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Effects of heating and the addition of seasonings on the anti-mutagenic and anti-oxidative activities of polyphenols

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Abstract

Polyphenols are functional compounds in edible plants. In this study, we investigated the effects of heating and addition of seasoning, with and without heating, on the DPPH radical scavenging activity and the anti-mutagenicity effect against benzo(α)pyrene of polyphenols. Anti-oxidative activity and anti-mutagenicity of polyphenols were hardly influenced by heating without seasoning. Radical scavenging activity was clearly promoted by the addition of soy sauce, rice soybean paste, barley soybean paste and consommé, and a similar tendency was observed on the anti-mutagenicity of polyphenols by adding two kinds of soybean pastes. Marked increases in the activities of caffeic acid, and catechin occurred and epicatechin was several-fold more active than the seasoning itself. Heating around the boiling point enhanced the radical scavenging and anti-mutagenic activities of catechin, epicatechin, naringenin and naringin with seasoning addition. However, analysis showed differences of increments in these three polyphenols. More active reaction products might have been formed by interaction of constituents in the model system.

1. Introduction

Though most of the creatures on the earth utilize oxygen to obtain energy for life, they are always exposed to the dangers of active oxygen and peroxide. Active oxygen and free radicals are thought to cause many different types of oxidative damage to biomolecules, such as aging of cells, cancer and other lifestyle-related diseases.

With the progress toward an aging society, the problem of adult diseases such as hypertension and cancer has been increasing steadily in recent years. Cancer became the most common cause of death in Japan in 1985 (Kuroda, 1995). Active oxygen and free radicals induce oxidative damage to DNA, to causing initiation, and also play a big role in promotion, the midstep of carcinogenesis. In addition, mutagens, such as heterocyclic amines (HCA) and polycyclic aromatic hydrocarbons (PAH), have been detected in foods as one of the major food-borne causes of the various mutations to the gene of the cell. On the other hand, epidemiological studies have shown that daily consumption of fresh vegetables and fruit reduces the risk of life-style-related diseases such as cancer and coronary heart disease (Ames, Shigenaga, & Hagen, 1993; Joshipura et al., 1999; Steinmetz & Potter, 1991). Juices of cabbage and broccoli restrains mutagenicity induced by 3-amino-1,4-dimethyl-5*H*-pyrido-[4,3-*b*]indole (Trp-P-1) in the Ames test (Morita, Hara, & Kada, 1978). The anti-mutagenic activities of the juices of 19 fruits and 25 vegetables commonly consumed in Germany were found to decrease the mutagenic activities induced by 2-amino-3-methyl-[4,5-f]-quinoline(IQ), 2-amino-3,4-dimethylimidazo-[4,5-f]quinoline (MeIQ), or 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx) in *Salmonella typhimurium* TA98 and TA100 (Edenharder, Kurz, John, Burgard, & Seeger, 1994). Diets with enough fruits and vegetables could reduce DNA damage according to the assay of urinary biomarkers (Simic & Bergtold, 1991). The anti-oxidative activities of 22 common vegetables, green tea and black tea were reported to be due to the autoxidised radical quenching ability (Cao, Sofic, & Prior, 1996).

Suppression of peroxidation in biomolecules from components of dietary foods, reduces the risk of

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oxidative damage in DNA and mutation, and also prevents of carcinogenesis. Many active ingredients, such as vitamins, chlorophyll, dietary fibres and polyphenol are distributed in vegetables, fruit, edible seaweeds and teas. They inhibit mutation and scavenge active oxygen and free radicals. Polyphenols, including more than 4000 related compounds, were found to have anti-mutagenic and anti-oxidative activities, and cholesterol-lowering effects.

Quercetin is one of the polyphenols existing most commonly in vegetables and rutin, the glucoside of quercetin, is also widely found in edible plants (Miean & Mohamed, 2001). Quercetin levels, in 1 kg of lettuce, onion, kale and apple, were found to be 911 mg, 284–486, 110, and 21–72 mg, respectively (Crozier, Lean, McDonald, & Black, 1997; Hertog, Hollman, & Katan, 1992). Quercetin levels varied from 4 to 16 mg/l and from 10 to 25 mg/l in red wine and black tea, respectively (Hertog, Hollman, & Putte, 1993). The naringin level in grapefruit juice was determined to be 100–800 mg/l (Galati, Chan, Wu, & O'Brien, 1999).

