Cooking Loss of Major Onion Antioxidants and the Comparison of Onion Soups Prepared in Different Ways

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After sautéing or frying onions as a first procedure in making onion soup, major onion antioxidants (quercetin 4'-glucoside and quercetin 3,4'-diglucoside) remained mostly intact. In the onion soup prepared with sautéed onions, the quantity of flavonoids remained high, but in the onion soup prepared with fried onions, a part of them was changed during cooking. The heating stability of these flavonoids was investigated through simple boiling and oven heating of these compounds. Major onion antioxidants were quite stable in a simple cooking model of boiling and oven heating at 100°C but considerably degraded in that of oven heating at 200°C. DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of sautéed onions was similar to that of raw onions, and fried onions exhibited much higher activity based on equivalent amounts of raw onion. Fried onions had much higher browning degree than sautéed onions, and the browning substance was found to contribute to the radical scavenging activity. The onion soup prepared with sautéed onions had DPPH radical scavenging activity similar to plain sautéed onions, but the onion soup prepared with fried onions had much lower activity than fried onions alone.

Keywords: onion, *Allium cepa*, onion soup, antioxidants, quercetin

Introduction

The onion (*Allium cepa*) is a vegetable rich in flavonoids (Tsushida and Suzuki, 1996) that are known to play an important role as antioxidants in reducing the risk of degenerative diseases of aging, such as cardiovascular disease and various cancers (Ames *et al.*, 1993). Organosulfur compounds such as sulfides and isochiocyanates in *Allium* vegetables also have been found to have beneficial effects regarding antioxidant property against several diseases including cancer (Bianchini and Vainio, 2001). In many countries, onions are often eaten after being cooked in a wide variety of ways. Recently, some results on functional ingredients of cooked vegetables have been reported (Hirota *et al.*, 1998; Ioku *et al.*, 2001; Makris and Rossiter, 2001). Most of these tests were made under simple cooking conditions, and there are few examples which investigated the changes of functional ingredients during practical cooking considering the complex handling or the influence of various additives like oils or seasonings.

Onion soup is a typical French dish eaten in many countries. We investigated major onion antioxidants in onion soups prepared both with sautéed onions and fried onions. Using sautéed onions is more traditional and requires a refined technique and long cooking times to create an appetizing brown color with proper finishing conditions. Fried onions are often used as a simple, quick substitute. To ensure constant, high quality, both types of onion soup

were prepared by a professional chef of the Association des Disciples d'Auguste Escoffier du Japon, an authoritative association of French chefs. In this study, we evaluated how well flavonoids of onions withstood cooking in onion soups prepared in different ways and how radical scavenging activity of onions shifted during the preparation of onion soup.

Materials and Methods

Materials Onions (*Momiji 3 gou*) were obtained from Tokushima Prefecture in Japan. Butter (Morinaga milk industry Co., Ltd., Japan), salad oil (Ajinomoto Co., Inc., Japan), beef bouillon powder (Ajinomoto Co., Inc.), and tap water were used for the additional components of onion soup.

Chemicals Quercetin 4'-glucoside (Q4'G), quercetin 3,4'-diglucoside (Q3,4'G), and isorhamnetin 4'-glucoside (I4'G) were separated from the onions and purified by preparative HPLC. Other chemicals were analytical grade.

Onion soup recipes Sautéed type: Sliced onions (15 kg) were sautéed in an aluminum pan with 120 g butter for a total of 2.75 hours. Onion samples sautéed for 1 and 2 hour(s) (early and middle stages) were saved for analyses. Nine hundred milliliters of beef bouillon solution (1.1%, powder/water, w/w) was added to 150 g sautéed onions, then heated until reduced to 900 ml total volume. Fried type: Four hundred grams of sliced onions was fried in salad oil for 10 min at 120°C. In the same way, 1080 ml beef bouillon was added to 90 g of the fried onions. These

were heated and reduced to 900 ml. Both onion soups were prepared in an aluminum pan heated by a gas cooker. In the final process, excess oil and surface scum were removed from the surface of the soup.

Extraction of flavonoids Each sample was freeze-dried. The samples, which corresponded to 10 g of raw materials, were homogenized in 10 ml of 80% MeOH and centrifuged. The precipitates were washed with 80% MeOH two more times. Each total supernatant was adjusted to 50 ml with 80% MeOH and filtered through a cartridge filter (0.45 μ m pore size, Nacalai Tesque, Japan). This solution was used for HPLC analysis, measurement of DPPH radical scavenging activity, and measurement of browning degree.

HPLC analysis HPLC analysis was conducted with a HPLC system (Jasco, Japan) using the $\phi 4.6 \times 150$ mm, COSMOSIL 5C₁₈-MS column (Nacalai Tesque, Japan). The samples were eluted with the linear gradient of 10 to 100% acetonitrile in 10 mM phosphoric acid solution at a flow rate of 1.0 ml/min. The detector was set at 280 nm. Q4'G, Q3,4'G, quercetin, and (I4'G) were quantified.

