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Changes in the chemical properties and anti-oxidant activities of curcumin by microwave radiation

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Abstract Curcumin is a dietary phenolic compound that has numerous beneficial health effects. In the present study, changes in the chemical properties and anti-oxidant activities of curcumin by microwave radiation were investigated. Degradation of curcumin dissolved in distilled water was accelerated according to the increase in radiation time or radiation intensity. Residual levels of curcumin after 5 min radiation at 500 W were 24-29%. Scavenging activities of curcumin against DPPH radical decreased by microwave radiation; those of curcumin against ABTS and AAPH radicals and nitrite were rather significantly enhanced. Conventional heating at 95°C also increased scavenging activities of ABTS, AAPH, and nitrite of curcumin but to a lesser extent. Fluorescence intensity of curcumin increased by regular heating but decreased by microwave heating. Among curcuminoids, bisdemethoxycurcumin was most resistant under microwave radiation as compared to curcumin or demethoxycurcumin.

Keywords: curcumin, curcuminoid, microwave radiation, anti-oxidant activity, heating

Introduction

Phytochemicals are plant-derived compounds and show many physiological effects, including anti-oxidant, anti-cancer, and anti-aging activities, as well as immunomodulatory effects (1). Among phytochemicals, curcuminoids [natural pigments derived from the rhizome of turmeric (*Curcuma longa*)] have received great attention because of their various health benefits. Curcuminoids, which are yellow coloring compounds, include demethoxycurcumin (DMC), bisdemethoxycurcumin (BMC), and curcumin as major compounds. Many previous reports have indicated that curcumin shows anti-oxidant, anti-inflammatory, and anti-carcinogenic activities (2-5). Experimental and epidemiological evidences have also indicated that curcumin has a protective effect on neurodegenerative diseases such as Alzheimer's disease (6,7).

Curcumin comprises two aromatic phenolic ring structures connected by two α,β-unsaturated carbonyl groups. The diketone structure is highly reactive; curcumin is unstable and undergoes decomposition at many conditions (8-10). Because of the potential health benefits of curcumin, it is widely used as a functional food ingredient and is added to a variety of processed foods such as turmeric rice and retort pouched foods (11,12). Accordingly, curcumin and its derivatives can be exposed to various processing and cooking conditions; changes in stability and bioactivities of curcumin under different conditions have not been well defined.

Microwave heating, also referred as dielectric heating, electronic heating, or high-frequency heating, has been commonly utilized for cooking, reheating, or thawing foods as it is a convenient method for consuming various types of processed and frozen foods. The heating method uses microwave that has a frequency of 300 MHz-300 GHz and wavelength of 1-1,000 mm. A common cooking oven utilizes a microwave with a frequency of 2.45 GHz (13-15). The microwave generated in an oven is absorbed by polar compounds of foods such as water, sugar, and amino acids. These polar molecules aligned along the electromagnetic field rapidly rotate their orientation as the electric field of microwave alternates between positive and negative. The molecular dipole rotation induces collision with adjacent molecules to generate heat energy (14-16).

Food industries have developed various curcumin-containing processed foods such as instant rice and retort pouched foods that need microwave heating for consumption. Several studies have investigated changes in curcumin stability under different conditions (17,18); effects of microwave radiation on chemical properties and bioactivities of curcumin have not been studied. In the present study, changes in stability by microwave radiation and the consequent changes in anti-oxidant activities were investigated.

Materials and Methods

Chemicals Curcumin (a mixture of curcumin, DMC, and BMC; 79.4, 16.8, and 3.8% (w/w), respectively; average molecular weight 361.05) was purchased from Acros Organics (Morris Plains, NJ, USA). HPLC grade solvents were obtained from J.T. Baker Co. (Phillipsburg, NJ, USA). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Curcumin treatment by microwave radiation and heating Curcumin (40 μM) dissolved in double distilled water (1 mL, DW) was divided into an open glass vial (5 mL size). Microwave radiation was performed on 5 vials located at each symmetric point of a pan simultaneously in a microwave oven (2.45 GHz, KR-A202B; Daewoo Electronics, Seoul, Korea) for different periods (0-5 min at 500 W) or with different radiation power intensity (0-500 W for 5 min). For regular heating, 0.5 mL of curcumin dissolved in DW (40 μM) was divided into 1.5 mL e-tube and heated at 95°C on a block heater (Type 17600; Thermolyne, Dubuque, IA, USA) for different periods (0-15 min). After the treatment, the samples were put on ice immediately and water loss of each sample was calibrated by adding extra DW based on weight difference before and after the treatment.