Quercetin has been demonstrated to have an inhibitory activity against some indirect mutagens, such as 2-amino-1-methyl-6-phenylimidazo-[4,5-*b*]pyridine (PhIP) and MeIQx (Malaveille et al., 1996; Oguri, Suda, Totsuka, Sugimura, & Wakabayashi, 1998). It was also reported that morin inhibited indirect mutagenicity of aflatoxin B_1 (AFB_1), the strongest carcinogen of natural origin, and the direct mutagen *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) (Francis, Shetty, & Bhattacharya, 1989a; Francis, Shetty, & Bhattacharya, 1989b). On the other hand, quercetin, morin and naringin were reported to have an anti-oxidative capacity (Burda & Oleszek, 2001). The hydroxyl radical scavenging activity was also detected in quercetin, morin, naringenin and catechin (Husain, Cillard, & Cillard, 1987).

Addition of seasoning is an indispensable process for cooking. Concerning public food safety, soy sauce is noted for its mutagenicity, generated when treated with nitrite under strongly acidic conditions in relation to the high incidence of stomach cancer in Japan (Wakabayashi et al., 1983). Tyramine was found to be one of the major mutagen precursors in soy sauce treated with nitrite and ethanol, and 1-methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (MTCCA) is a minor constituent (Higashimoto, Matano, & Ohnishi, 1988; Ochiai, Wakabayashi, Nagao, & Sugimura, 1984). Contrary to these reports, anti-oxidative and anti-carcinogenic activities were found in 4-hydroxy-5-methyl-3(2*H*)-furanone (HMF) and 4-hydroxy-2,5-dimethyl- 3-(2*H*)-furanone (HDMF), one of the soy sauce flavour components (Kataoka, Liu, Albright, Storkso, & Pariza, 1997).

The effects of heating and the addition of seasonings on the activity of polyphenol have not been well studied to date. We investigated the effect of heating, and that of seasoning addition with and without heating on the radical-scavenging activity and the anti-mutagenicity of commercially available polyphenols. This paper describes some interesting results.

2. Materials and methods

2.1. Reagent and test microbes

Eight authentic polyphenols (shown in Table 1) were purchased from Sigma Chemical Co. (Louis, USA). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), tris(hydroxymethyl)-amino-methane (Tris), ethanol and acetic acid (HPLC grade) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Methanol (HPLC grade)

Table 1
Polyphenols used in this study

Polyphenol	Scientific name	Purity (%)	Manufacturer	Concentration used in:	
				Anti-oxidant test (mM)	Anti-mutagenicity test (μg/plate)
Caffeic acid	3,4-dihydroxycinnamic acid	N/A[a]	Sigma	0.15[b]	11.7[c]
Morin	2′,3,4′,5,7-pentahydroxyflavone	N/A	Sigma	0.20	3.52
Quercetin	3,3′,4′,5,7-pentahydroxyflavone	N/A	Sigma	0.10	0.115
Rutin	3,3′,4′,5,7-pentahydroxyflavone-3-rutinoside	95	Sigma	0.15	0.205
Catechin	2-[3,4-dihydroxyphenyl]-3,4-dihydro-1-[2*H*]-benzopyran-3,5,7-triol	98	Sigma	0.10	46.9
Epicatechin	([2*R*,3*R*]-2-[3,4-dihydroxyphenyl]-3,4-dihydro-1[2*H*]-benzopyran-3,5,7-triol	N/A	Sigma	0.10	46.9
Naringenin	4′,5,7-trihydroxyflavanone	95	Sigma	50.0	1.71
Naringin	4′,5,7-trihydroxyflavanone 7-rhamnoglucoside	95	Sigma	30.0	6.84

[a] Not available.
[b] Final concentration of polyphenols in the reaction mixture (2 ml).
[c] Final concentration of polyphenols per plate.

was obtained from Kokusan Chemical Co. Ltd. (Tokyo, Japan). Benzo(α)pyrene (BaP) was purchased from Tokyo Kasei and dimethylsulfoxide (DMSO, fluorometry analysis grade) from Dojin Chemical (Tokyo, Japan). Crude enzyme, prepared from rat liver (S9), and its cofactor kit for activation of indirect mutagens were supplied frozen by Oriental Yeast (Tokyo, Japan). *Salmonella typhimurium* TM677 was developed as by Skopek, Liber, Kaden, and Thilly (1978) using an 8-azaguanine sensitive strain, *S. typhimurium* TA1535, by introducing a plasmid pKM101 of *S. typhimurium* TA2000 to improve the sensitivity of mutagen detection.

