertension, atherosclerosis and thrombosis [7]. Recently, the beneficial effects of resveratrol have been shown in \textit{in vitro} and \textit{in vivo} model [8] and also clinical studies of diabetes and cancer [9]. In addition, quercetin compounds are major dietary flavonoids in onions skins [10]. Generally, quercetin consists of two aromatic rings linked by an oxygen containing heterocycle. This molecular structure of quercetin may play crucial roles in its extensively proved biological activities. Quercetin has various human health benefits and shown to antioxidant, anti-inflammatory, anti-viral, and anti-cancer properties [11], [12]. Therefore, there is considerable interest in using resveratrol and quercetin as a nutraceutical ingredient in food and beverage products. In the mean times, \textit{in vitro} human digestion systems are widely used to study the structural changes, digestibility, and release of food components under simulated gastrointestinal conditions [13]. Although a number of studies reported that the phytochemicals such as quercetin and resveratrol have various bioavailabilities, the influence of \textit{in vitro} human digestion on bioavailability of quercetin and resveratrol during digestion remains to be
elucidated. Therefore, the purpose of this study was to determine the effects of in vitro human
digestion on the structural changes and digestibility of onion quercetin and grape resveratrol.

2. Materials and Methods

2.1. Materials

The grapes and onion were obtained from a local market (Seoul, Korea). Resveratrol,
quercetin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), α-amylase,
uric acid, mucin, bovine serumalbumin, pepsin, pancreatin, lipase, bile, acetonitrile were
purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of the
highest grade available commercially.

2.2. Preparation of sample and extracts

The grapes and onion (included skin) were washed with running tap water before being
chopped into pieces. Then, they were oven-dried at 45°C for 2 days and ground to a powder.
The samples were extracted with water and 95% ethanol. The 95% ethanol extracts were
prepared three times with 95% ethanol and filtered with Whatman #1 filter paper at room
temperature (R.T). The filtrate was evaporated using an evaporator (EYELA, Tokyo, Japan) at
40°C. Powder samples were suspended and extracted with 1.0 L of water at 75°C for 3 h.
Extracts were produced same filter paper and then centrifuging at 10,000 rpm for 30 min. In addition, a crude liquid were homogenized with same volume water. Concentrated using a rotary evaporator, and lyophilized for 3 days. The freeze-dried powder sample was stored at -30°C until use.

2.3. DPPH radical scavenging activity

The DPPH radical scavenging activity was measured using the method described by Yue and Xu [14]. An aliquot of 0.2 mL of the diluted BHT reacted with 1.8 mL of DPPH solution (0.1 mmol/L) in a spectrophotometer cuvette for 30 min at 25°C in the dark. The absorbance was measured at 0 and 30 min, respectively, at wavelength 517 nm. Then, the difference of the absorbance was calculated and converted to the scavenging DPPH free radical percentages below in order to measure of scavenging DPPH radical activity of each sample.

\[
\text{DPPH free radical scavenging percentage (\%)} = (1 - \frac{\text{absorbance of sample}}{\text{absorbance of control}}) \times 100
\]

2.4. In vitro digestion system and digestibility

The in vitro human digestion system was performed as described previously [15]. The small intestine digestion used a dialysis tubing method to simulate small intestine epithelium structure. Mouth step of grapes and onion were mixed with 6 mL of simulated saliva fluid (pH 6.8) and
stirred for 5 min at 37°C. Next, stomach step of 12 mL of simulated gastric fluid (pH 2) was added and the mixture was stirred for 2 h at 37°C. Final small intestine step of 12 mL of duodenal juice, 6 mL of bile juice, and 2 mL of sodium bicarbonate solution (pH 6.5-7) were added, and the mixture was stirred for 2 h at 37°C. During the *in vitro* human digestion, these samples were swirled at 60 rpm in a shaking water bath (Model HB-205SW, Hanbaek, Co., Korea) to simulate gastrointestinal tract motility (Table 1).