Analysis of chemical properties Chemical stability of curcumin was analyzed by measuring absorbance at 405 nm, and changes in absorbance spectrum were analyzed at 400-800 nm (Spectra Max M3; Molecular device, Sunnylvale, CA, USA). Fluorescence properties of curcumin were also measured at excitation 440 nm and emission 535 nm (Spectra Max M3; Molecular device). HPLC analysis for individual curcuminoid levels was performed with an LC equipped with an L-6200 intelligent pump (Hitachi, Tokyo, Japan), a UV-975 UV/Vis detector (Jasco Co., Tokyo, Japan), and a Shiseido C18 packed column (150×4.6 mm, 5 μm particle size) according to the previous method (18,19).

Determination of anti-oxidant activity For analyzing DPPH radical scavenging activity, 100 μL of 600 μM DPPH in MeOH was added to an equal volume of each sample solution and the absorbance was measured at 517 nm after 30 min (20). For measuring ABTS radical scavenging activity, ABTS radical solution containing 7.4 mM ABTS and 2.6 mM potassium persulfate was diluted five times with phosphate-buffered saline (PBS). The diluted solution (150 μL) was mixed with each sample (50 μL), and the mixture was incubated further for 30 min in a dark place (21). For nitrite scavenging activity, 50 μL of curcumin solution was mixed with 100 μL of 100 μM $NaNO_2$ and the mixture was incubated for 1 h in a dark place. The residual level of nitrite was then measured by the previous method (22). For analyzing AAPH radical scavenging activity, 1 μM curcumin (60 μL) was added to 100 μL of 0.5 μM fluorescein sodium salt in PBS and a reaction was initiated by adding 40 μL of 20 mM AAPH radical. The mixture was incubated in a dark place at room temperature. At different time points, the decreased fluorescence intensity of fluorescein of the reaction mixture was measured at excitation 485 nm and emission 535 nm using a microplate reader (Spectra Max M3; Molecular device) (23). The area under the curve (AUC) for the fluorescence changes was analyzed using the Image J software (ver. 1.46, NIH, Washington DC, USA).

Data analysis Statistical significance was evaluated by the Student's *t*-test. One-way ANOVA with Tukey's honestly significant difference (HSD) test was used for comparing multiple results using the SAS system (SAS Institute, Cary, NC, USA).

Results and Discussion

Changes in curcumin stability by microwave radiation Microwaves are widely used for cooking, reheating, and thawing of foods; they involve the use of magnetic waves that have a frequency of 300 MHz-300 GHz. A common cooking oven utilizes a microwave with a frequency of 2.45 GHz (13-15). Microwave radiation induces molecular movement in foods to generate heat; a water molecule in foods is an important mediator for converting its vibrating kinetic energy to thermal energy (14-16). This heating process could cause thermal degradation of various food constituents and affect their physiological activities. Several studies have reported the microwave-assisted extraction of polyphenols, including curcumin (24); the main focus of these studies was to enhance extraction yields of phyto-chemicals using microwave. Changes of polyphenol levels in several fruit juices by microwave heating have also been investigated, and the effects of microwave treatment were recently reviewed (25). A recent study also indicated that microwave treatment on 25 leafy vegetables resulted in different patterns of phytochemicals in different vegetables (26). However, few studies have determined the effect of microwave on stability and bioactivity of a specific phyto-chemical. Accordingly, in the present study we aimed to evaluate the changes in curcumin stability and anti-oxidant activities by microwave radiation with 2.45-GHz frequency.

