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Change in the Radical-Scavenging Activity of Quercetin and Epigallocatechin Gallate during Heat Treatment

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The effects of heat treatment on the radical-scavenging activity of the two phenolic compounds, quercetin and epigallocatechin gallate, were examined. The retained radical-scavenging activity of both polyphenols was 100% and 80% after respective 60- and 480-min heating at 100℃ suggesting that common boiling practices do not compromise the antioxidation effects. However, the content of epigallocatechin gallate was significantly decreased, although its radical-scavenging activity remained. With heating at 180℃, both the quercetin and epigallocatechin gallate contents decreased much more rapidly than their radical-scavenging activity, suggesting that the radical-scavenging activity remained in their degradation products. After 15 min of heating at 180℃, the retained radical-scavenging activity of quercetin and epigallocatechin gallate was more than 80%. Therefore, usual high-temperature cooking methods such as deep frying or oven heating would not severely compromise the antioxidation effects of these compounds.

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Keywords: radical-scavenging activity, quercetin, epigallocatechin gallate, heat treatment, total phenolic content.

INTRODUCTION

Many studies on the physiological functions of the polyphenolic compounds contained in dietary plants and foods have been performed. Polyphenolic compounds present in fruits, vegetables and tea can act as antioxidants because they are free-radical scavengers, or singlet-oxygen quenchers. The intake of these foods is associated with a reduced risk of such chronic ailments as cancer, coronary heart disease, and other lifestyle-related diseases (Shahidi and Wanasundara 1992; Rice-Evans *et al.* 1996; Kahkonen *et al.* 1999).

We have previously reported an evaluation of the antioxidative activity of polyphenolic compounds in foods (Murakami *et al.* 2002a), the radical-scavenging activity of Maillard-reaction products (Murakami *et al.* 2002b), and the effects of vitamins on the polyphenolic antioxidative activity. However, not all foods are eaten fresh; for instance, we generally eat vegetables after cooking by boiling or deep-frying. Less attention has been paid to the effects of heat treatment on the antioxidative activity in foods (Dewanto *et al.* 2002; Papetti *et al.* 2002).

In this work, we consider the effects of heat treatment on the radical-scavenging activity of polyphenolic compounds. We chose quercetin and (−)-epigallocatechin gallate (EGCG), which are found almost exclusively in vegetables and Japanese tea, both having high antioxidative activity.

MATERIALS AND METHODS

Reagents

DPPH, tris(hydroxymethyl)aminomethane (Tris), trichloroacetic acid, a phenol reagent solution, tetrahydrofuran, acetic acid, and phosphoric acid were obtained from Nacalai Tesque (Kyoto, Japan). Quercetin, ethanol, methanol (HPLC grade), and gallic acid were obtained from Wako Pure Chemical Industries (Osaka, Japan), and EGCG and (−)-gallocatechin gallate were obtained from Kurita Co. (Tokyo, Japan).

Heat treatment

Each sample was dissolved in ethanol and diluted in

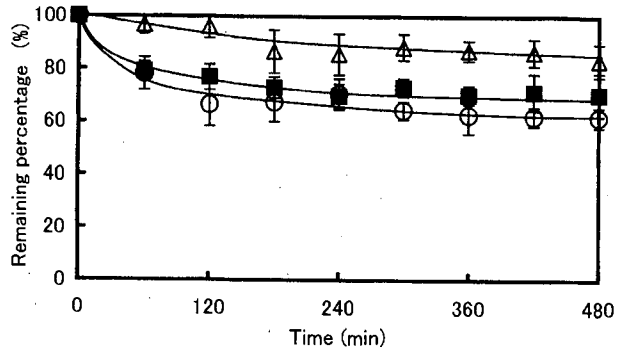

Fig. 1. Remaining percentages of radical-scavenging activity, total phenolic content and quercetin content after heating at 100℃

△, radical-scavenging activity; ■, total phenolic content; ○, quercetin content.

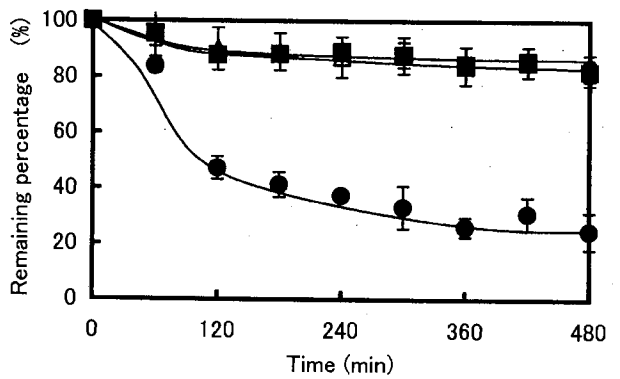

Fig. 2. Remaining percentages of radical-scavenging activity, total phenolic content and EGCG content after heating at 100℃

