Antioxidant Activity and Flavonoid Content of Welsh Onion (Allium fistulosum) and the Effect of Thermal Treatment

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Antioxidant activity and flavonoid content of Welsh onion (Allium fistulosum) (green-leafy and white-sheath varieties) and the effect of thermal treatment on them were studied by comparing with those of onion (Allium cepa) (yellow and red varieties). Antioxidant activity was measured by Trolox equivalent antioxidant capacity (TEAC) and ferric reducing antioxidant power (FRAP) assays. The order of these indices of antioxidant activity was red onion > yellow onion = green Welsh onion > white Welsh onion. The order of the total flavonoid content was red onion > yellow onion > green Welsh onion > white Welsh onion. Major flavonoid of yellow and red onions was quercetin, and that of green Welsh onion was kaempferol. Antioxidant activity of green Welsh onion was increased, but that of the other three vegetables was decreased during boiling for more than 15 minutes. Flavonoids in green Welsh onion were less stable than those in the other three vegetables during the boiling procedure. These results suggested that green Welsh onion, but not the white one, is a potent antioxidant food comparable to yellow onion, and is a good source of kaempferol. Increased antioxidant activity and decreased flavonoid content during boiling were characteristics of green Welsh onion.

Keywords: onion, Welsh onion, antioxidant activity, flavonoid, boiling

Introduction

Various studies on the antioxidant activity of Allium vegetables have been conducted as a result of the current interest in health foods (Helen et al., 2000; Wu et al., 2001; Moriguchi et al., 2001; Numagami and Ohnishi, 2001; Kawamoto et al., 2004). The results suggest that onion (Allium cepa L.) and garlic (Allium sativum L.) are sources of numerous kinds of antioxidants that could play important roles in physiological protection against reactive oxygen species generated in the body. Onion is a good source of quercetin, one of the most abundant flavonol-type flavonoids in fruits and vegetables. Antioxidant activity of quercetin has been demonstrated in in vivo (Duarte et al., 2001; Frémont et al., 1998), and in vitro (Kähkönen and Heinonen, 2003; Joyanovic et al., 1994) experiments. Onion contains other well-known antioxidant components, in that, volatile sulfur compounds are known to inhibit lipid peroxidation (Wu et al., 2001; Moriguchi et al., 2001; Numagami and Ohnishi, 2001). The Welsh onion (Allium fistulosum L.) is another Allium family that is a popular flavoring vegetable in Asian countries, however, few reports on its antioxidant activity have been available to present (Naito et al., 1981).

The evaluation of the impact of common domestic food processing on the health benefits of vegetables is of great practical importance. There are a number of reports on the changes of antioxidant activity of vegetables during heat processing (Gazzani et al., 1998a; Gazzani et al., 1998b; Makris and Rossiter, 2001), and the thermal stability of flavonoids such as quercetin and its glucosides (Makris and Rossiter, 2991; Price et al., 1997; Ewald et al., 1999; Hirota et al., 1998; Ioku et al., 2001). However, we have never seen any reports on the effects of heat treatment on the antioxidant activity and flavonoid content of the Welsh onion.

Vegetables and fruits contain a diversity of antioxidant components, and synergistic redox interactions may occur among these antioxidants in foods, and consequently, their total antioxidant activity is best measured by several independent methods of different assay principle (Halvorsen et al., 2002; Ou et al., 2002; Pellegrini et al., 2003). Among available assays for the estimation of antioxidant activity of food constituents, the Trolox equivalent antioxidant capacity (TEAC) assay is useful when monitoring lipophilic antioxidants such as carotenoids and phenolics. Moreover, it is appropriate for the estimation of lipophilic (and aqueous) systems (Pellegrini et al., 1999). In this experiment, the TEAC assay measured the ability to quench a stable radical cation (ABTS•⁺), and the ferric-reducing antioxidant power (FRAP) assay evaluated the reducing power of foods, were used to estimate the antioxidant activity of Welsh onion (green-leafy and white-sheath varieties) by comparing with those of onion (yellow and red varieties). In addition to the amounts of flavonoids, such as quercetin and kaempferol, in these

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Allium vegetables, the effects of heat treatment on the antioxidant activity and flavonoid contents were also studied.

Materials and Methods

**Chemicals** 2,2’-azinobis (3-ethyl)benzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,4,6-tripyridyl-s-triazine (TPTZ), quercetin dihydrate, kaempferol and acetone were purchased from Sigma-Aldrich.Com Japan, Merck Ltd., Japan. Myricetin, apigenin and luteolin were purchased from Wako Pure Chemical Industries, Ltd., Japan.

**Sample preparation** Onion (Allium cepa L.) (yellow and red varieties) and Welsh onion (Allium fistulosum L.) (green-leafy and white-sheath varieties) were purchased from local markets in Osaka City before undergoing analysis. Fresh onion and Welsh onion were washed, edible portions taken out, and cut into small pieces. For the homogenization of the homogenized sample, portions of extract B were applied on circular substances, portions of extract B were applied on filter paper, instead of filter paper, for comparison by Fisher’s protected least significant difference (PLSD) procedure. A p value of <0.05 is considered significantly different.