*Digestibility in small intestine:* The digestibility of quercetin and resveratrol during *in vitro* human digestion was determined by the rates of infiltration rate of quercetin and resveratrol through dialysis tubing and expressed inside and outside dialysis bags. Briefly, the dialysis tubing was purchased from Spectrum Laboratories Inc. (Rancho Dominguez, CA, USA) with a molecular weight cut-off of 12-14kDa. The dialysis tubing was cut into pieces of 10 cm and soaked in distilled water at 4°C before usage. After stomach digestion, the sample was then transferred to dialysis bags with duodenal, bile juice and bicarbonate solution. The dialysis tubes with digestive samples were placed into a sodium phosphate buffer (pH 6.9) and then incubated at 37°C for 2 h.

2.5. *Measurement of quercetin and resveratrol by HPLC*

The amount of onion quercetin and grape resveratrol were analyzed using high-performance
liquid chromatography (HPLC, HP Agilent 1100, Hewlett Packard Co) with a modified version of that described in previous studies (Lee, Lee, & Hur, 2015) on a Fortis H2O column (250 mm × 4.6 mm, 3 μm) using a solution A (25% acetonitrile) to solution B (H2O contain formic acid 0.1%) at a flow rate of 1.5 mL/min. The volume of sample injected for analysis was 20 μL, and the detection wavelength was set at 370 nm for quercetin. The resveratrol condition the following step, mobile phase was acetonitrile and water containing 0.2% formic acid (25:75, v/v). The UV detector wavelength was set at 306 nm, flow rate was 0.8 ml/min and the injection volume was 20 μL. All solutions were passed through a 0.45 μm Whatman membrane filter before injection onto the HPLC column.

2.6. LC-MS analysis

Electrospray Ionization Mass Spectrometry (ESI/MS) was performed with an Agilent 1100 Series LC/MSD VL model in positive scan mode interface for mass analysis and detection. Following optimization of the settings, negative ion mass spectra of the column eluate were recorded in the range of 10.1500 m/z/s. The instrument was operated with an ion source of electron multiplier, and the injection volume was 20 μL.

2.7. Statistical analysis
Statistical analyses were done for 3 times for all experimental items. The data are expressed as the mean ± standard error of mean (SEM). Statistical analyses were assessed by Student’s t-test for paired data. Graph Pad Prism software version 4.00 (Graph Pad Software Inc., San Diego, CA) was used.

3. Results and Discussion

3.1. Identification of resveratrol and quercetin from grapes and onion

The concentration of resveratrol and quercetin in skin of grapes and onion were identified using the HPLC and LC-MS method. The identified resveratrol are shown in Fig. 2, the chromatograms are shown in Fig. 2 (A, C, E and G) and the mass spectrums are shown in Fig. 2 (B, D, F and H). As shown in Fig. 2, we confirmed that the grape resveratrol decreased until stomach digestion with resveratrol main peak of m/z 102.2 [M]+ ion, however, main peak of resveratrol increased 344.2 [M]+ ion after small intestinal digestion. This result indicates that the structure of grapes resveratrol was influenced in small intestine digestion. The analysis of quercetin are shown in Fig. 3, the chromatograms are shown in Fig. 3 (A, C, E and G) and the mass spectrums are shown in Fig. 3 (B, D, F and H). The amount of onion quercetin decreased by in vitro human digestion with the main peak of m/z 64.1 [M]+ ion on LC/MS spectrums, however, the structure of onion quercetin did not changed during in vitro human digestion. This
result indicates that the structural change of onion quercetin is less influenced by *in vitro* human digestion.