2.2. Preparation of samples for DPPH-HPLC

Eight authentic polyphenols were dissolved in ethanol. To detect variations in the activity on either the positive or negative side, the concentration of each polyphenol was adjusted to show 50% scavenging activity against 250 μM DPPH, as shown in Table 1. Soy sauce, rice soybean paste, barley soybean paste and consommé (shown in Table 2) were dissolved in water to bring their concentrations to 2%, 24%, 12% and 5%, respectively. Polyphenol ethanol solutions (500 μl each) were put into three test tubes with screw caps. One was treated with seasoning solution (500 μl) to be the sample with seasoning added without heating. The remaining two were mixed with water or seasoning solution (500 μl each), and were then heated in a boiling water for 10 min to be the samples of heated polyphenols and that with seasoning added with heating.

2.3. Measurement of DPPH radical-scavenging activity

The DPPH-HPLC method was carried out according to Yamaguchi, Takamura, Matoba, and Terao (1998). An aliquot of sample solution (200 μl) was mixed with the 100 mM Tris–HCl buffer (pH 7.4, 800 μl) and then added to 1 ml of 500 μM DPPH in ethanol (final concentration of 250 μM). The mixture was shaken vigorously and left to stand for 20 min at room temperature in the dark. A blank test was run with the Tris–HCl buffer instead of the sample solution. The absorbance at 517 nm by DPPH was measured by reversed-phase HPLC analysis. The HPLC equipment consisted of a Shimadzu LC-6A pump, a Rheodyne injector fitted with a 20 μl sample loop and a Shimadzu SPD-6AV UV-VIS detector set at 517 nm. Analyses were performed with a TSK gel Octyl-80Ts column (4.6 × 150 nm; Tosoh, Tokyo, Japan) at ambient temperature and mobile phase of methanol/water (70:30, v/v) at a flow rate of 1 ml/min. The DPPH radical-scavenging activity was calculated by the following equation:

$$\text{radical scavenging activity } (\%) = (A_b - A_s)/A_b \times 100,$$

where A_b is the peak area of the blank and A_s is the peak area of the sample.

2.4. Preparation of samples for anti-mutagenicity test

Polyphenol standards were dissolved in DMSO, and then diluted to make 5% DMSO solution by water addition. The highest dose showing no growth inhibition was employed as a sample dose (shown in Table 1). The other three types of samples were prepared in the same manner as described in the DPPH-HPLC method.

2.5. Measurement of anti-mutagenicity test

We employed the FM assay method proposed by Skopek et al. (1978) modified by Takagi et al. (1988) and reduced in size by Ren, Endo, and Hayashi (2001). According to the results of the preliminary examination, the highest concentration of BaP was 32 ng per plate which showed sufficient mutagenicity with no growth inhibition. The test samples and BaP were mixed in each well of the microtitre plate (Corning, 96 wells, NY, USA). They were incubated on a rotary shaker (Tuple mixer, Iwaki Glass Co. Ltd., Funabashi, Japan) for 2 h at 37 °C and treated in the same manner reported by Ren et al. (2001). The mutation frequency and the mutagenicity suppression ratio were obtained by the formula shown below.

$$\text{Mutation frequency ratio} = (C_m - C_n)/C_v,$$

where C_m was the number of colonies of mutants, C_n was the number of spontaneous colonies for negative control, and C_v was the number of colonies for viable cells

$$\text{Mutagenicity suppression ratio } (\%) = (1 - M_{b+s}/M_b) \times 100,$$

Table 2
Seasonings used in this study

Seasoning	Japanese name	Major constituents	Manufacturer
Soy sauce	Shoyu	Defatted soybean, soybean, wheat, salt, alcohol	Kikkoman
Rice soybean paste	Kome Miso	Rice, soybean, salt, alcohol, vitamin B_2	Saikyo Miso
Barley soybean paste	Mugi Miso	Barley, soybean, salt, alcohol, Vitamin B_2	Daiei
Consommé	Kansou Supu	Salt, lactose, sugar, vegetable oil, spice, beef extract, chicken extract, vegetable extract, soy sauce, yeast extract, caramel	Ajinomoto

where M_{b+s} was the mutation frequency of BaP upon treatment of test samples and M_b was mutation frequency of BaP alone.