**Results**

Antioxidant activity of Welsh onion The antioxidant activity of two varieties of Welsh onion was compared with that of two varieties of onion (Table 1). TEAC and FRAP assays of both varieties resulted in a similar trend.
on a ranking order of antioxidant activity of onion and Welsh onion. Red onion exhibited the highest levels, followed by yellow onion and green Welsh onion. White Welsh onion had the lowest antioxidant activity. The TEAC and FRAP values of red onion were approximately 2-fold higher than those of yellow onion, and the values of green Welsh onion were roughly 2.5-fold higher than those of white Welsh onion. The percentages of the activity in lipid-soluble extract of green Welsh onion were the highest (about 42 and 60% of TEAC and FRAP, respectively) among these four vegetables.

The TEAC and FRAP values (vegetable tissue boiling water) of yellow, red, and white Welsh onion were decreased to about 20% of original activity after boiling for 15, 30 or 60 min (Fig. 1). In green Welsh onion, TEAC at 30 min and FRAP at 30 min were higher than those of the raw sample. About 20-40% of original activity of both TEAC and FRAP of yellow and red onions was transferred into the boiling water. Approximately 50% of TEAC, as opposed to roughly 20% of FRAP, were detected in the boiling water of white Welsh onion, suggesting the different solubility of radical scavengers and reducing agents in the boiling water. In green Welsh onion, the percentages of this activity in the boiling water was about 60-80% of the original activity.

Effect of thermal treatment on antioxidant activity
The TEAC and FRAP values (vegetable tissue + boiling water) of yellow, red, and white Welsh onion were decreased to 60-80% of original activity after boiling for 15, 30 or 60 min (Fig. 1). In green Welsh onion, TEAC at 15 and 30 min and FRAP at 30 min were higher than those of the raw sample.

Thermal stability of antioxidant components in green Welsh onion
Thermal stability of antioxidant components of green Welsh onion was compared with that of yellow onion (Table 2). In both yellow and green Welsh onion, the TEAC value of the extract B was higher, and the value of the extract C-1 and C-2 was lower than that of the extract A. These results suggest that antioxidant components might be activated or produced during the heating of the water-soluble extract of yellow and green Welsh onion, and the molecular weight of these new components might be less than 5,000. While results of green Welsh onion coincided with the increased activity in the boiling experiment of the vegetable, the results of yellow onion were contradictory. In yellow onion, the
destruction of antioxidant components may be precursors to the production or activation of them during the boiling of vegetable tissue.

The TEAC values of the extract D-1 and D-2 in green Welsh onion were 1.37 and 1.50 times that of extract C-1 and C-2, respectively. In yellow onion, the values of the extract D-1 and D-2 were 1.23 and 1.07 times that of extracts C-1 and C-2, respectively. These results suggest that much more water-soluble antioxidant components were produced in extract D-1 and D-2 in green Welsh onion than in yellow onion. This high increase during the second boiling procedure suggested that the increase in antioxidant components might continue when the boiling time is increased in green Welsh onion. In yellow onion, such increase in the antioxidant components would be of a much lesser extent.

Flavonoid content and the effect of thermal treatment
Myricetin, apigenin, and luteolin, were not detected in onions and Welsh onions. Quercetin, but not kaempferol, was detected in yellow and red onions. Kaempferol, but not quercetin, was detected in white Welsh onion (Table 3). Both quercetin and kaempferol were detected in green Welsh onion. The order of total flavonoid contents was red onion > yellow onion > green Welsh onion > white Welsh onion. The flavonoid content of white Welsh onion was much lower than that of the other three vegetables.

Thermal stability of quercetin and kaempferol in vegetable tissue and boiling water are shown in Fig. 2. Quercetin in yellow and red onion decreased to 50–60% of that of the original raw vegetables during boiled for 60 min. Total flavonoids in green Welsh onion significantly decreased following boiling for 15 min or more, and the residue of flavonoids was less than 15% of that of the original raw vegetables.

The distribution of total flavonoids in vegetable tissue and boiling water after thermal treatment is shown in Fig. 3. Total flavonoid contents were expressed as percentages with an assumption that the contents in the original raw vegetables were 100. The percentages of total flavonoid in boiling water of all of the four vegetables examined were less than 5% of the original raw vegetables. The percentages of total flavonoid in boiling water in yellow and red onions slightly increased during the boiling time from 15 min to 60 min.

Table 3. Flavonoid contents in onions and Welsh onions.