Changes in curcumin stability by microwave heating were evaluated in an aqueous solution. Proximate levels of curcumin after microwave radiation were first determined by measuring absorbance at 405 nm as previous studies have confirmed that the absorbance value at 405 nm is proportional to total amounts of the three curcuminoids (18,27). Degradation of curcumin occurred more prominently according to the increase in radiation time (at 500 W) and power intensity (for 5 min) (Fig. 1A and 1B). Residual curcumin level significantly decreased by microwave radiation from 0.5 min (at 500 W) or from 200 W (at 5 min). Exposure to microwave radiation for 5 min at 500 W resulted in approximately 70% of curcumin degradation based on the absorbance valued at 405 nm (Fig. 1A and 1B). Curcumin as a yellow pigment exhibits strong absorption spectrum with the maximum absorption wavelength ranging from

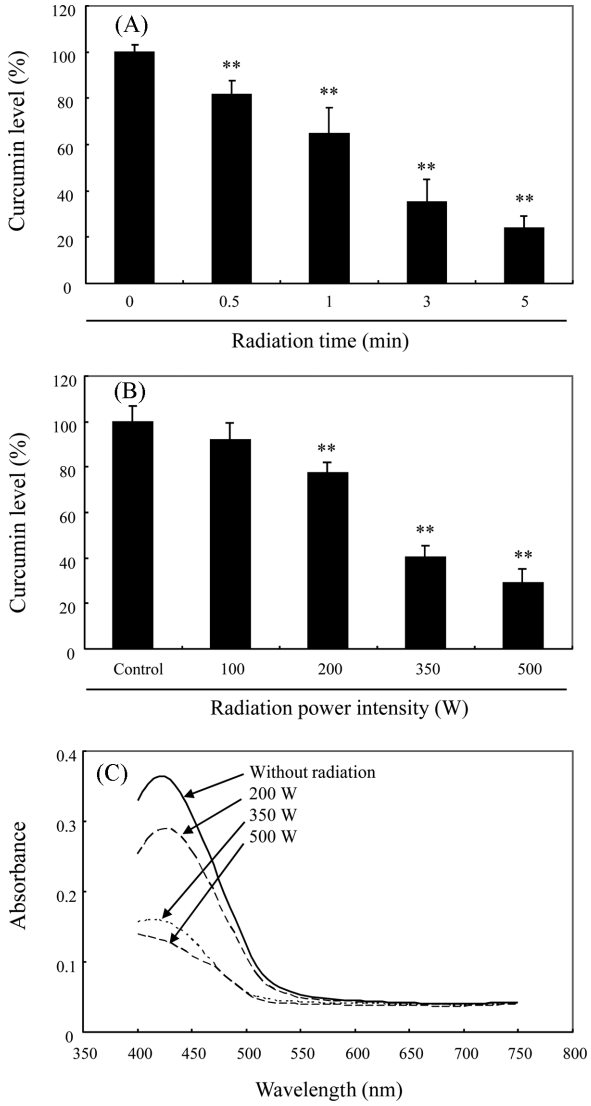

Fig. 1. Changes in residual curcumin level and absorbance spectrum by microwave radiation. Curcumin (40 μM) in DW was treated by microwave radiation with a different time (A) at 500 W or different power intensity (B) for 5 min. After the treatment, the absorbance values at 405 nm were measured. Changes in absorbance spectrum were also analyzed (C). Each value represents the mean±SD (n=5-10, A and B). **Significantly different from control according to Student's t-test (**p<0.01).