2.6. LC-MS analysis

Three polyphenol samples were prepared in the same manner as used for the DPPH-HPLC sample preparation. The LC-MS system (Shimadzu, Kyoto, Japan) consisted of two LC-10ADVP pumps, a DGU-14AM degasser, a CTO-10AVP column oven, a SPD-10AVP UV-VIS detector, and a QP8000α mass spectrometer controlled by a FMV-6500DX3 personal computer (Fujitsu, Tokyo, Japan). Mobile phase consisted of 0.2% (v/v) acetic acid (A) and methanol (B) (B: 0–10 min; 20 → 50%, 10–11 min; 50 → 90%, 11–14 min; 90%, 14–20 min; 20%). Analyses were performed with a STR ODS-II Column (5 μm, 150 × 2.0 mm, Shinwa Chemical, Kyoto, Japan) at a rejection volume of 5 μl and a flow rate of 0.2 ml/min. Peak areas of the 3 polyphenol samples were detected at 254 nm. Each peak was confirmed qualitatively by MS using selected ion monitoring (SIM) mode and then quantitatively determined by UV detector.

3. Results

3.1. Changes in DPPH radical-scavenging activity

Eight polyphenols showed 29–57% changes of DPPH radical-scavenging activity (shown in Table 3) and heating gave only a little change in their activities.

When mixed with soy sauce, the activities of all samples, except for naringenin, increased by 11–25%. The ratios of increase in caffeic acid and epicatechin were 1.6 and 1.8 times bigger than that of soy sauce itself, respectively.

Polyphenols increased the activity by 12–41% after addition of rice soybean paste. Among them, anti-oxidative activities of caffeic acid and epicatechin rose 2.3- and 1.3-fold that of the seasoning itself, respectively. After being heated, the scavenging activities of catechin, epicatechin, naringenin and naringin increased by more than 7–16%.

In the case of barley soybean paste addition, the activity increase of the polyphenols except for naringenin was 11–31%. In particular, the gain of the abilities in caffeic acid and catechin were 1.6 and 1.3 times that of the seasoning itself, respectively. The supplementary 10 point increase was seen in naringenin, and 5 points in naringin, by heating after the addition of each compound.

After adding consommé, increaments of 8–19% were found in the samples, except for naringenin. The augmentations of scavenging activities in caffeic acid and

Table 3
Change of DPPH radical scavenging activity (%) of the polyphenol samples before and after heating

Polyphenol	Untreated	Heated	Seasoning added							
			Soy sauce		Rice soybean paste		Barley soybean paste		Consommé	
			Unheated	Heated	Unheated	Heated	Unheated	Heated	Unheated	Heated
Caffeic acid	29 ± 3.3[b]	30 ± 3.6	54 ± 3.6	55 ± 3.5	70 ± 2.5	75 ± 1.0	60 ± 4.8	63 ± 4.4	48 ± 3.9	51 ± 3.4
Morin	37 ± 2.9	39 ± 4.5	49 ± 2.8	46 ± 3.1	51 ± 2.9	54 ± 5.3	55 ± 2.9	55 ± 4.1	45 ± 2.8	45 ± 4.8
Quercetin	43 ± 1.8	45 ± 1.9	55 ± 3.9	56 ± 3.3	62 ± 3.6	65 ± 3.1	58 ± 2.8	61 ± 4.2	56 ± 4.4	58 ± 3.0
Rutin	48 ± 1.0	47 ± 0.8	64 ± 4.4	55 ± 2.7	65 ± 2.1	65 ± 1.0	62 ± 4.3	62 ± 3.5	57 ± 2.2	58 ± 1.6
Catechin	42 ± 1.9	41 ± 2.9	58 ± 5.1	59 ± 4.2	61 ± 3.3	77 ± 3.5	68 ± 5.1	70 ± 3.5	58 ± 3.0	60 ± 2.0
Epicatechin	44 ± 2.5	45 ± 1.1	67 ± 3.9	67 ± 3.1	68 ± 3.7	75 ± 3.1	64 ± 2.8	64 ± 4.7	56 ± 4.4	57 ± 3.6
Naringenin	57 ± 4.0	59 ± 3.6	60 ± 3.6	69 ± 3.2	69 ± 4.9	82 ± 5.0	62 ± 3.6	67 ± 2.4	61 ± 3.3	67 ± 3.8
Naringin	41 ± 2.8	42 ± 3.3	52 ± 5.1	56 ± 3.5	55 ± 3.5	65 ± 2.7	52 ± 3.2	62 ± 3.2	49 ± 2.6	57 ± 2.6
Seasoning alone[a]			14 ± 1.9	13 ± 1.6	18 ± 2.4	22 ± 4.0	20 ± 2.7	21 ± 4.4	12 ± 2.8	11 ± 1.1