△, radical-scavenging activity; ■, total phenolic content; ●, EGCG content.

distilled water. Aliquots of each sample solution (1 ml) were placed in screw-capped vials and heated in an oil bath at a temperature of 100℃ for 60, 120, 180, 240, 300, 360, 420 or 480 min, or at 180℃ for 15, 30, 45, 60, 120 or 180 min. The quercetin or EGCG remaining after the heat treatment was analyzed by HPLC. These heating conditions, which are not usual in home cooking, were used in order to clearly determine the heat degradation of polyphenols.

Radical-scavenging activity assay

The radical-scavenging activity was determined by the DPPH-colorimetric method. An aliquot of a sample solution (200 μl) was mixed with a 100 mM Tris-HCl buffer (pH 7.4, 800 μl) and added to 1 ml of 500 μM DPPH in ethanol (a final concentration of 250 μM). The mixture was shaken vigorously and left to stand for 20 min at room temperature in the dark. A control or blank was without the sample solution. The absorbance at 517 nm by DPPH was measured by a UV-2100 PC UV-VIS spectrophotometer (Shimadzu, Kyoto, Japan). The radical-scavenging activity was calculated as the difference in the DPPH-radical absorbance detected at 517 nm between the blank and sample. The following equation was used:

Radical-scavenging activity (%)$=(C-A)/C\times 100$

where A=the difference in absorbance at 517 nm between the blank and sample and C=the absorbance of the blank.

Determination of total phenols, quercetin and EGCG

The total phenolic content was determined by the Folin-Ciocalteu method as modified by Singleton and Rossi (1965) and Hoff and Singleton (1977). An aliquot of a sample solution (200 μl) was mixed with 7.5% sodium carbonate (800 μl) and then added to 1 ml of the phenol reagent solution. The mixture was shaken vigorously. The absorbance was measured at 765 nm after shaking for 30 min at room temperature. A mixture of water and the reagent was used as a blank. The total phenolic content is expressed as gallic acid equivalents.

The quercetin content was determined by the HPLC method according to Mizuno *et al.* (1992). A sample solution was applied to a reversed-phase HPLC analysis. The HPLC equipment consisted of a Hitachi L-7110 pump, a Rheodyne injector fitted with a 20-μl loop and a Hitachi L-7420 UV-VIS detector set at 254 nm. Analyses were performed in a Lichrospher RP-18(e) column (4.6×250 mm, E. Merck Darmstadt, Frankfurt, Germany) at ambient temperature with a mobile phase of 40% tetrahydrofuran containing 1% acetic acid at a flow rate of 0.8 ml/min.

The EGCG content was determined by HPLC according to Iwai *et al.* (2000). A sample solution was applied to a reversed-phase HPLC analysis. The HPLC equipment consisted of the Hitachi L-7110 pump, Rheodyne injector fitted with the 20-μl loop and the Hitachi L-7420 UV-VIS detector set at 280 nm. Analyses were performed in a Cosmosil $5C_{18}$-AR-II column (4.6×150 mm, Nacalai Tesque, Kyoto, Japan) at ambient temperature with a mobile phase of methanol/0.5% phosphoric acid (25:75, v/v) at a flow rate of 0.8 ml/min.

RESULTS AND DISCUSSION

Effects of heating at 100℃

The effects of heating at 100℃ are shown in Figs. 1 and 2. The remaining percentages were calculated

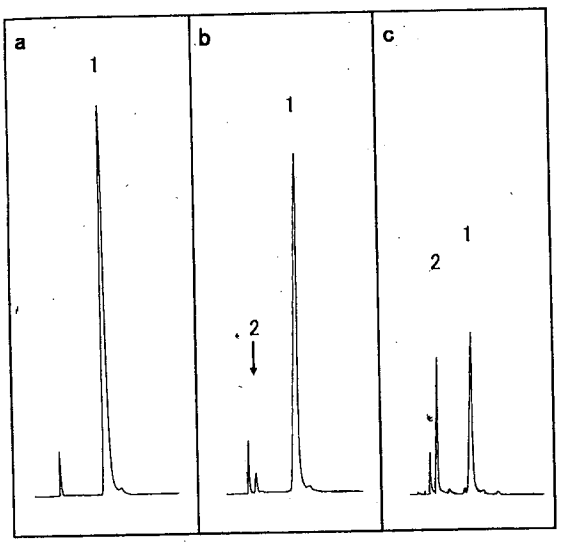

Fig. 3. HPLC chromatograms of quercetin before and after heating

HPLC analysis was carried out as described in Materials and Methods. a, before heating; b, after heating for 60 min at 100℃; c, after heating for 15 min at 180℃. 1, quercetin; 2, unknown product.