Reproducing onion soup with fried onions Onion soup with fried onions was prepared with the same composition of materials along with the recipe described above. Sliced onion (130 g) was fried in salad oil for 10 min at 120°C to 33 g. The fried onion (20 g) and beef bouillon (1.1%, powder/water, w/w, 245 ml) was heated for 13 min. The onion soup was separated to onion and soup parts by decanting without removing oil. The onion part was rinsed with distilled water (20 ml), which was added to the soup part. The soup part was then put in a separating funnel, and oil and water layers (15 and 200 ml, respectively) were separated.

Extraction and HPLC measurement of flavonoids in each part of the reproduced onion soup The onion part was freeze-dried and extracted by 80% MeOH in the same way as described above. The oil part was extracted by MeOH, and the extract was adjusted to 100 ml. The water part was adjusted to 250 ml with distilled water, then 10 ml was freeze-dried. The dried sample was extracted by 80% MeOH and adjusted to 10 ml. These extracts were measured to determine the contents of flavonoids by HPLC using the same conditions as described above.

Model boiling One milligram of each of Q4'G and Q3,4'G was dissolved in 10 ml of water. The solution was put in a glass flask, which was then stoppered and heated for up to 60 min in a water bath set at 100°C. One hundred and fifty microliters of portions of the solution were periodically collected during heating. The collected solutions were immediately cooled in ice and used for HPLC measurement.

Model oven heating Five milligrams of each of Q4'G and 3,4'G was put into $\phi 1.2 \times 3.5$ cm glass bottles and heated for 30 and 60 min in a convection oven set to 100, 120, and 200°C. After heating, the sample was dissolved in MeOH (1 ml) and used for HPLC measurement.

Measurement of DPPH radical scavenging activity This followed Yan's method (Yan *et al.*, 1999) with the 80% MeOH extracts.

Measurement of browning degree Browning degree was evaluated as absorbance at 500 nm of the 80% MeOH extract of each sample. The absorbance was measured by a UV-VIS spectrophotometer, UVmini 1240 (Shimadzu, Japan). The optical path length was 1 cm.

Results and Discussion

The contents of major onion antioxidants Q4'G, Q3,4'G, and I4'G in raw onion, cooked onions, and onion soups prepared in different ways are shown in Fig. 1. Quercetin was not detected in any sample. Most of those flavonoids were retained in the onions after sautéing, frying, and preparing onion soup with sautéed onions. However, more than half of the flavonoids were lost when soup was prepared with fried onions. One possible reason for the decrease of flavonoids in the onion soup made with fried onions was that flavonoids were removed with surface oil during preparation of the soup because fried onions had much more oil than sautéed ones. To investigate this possibility, onion soup with fried onions was reproduced with the same recipe in a laboratory, where the onion, oil, and water parts were separated. The contents of major onion flavonoids corresponding to the total amount of each part in a pot of onion soup are shown in Fig. 2. This result, with the exception of some difference of degree, was consistent with the previous result that used onion soup prepared by a chef with fried onions. In the reproduced onion soup, quite a few flavonoids (0.4% of total flavonoids in fried onions) were detected in the oil part, 44% in the water part, and 16% in the onion part. Quercetin was not detected in any part. In the onion part, a composition of Q3,4'G, Q4'G, and I4'G was changed from raw and fried onions, and the loss rate of Q3,4'G was much higher than that of Q4'G and I4'G. The result of the reproduction test indicates that major flavonoids in fried onions were partly dissolved in the oil and removed with it, and some part was lost by degradation or aggregation with other components during heating with bouillon.

Fig. 1. Amount of major flavonoids in raw onion, cooked onions, and onion soups based on 1 g of raw onion. ■: Q3,4'G; □: Q4'G; □: I4'G.

Fig. 2. Contents of major onion antioxidants before and after preparing onion soup (reproducing test). ■: Q3,4'G; ▨: Q4'G; □: I4'G.

In the practical cooking of onions, flavonoids showed different behavior depending on cooking ways. To investigate the influence of heat, some models of cooking used Q4'G and Q3,4'G which are major onion antioxidants. Sautéing and frying are often used as onion cooking methods, and they are classified as dry heating. As a simple model of dry heating, oven heating (100, 120, and 200°C) was performed. For comparison, boiling (100°C) was performed as a simple model of wet heating. In the simple cooking model of Q4'G and Q3,4'G, they were quite stable when boiled for 60 min (Fig. 3, A and B). Similarly, they were stable when heated by oven for 60 min at 100°C (Fig. 3, C and D). At 120°C of oven heating, they were degraded to about 90% in 60 min (Fig. 3, E and F). In the practical