The polyphenols of flavonoid group is known to be affected by temperature, pH, and various enzymes. In this study, the changes of bioavailability of resveratrol and quercetin can be influenced due to structure destroyed or changed as the liberated from the strong bond of phenolic compound with surrounding tissue of hydrogen and carbon bond form hydroxyl or phenyl group by temperature, ion strength, pH, or digestive enzymes during *in vitro* human digestion. We assume that the structural changes of hydroxyl or phenyl group during *in vitro* human digestion might be influenced the changes of pH or hydrolysis enzymes. In the model of digestion used in this study, the pH shifts dramatically between the stomach and the small intestine, from pH 1.5 to pH 7.5, mainly because bile salt has a higher pH. This change in pH is the primary factor involved in the irreversible breakdown of quercetin or resveratrol, moreover, their hydrogen bonds could be cleaved by the hydrolysis enzymes such as amylase, pancreatin or pepsin. Although this study found that an *in vitro* human digestion affects the structure of bioavailability in onion and grape, there is clearly an urgent need for more research into correlations between structural changes or bioavailability and influence factors of human digestion in onion and grape.
3.2. Changes of onion quercetin and grape resveratrol concentration during in vitro human digestion.

The changes of the onion quercetin and grape resveratrol concentration during in vitro human digestion are shown in Table 2. The amount of grape resveratrol in homogenized, water and ethanol extract samples were reduced with 1.56 to 0.89 mg, 1.15 to 1.09 mg, and 1.24 to 1.09 mg after in vitro human digestion, respectively. In addition, onion quercetin in homogenized, water and ethanol extract samples were reduced with 5.23 to 3.90 mg, 5.22 to 4.18 mg, and 5.99 to 5.33 mg, respectively.

3.3. Digestibility of onion quercetin and grape resveratrol during in vitro human digestion.

The digestibility of the onion quercetin and grape resveratrol during in vitro human digestion are shown in Table 3. In this study, the digestibility of onion quercetin and grape resveratrol during in vitro human digestion (in small intestine step only) was determined by the rates of infiltration rate of quercetin and resveratrol through dialysis tubing and expressed inside and outside dialysis bags. The digestibility of onion quercetin from homogenized, water and ethanol extract showed 45.68, 40.14 and 46.48%, respectively. The grape resveratrol was 52.45, 51.08 and 49.54%, respectively. This result revealed that the digestibility was shown to have similar
between onion quercetin and grape resveratrol during *in vitro* human digestion (no significantly different). In previous studies, we found that the quercetin concentration in onion extracts was increased by *in vitro* human digestion, and antioxidant activity was increased by *in vitro* human digestion of both onion extract and/or quercetin standard [13], [16]. This is because quercetin aglycone was cleaved from rutin glucoside in onion [13]. Indeed, quercetin aglycone has strong antioxidative activity compared to rutin glucoside. Our previous study found that the polyphenols are obviously stable through gastric digestion [16]. Over the left, these polyphenols are great sensitive to the mild alkaline conditions in the small intestine, some compounds may be form of other components and structure via duodenum. These results suggest that the pH, enzyme, temperature, oxygen or carbon may impact the phenolic compositions. In our study, the change in quercetin observed during small intestine of *in vitro* human digestion may have been due to the influence in pH or enzymes between the stomach and small intestine.

3.3. *DPPH radical scavenging activity during in vitro human digestion*

The homogenized, water and ethanol extracted grapes and onion were shown to have high scavenging activities in DPPH radicals during *in vitro* human digestion in this study. Particularly, grapes showed higher DPPH radical scavenging activities compared to onion (Fig. 1). During *in vitro* human digestion, the DPPH radical scavenging activities of homogenized grape were 72.96, 74.32, 75.20, and 75.81%, respectively. The DPPH radical scavenging activities of water
extracted grapes were 66.60, 67.12, 67.49, and 68.21%, and ethanol extract showed 67.36, 68.26, 68.76, and 69.60%, respectively. However, the DPPH radical scavenging activities were no significantly different among the homogenized, water and ethanol extract samples. (Fig. 1A).

In onion, the DPPH radical scavenging activities during \textit{in vitro} human digestion were 58.43, 61.67, 62.55, and 63.58%, respectively. During \textit{in vitro} human digestion, water extract showed 58.43, 59.83, 60.94, and 61.95%, and ethanol extract were 57.55, 61.79, 62.42, and 63.18% in DPPH radical scavenging activities, respectively.