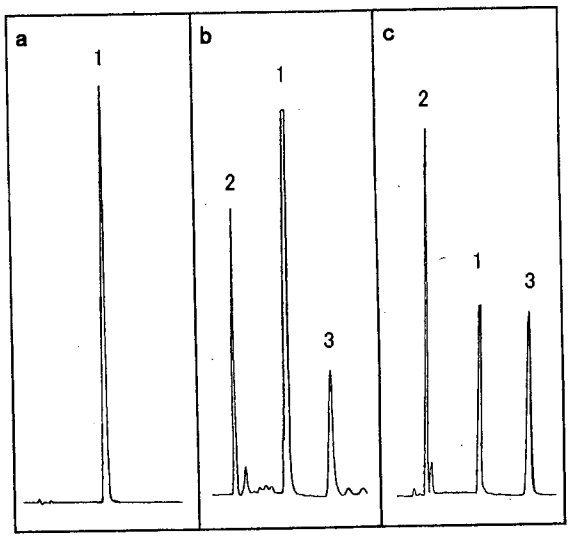

Fig. 4. HPLC chromatograms of EGCG before and after heating

HPLC analysis was carried out as described in Materials and Methods. a, before heating; b, after heating for 60 min at 100℃; c, after heating for 15 min at 180℃. 1, EGCG; 2, gallic acid; 3, gallocatechin gallate.

against standards of the radical-scavenging activity, phenolic content, and quercetin or EGCG level without heating. As shown in Figs. 1 and 2, even when quercetin was heated for 120 min and EGCG for 60 min, the retained radical-scavenging activity was about 100%. When heated for a total of 480 min, the retained activity was 80%. The total phenolic content of quercetin remained in the range of 70–80%. After 60 min of heating, the quercetin content was 80%; even after 480 min of heating, 60% was retained. As shown in Fig. 3a and b, a new peak appeared after heating. This peak is thought to have been a degradation product containing phenolic OH group(s), since the retained total phenolic content was 80% after 60 min of heating. However, this peak could not be identified. It has been reported that quercetin was stable to heating (Mizuno *et al.* 1992) which is in agreement with our results. However, Ioku *et al.* (2001) have shown that quercetin in boiled onion decreased to 25% of the fresh value, which is not in agreement in our results. Heating in a screw-capped vial may have inhibited the oxidization of quercetin in our work.

The retained total phenolic content in EGCG was 80–90%, which is correlated with the radical-scavenging activity level. However, while the EGCG content was 80% after 60 min, it decreased rapidly to 50% after 120 min and continued to decrease thereafter. The remaining percentage after 480 min was about 25%. This shows that the heating-degradation products of EGCG demonstrated radical-scavenging activity. As shown in Fig. 4a and b, by the HPLC analysis, the standard solution showed only one peak, while three peaks appeared after heating. One of them was in agreement with the retention time for gallic acid; another was in agreement with that of gallocatechin gallate. It is thought that the retention of radical-scavenging activity resulted from this gallic acid, gallocatechin gallate and the fraction of EGCG that remained. It has been reported that heating caused EGCG in Japanese tea to generate gallocatechin gallate through an isomerization reaction (Komatsu *et al.* 1993; Saijo and Takeda 1999); our result is in agreement with these reports. It was also clear that gallic acid had been generated. In addition, Komatsu *et al.* (1993) have shown that 50% of EGCG in a green tea infusion remained after heating for 15 min at 95℃. The foregoing results show that, even if heating was continued at 100℃ for 60 min, quercetin and EGCG remained and their radical-scavenging activity was retained. Moreover, even if heating was continued for longer, the scavenging activity was retained from the coexistence of these compounds with their heat-degraded products. Furthermore, luteolin, luteolin-7-glucoside and rutin also remained and their radical-scavenging activity was retained at 100℃ (data not shown). It can therefore be concluded that

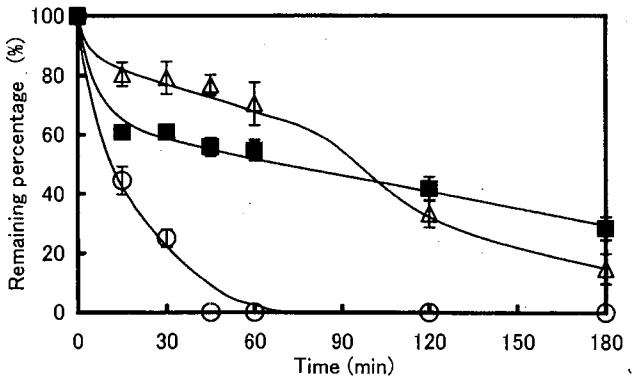

Fig. 5. Remaining percentages of radical-scavenging activity, total phenolic content and quercetin content after heating at 180℃

△, radical-scavenging activity; ■, total phenolic content; ○, quercetin content.