[a] Final concentrations of each seasoning in the reaction mixture (2 ml) of samples and DPPH: soy sauce 2 mg, rice soybean paste 24 mg, barley soybean paste 12 mg, consommé 5 mg.
[b] Values are means ± SD.

catechin were 1.6 and 1.3 times as much as that of consommé itself, respectively. When heated, the increases in naringenin and naringin were 8 and 6%, severally.

Putting together the results of the test, we found that the activities of polyphenols examined were hardly influenced by heating. It also became clear that the activities were reinforced by the addition of soy sauce, rice soybean paste, barley soybean paste or consommé. Particularly, the rises in caffeic acid, catechin and epicatechin were 1.3–2.3 times higher than those of the seasonings themselves. It was found that the increases of anti-oxidative capacities of catechin, epicatechin, naringenin and naringin with seasoning addition became more marked by cooking around the boiling range.

3.2. Changes in anti-mutagenicity

According to the FM assay (shown in Table 4), morin, quercetin, rutin, catechin, epicatechin and naringenin inhibited 12–42% of the mutation frequency induced by BaP. Heating, without the presence of seasoning, scarcely changed the anti-mutagenicities.

After soy sauce addition, 29% of the inhibition ratio was identified in caffeic acid. The repressive abilities of morin, quercetin, rutin and naringenin against BaP decreased by 12–38%, but catechin and epicatechin increased their activities by 23% and 9%, respectively. Particularly, the increases in caffeic acid and catechin were about 4.1 and 3.3 times larger than that of soy sauce itself.

When mixing with rice soybean paste, the increments of inhibition ratios, in 6 polyphenols except for naringenin and naringin, were by 29–73%, and the increase in caffeic acid was 2.9 times the repressive ability found in the seasoning itself. The suppressive percentages of morin, rutin, epicatechin and naringin increased by 6–21% upon heating.

After adding barley soybean paste, with the exception of naringenin and naringin, the other six compounds gained 22–62 percentage points. The gain of inhibitory action in caffeic acid was 2.3-fold of that of barley soybean paste. The inhibitory ratios, against BaP, of epicatechin and naringin with addition of the seasoning, increased by 11% and 17% after being heated, respectively.

Caffeic acid and naringin showed no variation after mixing with consommé, but the repression rate of epicatechin grew by 16% and disappeared in the other five polyphenols. Only epicatechin increased by 26 points upon heating.

Rounding off the result of this study, it was clear that the mutation inhibitory ratios of several polyphenols decreased after mixing with soy sauce and consommé. On the other hand, the increase of anti-mutagenic

Table 4
Result of anti-mutagenicity test: Suppression ratio (%) the mutagenicity of BaP[a] in the polyphenol samples