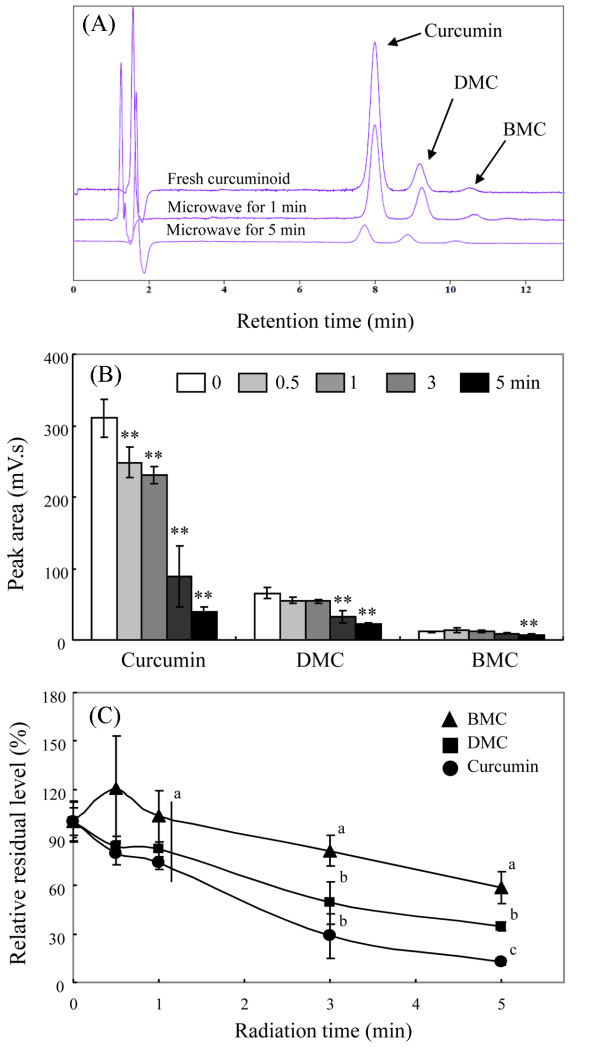

Fig. 2. Changes in individual curcuminoid level by microwave radiation. Changes in chromatograms (A) and residual levels (B) of each curcuminoid by microwave radiation for different treatment time (at 500 W) were analyzed using HPLC. Relative amount of residual curcuminoid levels was also calculated (C). Each value represents the mean±SD (n=5 in B and C). *,** Significantly different from control according to Student's t-test (*p<0.05, **p<0.01 in B). Different letters indicate a significant difference (p<0.05) based on one-way ANOVA and Tukey's HSD test (in C).

400 to 450 nm. Changes in the absorbance spectrum also indicate that microwave induced the radiation intensity-dependent degradation of curcumin showing the broadly-decreased spectrum of yellow color range without considerable shift of absorption peak (Fig. 1C). Although the precise mechanism underlying the color fading of curcumin by microwave radiation should be further elucidated, the radiation might cause decomposition of β-diketone moiety primarily because the structure is responsible for the yellow color of curcumin (28).

The present curcumin preparation included curcumin, DMC, and BMC; these comprised 79.4, 16.8, and 3.8% w/w, respectively. Accordingly, the changes in degradation patterns of individual curcuminoid by microwave radiation were also analyzed using HPLC. In the current HPLC system, three peaks of curcuminoids were detected, and microwave radiation decreased the response of all peaks (Fig. 2A). Curcumin was most unstable after microwave radiation; the residual levels of curcumin were 25 and 15% after 3 and 5 min radiation at 500 W, respectively (Fig. 2B and 2C). The residual levels of BMC and DMC also decreased according to the increase in radiation time; their decomposition rates by microwave were much slower than those of curcumin. Among curcuminoids, DMC was most resistant to microwave radiation; its residual level was 71% even after 5 min radiation (Fig. 2C). Degradation of total

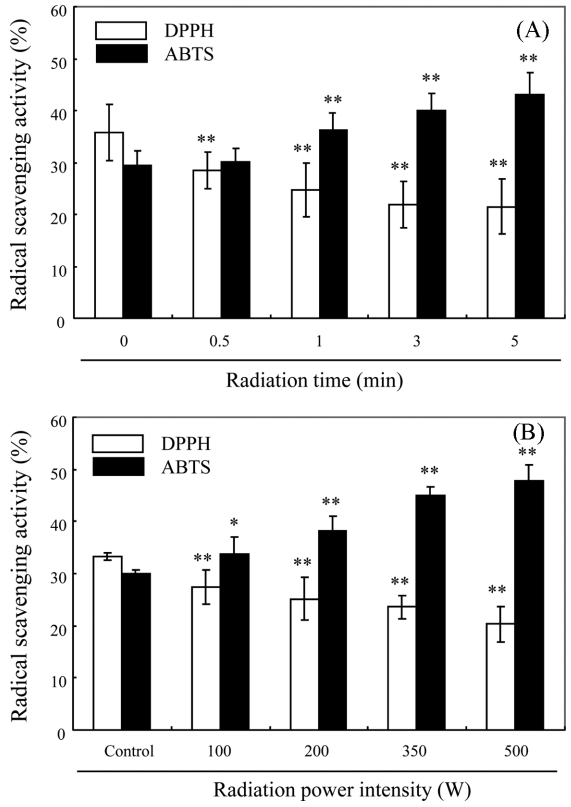

Fig. 3. Changes in DPPH and ABTS radical scavenging activities of curcumin by microwave radiation. Scavenging activities of DPPH and ABTS radicals of curcumin (40 μM) after microwave radiation with different time (A) or different power intensity (B) were analyzed. Each value represents the mean±SD (n=5–10). *,** Significantly different from each corresponding control according to Student's t-test (*p<0.05, **p<0.01).