<table>
<thead>
<tr>
<th></th>
<th>Quercetin (mg/kg FW)</th>
<th>Kaempferol (mg/kg FW)</th>
<th>Total Flavonoids (mg/kg FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yellow onion</td>
<td>414±22</td>
<td>ND</td>
<td>414±22</td>
</tr>
<tr>
<td>Red onion</td>
<td>680±55</td>
<td>ND</td>
<td>680±55</td>
</tr>
<tr>
<td>Green Welsh onion</td>
<td>12±1</td>
<td>269±21</td>
<td>283±20</td>
</tr>
<tr>
<td>White Welsh onion</td>
<td>ND</td>
<td>6±0.4</td>
<td>6±0.4</td>
</tr>
</tbody>
</table>

Values are mean±SEM of results determined three times in duplicate. Values within a column with different superscript letters are significantly different from each other (p<0.05). ND: not detected.

Discussion
Among the available assays for the estimation of antioxidant activity of food constituents, the TEAC assay is both useful for monitoring lipophilic antioxidants such as carotenoids and phenolics, as well as appropriate for the estimation of lipophilic (and aqueous) systems (Pellegrini et al., 1999). In this experiment, the TEAC assay was used to estimate the antioxidant activity of flavonoid rich Allium vegetables. The FRAP assay was also applied to evaluate the reducing power of these vegetables. Both TEAC and FRAP assays resulted in a similar trend on a ranking order of antioxidant activity of Welsh onions and onions. The activity of green Welsh onion was at the same level as that of the yellow onion, but lower than that of red onion. The activity of white Welsh onion was much weaker than that of the other three vegetables. Naito et al. (1981) reported that the antioxidant activity of...
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Welsh onion was lower than that of onion, but they did not comment on which variety of Welsh onion was used. In this experiment, the estimation of antioxidant activity suggested that green Welsh onion, but not the white one, was a potent antioxidant food comparable to yellow onion.

The TEAC and FRAP values of water-soluble extracts were much higher than those of lipid-soluble extracts of red onion, suggesting that the vegetable is rich in water-soluble antioxidant components. On the other hand, the relatively high value of the TEAC and FRAP in lipid-soluble extracts of green Welsh onion suggested that the vegetable is rich in lipid-soluble antioxidant components. In water-soluble components, flavonoid glucosides are well known antioxidant components in *Allium* vegetables. Other water-soluble antioxidant components may include ascorbic acid and antocyanin (Kähkönen and Heinonen, 2003; Chaovanaliki and Wrolstad, 2004). Antocyanins in the water-soluble extract of red onion might contribute to its high antioxidant activity. Volatile sulfur compounds are well-known to be water-soluble components of *Allium* vegetables, however, their antioxidant properties are not well understood. Lipid-soluble antioxidants including carotenoids, flavonoids (aglycons), chlorophylls, and tocopherols might contribute to the antioxidant activity of green Welsh onion.

Sakakibara *et al.* reported that only kaempferol was detected in Welsh onion (Sakakibara *et al.*, 2003), but provided no comments with respect to whether or not they used green or white types of Welsh onion. No other previous reports were found on flavonoid contents in Welsh onion. In this experiment, considerable amounts of kaempferol and small amounts of quercetin were detected in green Welsh onion. Trace amounts of kaempferol were detected in white Welsh onion. In yellow and red onion, only quercetin was detected in this experiment and other reports (Makris and Rossiter, 2001; Sakakibara *et al.*, 2003), however, some previous studies detected quercetin with a small amount of kaempferol (Price *et al.*, 1997; Bilyk *et al.*, 1984). From these results, we suggested that green Welsh onion, but not the white one, is a good source of kaempferol, and yellow and red onions are sources of quercetin.

During thermal treatment, the antioxidant activity of white Welsh, yellow, and red onions decreased, while that of green Welsh onion increased. Thermal instability of antioxidant activity of the former three vegetables was comparable to previous reports that the antioxidant activity of onion juice decreased to 63% of the activity before boiling (Gazzani *et al.*, 1998a). On the other hand, the antioxidant activity of green Welsh onion increased after boiling for more than 15 min, and was the highest after boiling for 30 min. The study on the thermal stability of antioxidant activity suggested that there was a greater increase of antioxidant activity during boiling in green Welsh onion than in yellow onion, and that antioxidant components might be continually produced during heating of green Welsh onion. The increase in the water-soluble antioxidant components, such as melanoidin, could account for the increased activity of green Welsh onion.

Total flavonoids in green Welsh onion decreased significantly following boiling for 15 min or more, and the residue of flavonoids was less than 15% of that in the original raw vegetables. On the contrary, flavonoids in white Welsh, yellow, and red onions were more stable than those in green Welsh onion during the boiling procedure. The increased antioxidant activity and the decreased flavonoid (mainly kaempferol) content during boiling in green Welsh onion may have some relationship. Consequent studies should be conducted to clarify the reason for the thermal instability of flavonoids during heating, and the mechanism of the thermal enhancement of antioxidant activity in green Welsh onion.

References


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