Polyphenol	Untreated	Heated	Seasoning added							
			Soy sauce		Rice soybean paste		Barley soybean paste		Consommé	
			Unheated	Heated	Unheated	Heated	Unheated	Heated	Unheated	Heated
Caffeic acid	nd[c]	nd	29 ± 6.3	nd	73 ± 4.7	72 ± 8.1	62 ± 5.1	63 ± 7.6	nd	nd
Morin	38 ± 4.4[d]	42 ± 5.1	26 ± 1.2	nd	72 ± 6.4	78 ± 2.6	60 ± 3.8	56 ± 6.5	nd	nd
Quercetin	14 ± 3.2	13 ± 2.8	nd	nd	62 ± 4.5	64 ± 9.1	58 ± 4.3	28 ± 3.8	nd	nd
Rutin	12 ± 2.7	13 ± 1.3	nd	nd	42 ± 3.8	62 ± 6.7	57 ± 4.7	54 ± 5.8	nd	nd
Catechin	42 ± 4.6	42 ± 6.5	65 ± 2.8	65 ± 4.3	78 ± 5.1	78 ± 5.3	75 ± 6.1	75 ± 1.4	nd	nd
Epicatechin	22 ± 4.1	23 ± 2.6	31 ± 5.6	43 ± 2.9	51 ± 4.2	62 ± 2.6	59 ± 4.1	70 ± 4.6	38 ± 2.7	65 ± 13.3
Naringenin	38 ± 3.8	36 ± 4.1	nd	nd	nd	nd	nd	nd	nd	nd
Naringin	nd	nd	nd	nd	nd	21 ± 6.1	nd	17 ± 6.3	nd	nd
Seasoning alone[a]			7 ± 3.6	20 ± 3.6	25 ± 3.5	23 ± 3.0	27 ± 7.1	23 ± 3.6	nd	nd

[a] Mutation ratio of BaP: 3.7×10^{-5}/32.5 ng BaP/plate.
[b] Final quantity (μg) of each seasoning per plate: soy sauce 113., rice soybean paste 1350, barley soybean paste 675, consommé 281.
[c] Not detectable.
[d] Values are means ± SD.

Table 5
Quantitative LCMS analysis of polyphenols before and after treatment

Polyphenol	Untreated (μM)	Seasoning added (μM)							
		Soy sauce		Rice soybean paste		Barley soybean paste		Consommé	
		Unheated	Heated	Unheated	Heated	Unheated	Heated	Unheated	Heated
Caffeic acid	150	149[a]	141	126	122	109	89	125	115
Catechin	100	116	63	99	83	NQD[b]	NQD	34	88
Epicatechin	100	123	114	151	118	133	127	102	99

[a] Average of three times.
[b] Not quantitatively determined (peak detected as a shoulder on a tailing of the big peak).

activity in polyphenol by the addition of two kinds of soybean paste was obvious in a series of these experiments against BaP. Particularly, the rise in the anti-mutagenic activities of caffeic acid and catechin were 2.3–4.1-fold those of the seasonings themselves. The increment of inhibiting activity in epicatechin with the addition of seasonings was more marked by heating.

3.3. LC-MS analysis

Quantitative analysis by LC-MS was done to examine the fluctuations of three polyphenols (shown in Table 5). This showed the most marked change in their activities in radical-scavenging or anti-mutagenic studies. The concentrations of caffeic acid and catechin decreased to levels ranging from 89 to 149 μM (average: 122 ± 18.9, untreated, 150 μM) and from 34 to 116 μM (average: 81 ± 28.7, untreated, 100 μM) by addition of seasonings with and without heating, in contrast to the increment of epicatechin, ranging from 99 to 151 μM (average: 121 ± 16.7, untreated, 100 μM).

3.4. pH test

The pH, under all experimental conditions applied for three polyphenol samples, ranged from 5.0 to 5.4. The pH was very stable when mixed with seasonings, regardless of heating, and no chemical reaction was expected.

4. Discussion

The concentration of quercetin used in this DPPH-HPLC test was about 34 mg/l, and was very close to the level in apple juice and in black tea. However, the daily intake of vegetables and fruits varies according to regions and cooking habits, and the average intake of polyphenol varies. Therefore it is difficult to compare the aforementioned analytical data directly with the concentration used in this study.

Epicatechin, catechin, quercetin and caffeic acid were reported to scavenge DPPH radicals (Murakami, Yamaguchi, Takamura, & Matoba, 2002). The report agrees with the result of this paper upon comparison of the sample concentrations employed. Our experimental results show that morin and quercetin inhibited, not only the mutagenicity of BaP, but also showed DPPH radical scavenging activity, suggesting that several polyphenols could contribute to anti-mutagenicity by obstructing the working mechanism of P450 enzyme, and suppressing the oxidative damage of DNA. From the viewpoint of utilization of functional activities of food components, anti-mutagenic and anti-oxidative activities are desirable, and it is important to understand their behaviour in the process of cooking.