Fig. 3. Residual rates after model cooking of major onion antioxidants. A. Boiling of Q3,4'G, B. Boiling of Q4'G, C. Oven heating (100°C) of Q3,4'G, D. Oven heating (100°C) of Q4'G, E. Oven heating (120°C) of Q3,4'G, F. Oven heating (120°C) of Q4'G, G. Oven heating (200°C) of Q3,4'G, H. oven heating (200°C) of Q4'G. ○: Q3,4'G; ●: Q4'G; x: Q.

cooking of onions, about 80% of total flavonoids was retained after frying at 120°C (Fig. 1 and 2). It is quite difficult to measure the temperature during sautéing, but it is assumed that the temperature is around 100°C while sautéed foods have enough water in themselves, and as the water content was decreased, the temperature rises to much higher than 100°C, and it is just like baking when sautéed foods contact a pan. Incidentally, the water contents of raw and sautéed onions estimated from the weight loss by freeze-drying were 90, 83, 62, and 49% (raw, sautéed for 1, 2, and 2.75 h). Additionally, sautéing involves the handling of stirring, so it is thought that the temperature during sautéing is constantly changing and rising overall. Consistently, 25% of total flavonoids was degraded in sautéing onion (Fig. 1). In the simple dry heating at 200°C, Q3,4'G was considerably degraded, to 16 and 3% in 30 and 60 min, simultaneously, small amounts of Q4'G and quercetin were detected (Fig. 3, G). Q4'G was degraded to 68% by oven heating at 200°C in 60 min, and a small amount of quercetin was detected (Fig. 3, H). In the cooking model of dry heating, Q3,4'G tended to have lower heating stability than Q4'G, and this tendency was found in sautéed and fried onions. These results indicate that Q4'G and Q3,4'G are stable in common cooking because the temperature of foods during cooking must not exceed 100°C unless water is completely lost. Q4'G and Q3,4'G were stable in simple heating at 100°C under both wet and dry conditions. In dry heating at around 200°C, such as baking or frying, those compounds may be degraded only around the food surface. From these results, it is indicated that in the practical cooking, quercetin glycosides are relatively resistant to heat itself, and their loss from material to final product may be due to coexisting substances or handling.

The DPPH radical scavenging activity of cooked onions is shown in Fig. 4. Sautéing tended to increase the activity gradually, but the activity was a little lower than for raw onions. After frying, the activity increased to approximately twice that of raw onions. Brown substances, such as Maillard reaction products are known to have higher antioxidant activity (Bressa *et al.*, 1996; Friedman *et al.*, 1996). When onions were heated, a brown product developed, which may have higher antioxidant activity. It was reported that the main browned compounds of heated onion juice are a kind of melanoidin and have lower molecular weight compared with model melanoidin prepared with glycine and glucose (Tamaki and Ukai, 2003; Tamaki *et al.*, 1996), and that raising of the temperature enhanced the browning reaction (Mizoi *et al.*, 1992). As shown in Fig. 1, major onion flavonoids were retained by 75 and 79% after sautéing and frying, respectively. Considering this result, in Fig. 4, 0.75 and 0.79 of total activity of sautéed and fried onions were from flavonoids, and the remaining 0.19 and 1.05 were probably from a browning substance, i.e., the contribution ratio of flavonoids and browning substance to DPPH radical scavenging activity is estimated at 80:20 and 43:57 in sautéed and fried onions, respectively. Consistently, the fried onions had a much higher degree of browning compared to sautéed onions (Fig. 5). This may

Fig. 4. DPPH radical scavenging activity of sautéed and fried onions based on 1 g of raw onion (the activity for raw onion = 1).

Fig. 5. Browning degree of 80% MeOH extract of cooked onions and onion soups.

be related to fried onions being uniformly heated at higher temperature than sautéed onions from the early stage of heating, and the reaction rate of the components relating to browning in fried onions being faster than in sautéed onions.

The antioxidant activity of sautéed onions was retained in the onion soup at a ratio of 85%, but for fried onions, the ratio was only 35% (Fig. 6). While fried onions had much higher antioxidant activity than raw onions, which came from the browning substance, they lost much activity by losing flavonoids during soup preparation.

Conclusions

The major onion antioxidants themselves were quite stable in simple cooking. They were retained well in simple sautéing or frying. DPPH radical scavenging activity rose with the browning degree of the cooked onion sample. The onion antioxidants were retained well in onion soup prepared with sautéed onions. The onion antioxidants and the DPPH radical scavenging activity significantly

Fig. 6. Ratio of DPPH radical scavenging activities (onion soup/cooked onion, onion soup/raw onion, based on equivalent amounts of onion). ■: onion soup/cooked onion; □: onion soup/raw onion.

decreased during preparing onion soup with fried onions. We should thus select the food processing method not only for taste but also for how well functional ingredients are retained.

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