As shown Fig. 4, The DPPH radical scavenging activities of quercetin standard was significantly reduced with \textit{in vitro} human digestion (58.43% $\rightarrow$ 9.33%) (Fig. 4B). Whereas, DPPH radical scavenging activities of resveratrol standard were slightly reduced with \textit{in vitro} human digestion (72.96% $\rightarrow$ 55.99%) (Fig. 4A). Numerous studies reported that quercetin and resveratrol have radical scavenging activity \cite{13}, \cite{17}. The antioxidant activity of flavonoids e.g. quercetin and resveratrol depends on the presence and number of the free hydroxyl groups in their skeleton. Therefore, the changes of antioxidative activity may be closely related with the structural changes of quercetin and resveratrol in this study. As result of this found that DPPH radical scavenging activity of quercetin and resveratrol standard was reduced by \textit{in vitro} human digestion. In structure, quercetin standard was more sensitive than resveratrol standard against \textit{in vitro} human digestion. In contrast, onion quercetin was shown to more stable than grape
resveratrol during \textit{in vitro} human digestion in this study. This different result may be due to the purity of quercetin and resveratrol, and also other ingredients such as water, other phenolic compounds in onion and grape could have intervention. Thus, more researches are needed to define

4. Conclusions

This study is the primary investigation to analysis of the relationship between free radical scavenging activities with structural changes in onion quercetin and grape resveratrol during \textit{in vitro} human digestion. As result of this study, we found that \textit{in vitro} human digestion was influenced the structural changes of onion quercetin and grape resveratrol, and also free radical scavenging activity was decreased by \textit{in vitro} human digestion. The onion quercetin showed more stable than grape resveratrol against \textit{in vitro} human digestion, whereas resveratrol standard showed more stable than quercetin standard. These different of structure could be influenced the change of digestibility and DPPH radical scavenging activities.

Acknowledgments

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Conflict of interest statement

We declare that we have no conflict of interest.

References


Figure Captions

**Figure 1.** Chang of DPPH radical scavenging activities of grapes (A) and onion (B) by *in vitro* digestion system. Values are expressed as the mean ± SD of determinations made in triplicate experiments. *p<0.05, **p<0.01.

**Figure 2.** HPLC chromatograms (left figure) and LC/MS spectrum (right figure) of grapes and resveratrol by *in vitro* digestion system. A & B; standard resveratrol, C & D; mouth digestion, E & F; stomach digestion, G & H; intestine digestion. HPLC trace on a Fortis H2O column (250 mm × 4.6 mm, 3 μm) of resveratrol peak from grapes or standard. All HPLC operation was carried out with a linear acetonitrile gradient (0–70%) at a flow rate of 1.5 ml/min using a UV detector at 340 nm.