Heat-unstable and biologically active compounds, such as enzymes and vitamins, might be lost during cooking, and the relative activities could be decreased as a result of food preparation. Superoxide anion radical-scavenging abilities, of the juices prepared from carrots, tomatoes and onions, decreased considerably in the process of boiling, but the juices of vegetables rich in chlorophylls still retain strong abilities (Nishibori & Namiki, 1998). The radical scavenging activities of broccoli, cabbage and several other vegetables increase a lot after boiling, and also, decrease of the activities of Chinese cabbage and Japanese radish have been observed (Yamaguchi et al., 2001). In the study of polyphenol levels in several vegetables during the heating process, the quercetin concentration in tomatoes and onions were reduced by 82% and 75%, respectively, after boiling for 15 min (Crozier et al., 1997). On the other hand, when spinach is heated at 90 °C for 10 min, nearly 50% of the polyphenol is leached into the boiling water (Gil, Ferreres, & Tomas-Barberan, 1999). In summary, major changes of polyphenol levels are explained by the outflow from plant tissue, but the structural transformation of the compounds is not clear. In our study, all the samples were heated in a closed system using test tubes with sealed caps, and structural transition of compounds was suspected. It was concluded that all of the nine polyphenols were themselves stable on boiling under ordinary cooking conditions and revealed little possibility of structural transformation.

Soy sauce and soybean paste are both made from soybean, and used in Asian cooking as one of the traditional seasonings with characteristic ethnic flavour. Powdered or cubed consommé, made up with several different types of natural extract, various individual seasonings and several more food additives, such as caramel pigment, has been widely used in recent years, with the additional use of natural seasonings. This study shows the anti-oxidative and anti-mutagenic activities of soy sauce, rice soybean paste and barley soybean paste, except for the anti-oxidative quality of consommé at the concentration commonly used in daily cooking.

As for the interaction with polyphenols and cooking oil, mutagenicities of oil formed after cooking were significantly reduced by adding catechin to the oil before heating (Chiang, Wu, Liao, Wang, & Ko, 1999). In this paper, the anti-mutagenicities of morin, quercetin and rutin decreased after the addition of soy sauce, suggesting that these polyphenols interacted with some anti-mutagenic components in soy sauce, to suppress the anti-mutagenicity of the admixture.

As another impressive result, the increment of the anti-mutagenic and anti-oxidative activities of caffeic acid and catechin, were several times higher than those of themselves. But, in quantitative LC-MS analysis, the concentrations of caffeic acid and catechin decreased upon addition of seasonings. Some novel components could have been formed by the interaction of constituents in the test systems. Some polyphenols, like rutin, could be hydrolyzed to quercetin by glycosidase at pH7 (MacDonald, Mader, & Bussard, 1983). But, in this study, pH was very stable in caffeic acid and catechin, even after addition of seasonings.

When the reaction system composed of multi-food ingredients was heated, heat-labile ingredients might be lost to some extent, and promote mutual reaction at the same time. In our result, the anti-mutagenicities found in the mixture of soy sauce and caffeic acid or morin, and that of barley soybean paste and quercetin, and the radical-scavenging activity detected in the admixture of soy sauce and rutin, were diminished to some extent, mostly by heating. Because of the heat-stability of these polyphenols, anti-mutagenic substances in the mixtures tested could be heat-labile around 100 °C. The activation rise of the mixtures of seasonings and catechin and epicatechin by heating was thought to be a result of the promoting of the mutual reaction by boiling.

In this paper, we have discussed the influence of heating around the boiling temperature, and that of the presence of 4 seasonings, on the activities of 8 polyphenols, and the interactions of some compounds have also been studied. We are planning to pursue studies of the active components in seasonings, and to analyze products in the mixtures showing marked change in activities using LC-MS. In the final step of the study, an in vivo test would also be carried out to determine the metabolism of each polyphenol and its related compounds obtained during and after cooking.

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