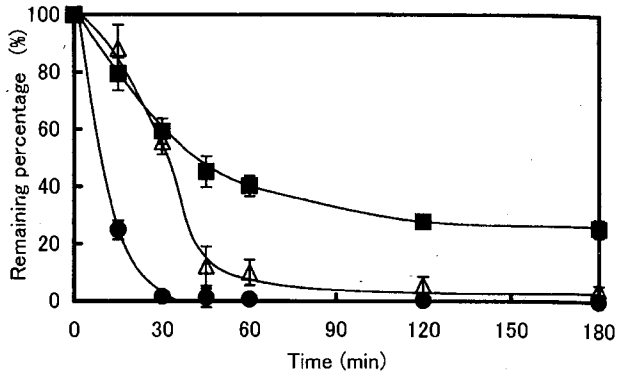

Fig. 6. Remaining percentages of radical-scavenging activity, total phenolic content and EGCG content after heating at 180℃

△, radical-scavenging activity; ■, total phenolic content; ●, EGCG content.

the polyphenolic compounds in food can withstand boiling temperature well.

Effects of heating at 180℃

The effects of heating at 180℃ are shown in Figs. 5 and 6. Figure 5 shows that the retained radical-scavenging activity of quercetin was 80% after heating for 45 min, but decreased gradually to 70% after 60 min and then to 40% after 120 min. After 180 min, only about 15% of the original scavenging activity remained. The total phenolic content remained at 60% after heating for 45 min, and was 40% after 180 min. One new peak appeared in the HPLC chromatogram, as was the case with 100℃ heating (Fig. 3c). However, the quercetin content decreased rapidly to 50% after 15 min, to about 30% after 30 min, and had disappeared completely after 45 min.

As shown in Fig. 6, although the retained radical-scavenging activity of EGCG was 90% after heating for 15 min, it decreased to 55% after 30 min, and then decreased rapidly to 10% after 45 min. The total phenolic content decreased from 80% to 60% from 15 min to 45 min and continued to decrease thereafter. After 180 min, the remaining percentage was about 30%. Although the total retained phenolic content was 30% after 180 min, it is thought that the OH groups of the heat-degraded products whose radical-scavenging activity was not evident had already reacted. The EGCG content was decreased rapidly to 10% by heating for 15 min, and had disappeared after 30 min. Peaks for gallic acid and gallocatechin gallate appeared, as with the case for 100℃ heating, from 15 min to 45 min (Fig. 4c), but these peaks had disappeared after 60 min. The disappearance of these peaks suggests the absence of radical-scavenging activity by EGCG and its products. Quercetin was more stable to heating at high temperature than EGCG. The foregoing results make clear that decomposition took place in both compounds due to high-temperature heating at 180℃. However, high-temperature heating such as deep frying and oven cooking is not often conducted for more than 15 min at home. It is therefore thought that these two antioxidants would retain their polyphenolic content and radical-scavenging activity relatively well with the usual high-temperature cooking methods. Moreover, since various antioxidants are contained in foods, other interactions can take place.

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ケルセチンおよびエピガロカテキンガレートのラジカル捕捉活性に対する加熱処理の影響

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ケルセチンおよびエピガロカテキンガレートのラジカル捕捉活性に及ぼす加熱の影響について検討を行った．100℃加熱において60分後および480分後のラジカル捕捉活性は，両者とも100%および80%保持されていた．このことは，通常のゆで調理では抗酸化作用が保持されることを示す．また，加熱によりエピガロカテキンガレート量は減少していたが，ラジカル捕捉活性は残存していた．180℃加熱においても，ケルセチンおよびエピガロカテキンガレートの含量はラジカル捕捉活性よりも速やかに減少した．このことから，ラジカル捕捉活性は，加熱分解生成物によって保持されていることが明らかとなった．15分間の180℃加熱では，ラジカル捕捉活性は80%以上保持されていた．従って，揚げ調理やオーブン調理のような通常の高温調理操作ではラジカル捕捉活性は保持されていると考えられる．

キーワード：ラジカル捕捉活性，ケルセチン，エピガロカテキンガレート，加熱処理，ポリフェノール量．