**Figure 3.** HPLC chromatograms (left figure) and LC/MS spectrum (right figure) of onion and quercetin by *in vitro* digestion system. A & B; standard quercetin, C & D; mouth digestion, E & F; stomach digestion, G & H; intestine digestion. HPLC trace on a Fortis H2O column (250 mm × 4.6 mm, 3 μm) of quercetin peak from onion or standard. All HPLC operation was carried out with a linear acetonitrile gradient (0–70%) at a flow rate of 1.5 ml/min using a UV detector at 340 nm.
**Figure 4.** Changes of DPPH radical scavenging activities of grapes (A) and onion (B) by dialysis system of *in vitro* digestion system. Values are expressed as the mean ± SD of determinations made in triplicate experiments. *p<0.05, **p<0.01.
<table>
<thead>
<tr>
<th></th>
<th>Saliva</th>
<th>Gastric juice</th>
<th>Duodenal juice</th>
<th>Bile juice</th>
</tr>
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<tbody>
<tr>
<td><strong>Organic and inorganic components</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Organic components</td>
<td>1.7ml NaCl$^a$ (175.3g/L)$^b$</td>
<td>6.5ml HCl (37g/L)</td>
<td>6.3ml KCl (89.6g/L)</td>
<td>68.3ml NaHCO$_3$ (84.7g/L)</td>
</tr>
<tr>
<td>and inorganic components</td>
<td>8ml urea (25g/L)</td>
<td>18ml CaCl$_2$·2H$_2$O (22.2g/L)</td>
<td>9 ml CaCl$_2$·2H$_2$O (22.2 g/L)</td>
<td>10 ml CaCl$_2$·2H$_2$O (22.2 g/L)</td>
</tr>
<tr>
<td>15 mg uric acid</td>
<td>1 g bovine serum albumin</td>
<td>1 g bovine serum albumin</td>
<td>1.8 g bovine serum albumin</td>
<td>30 g bile</td>
</tr>
<tr>
<td><strong>Enzymes</strong></td>
<td>290 mg $\alpha$-amylase</td>
<td>2.5 g pepsin</td>
<td>9 g pancreatin</td>
<td></td>
</tr>
<tr>
<td></td>
<td>25 mg mucin</td>
<td>3 g mucin</td>
<td>1.5 g lipase</td>
<td></td>
</tr>
<tr>
<td><strong>pH</strong></td>
<td>6.8 ± 0.2</td>
<td>1.50 ± 0.02</td>
<td>8.0 ± 0.2</td>
<td>7.0 ± 0.2</td>
</tr>
</tbody>
</table>

$^a$ The numbers are the concentration of chemicals to make digestive juices.

$^b$ The number in parentheses are the concentration of inorganic or organic components per liter distilled water. After mixing all ingredients (inorganic components, organic components and enzymes), the volume was increased to 500 ml with distilled water.
Table 2. Changes of onion quercetin and grape resveratrol concentration of different extraction methods during in vitro digestion system.

<table>
<thead>
<tr>
<th></th>
<th>Resveratrol (mg)/Grape (g)</th>
<th>Quercetin (mg)/Onion (g)</th>
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<tbody>
<tr>
<td><strong>Homogenized</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before digestion</td>
<td>1.87 ± 0.64&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.23 ± 0.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mouth</td>
<td>1.56 ± 0.51&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.05 ± 0.22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Stomach</td>
<td>1.54 ± 0.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.77 ± 0.11&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Small intestine</td>
<td>0.89 ± 0.47&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.90 ± 0.26&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Water extract</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before digestion</td>
<td>1.47 ± 0.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.22 ± 0.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mouth</td>
<td>1.40 ± 0.47&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.41 ± 0.30&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Stomach</td>
<td>1.15 ± 0.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.37 ± 0.44&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Small intestine</td>
<td>1.09 ± 0.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.18 ± 0.27&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Ethanol extract</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before digestion</td>
<td>1.97 ± 0.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.02 ± 0.28&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mouth</td>
<td>1.66 ± 0.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.99 ± 0.17&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Stomach</td>
<td>1.24 ± 0.48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.82 ± 0.29&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Small intestine</td>
<td>1.09 ± 0.64&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.33 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are represented as the mean ± standard deviation. <i>n = 3</i>. <sup>a-c</sup> means with different superscript letters in a column within each treatment differ significantly (<i>P < 0.05</i>).
**Table 3.** Changes of digestibility in onion quercetin and grape resveratrol of different extraction methods during *in vitro* human digestion.

<table>
<thead>
<tr>
<th></th>
<th>Quercetin (%)</th>
<th>Resveratrol (%)</th>
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<tbody>
<tr>
<td>Homogenized</td>
<td>45.68 ± 6.45</td>
<td>52.45 ± 3.48</td>
</tr>
<tr>
<td>Water extract</td>
<td>40.14 ± 4.65</td>
<td>51.08 ± 5.54</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>46.48 ± 6.04</td>
<td>49.54 ± 3.47</td>
</tr>
</tbody>
</table>

Data are represented as the mean ± standard deviation. *n* = 3.
Figure 1. Lee et al.
Figure 3. Lee et al.
Figure 4. Lee et al.