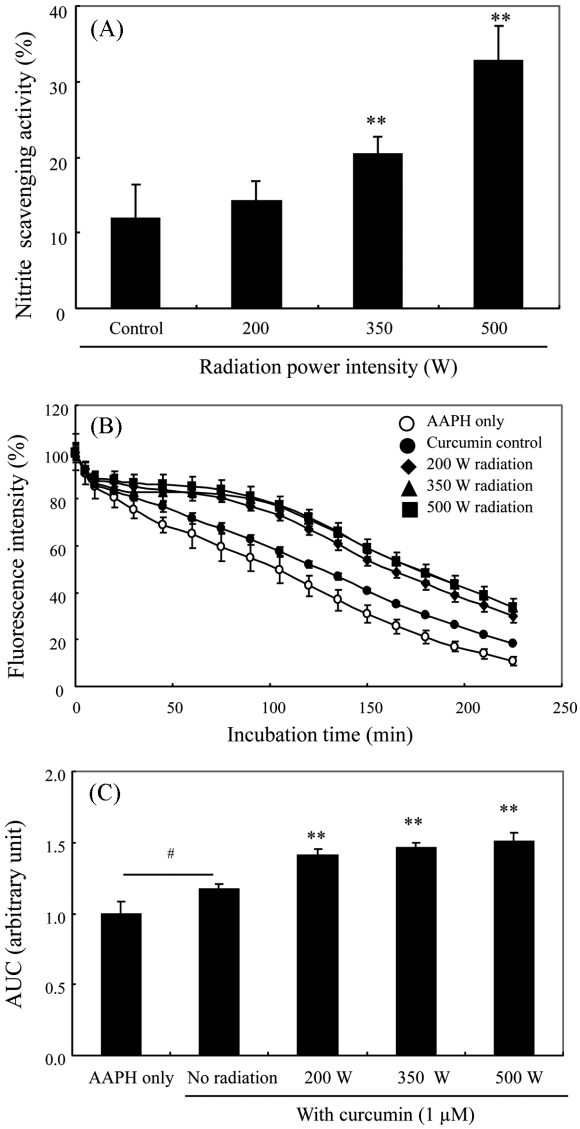

Fig. 4. Changes in nitrite and AAPH radical scavenging activities of curcumin by microwave radiation. Scavenging activities of nitrite (A) and AAPH radicals (B) of curcumin after microwave radiation with different power intensity were analyzed. AUC of the fluorescence changes was also calculated (D). Each value represents the mean±SD (n=4-8). **Significantly different from fresh control according to Student's t-test (**p<0.01). #Significantly different from control without curcumin according to Student's t-test (#p<0.05 in C).

curcuminoids by microwave radiation appeared to be in agreement with the results analyzed by absorbance at 405 nm. Although β-diketone moiety is responsible for the yellow color of each curcuminoid, it is believed that the absence of methoxy group on phenolic ring might also be important for the stabilization under microwave radiation.

Changes in anti-oxidant properties of curcumin by microwave radiation Microwave radiation effectively induced the curcumin decomposition; it might also cause changes in physiological activities of curcumin. To confirm whether the destruction of curcumin was followed by changes in its bioactivities, changes in anti-oxidant properties of curcumin by microwave treatment were analyzed. First, scavenging activities of DPPH and ABTS radicals of curcumin treated with different microwave radiation time (at 500 W) were analyzed (Fig. 3A). The scavenging activity of DPPH radical decreased as duration of radiation increased, and microwave radiation for 30 s induced significant reduction of the activity (p<0.05). The scavenging activity of ABTS radical, however, was significantly enhanced starting from 1 min radiation, and the activity increased according to the increase in radiation time (Fig. 3A). A similar pattern of activity changes was also observed in curcumin treated with different radiation intensity. As radiation intensity increased, DPPH radical scavenging activity of curcumin gradually reduced; the increased intensity of microwave radiation rather caused the enhancement of ABTS radical scavenging activity (Fig. 3B). When curcumin was treated with microwave radiation for 5 min at 500 W, the scavenging activity of DPPH radical was reduced by 40% but that of ABTS radical was enhanced by 46-57% (Fig. 3).

Changes in scavenging activities of nitrite and AAPH peroxyl radical of curcumin by microwave radiation were also evaluated. The

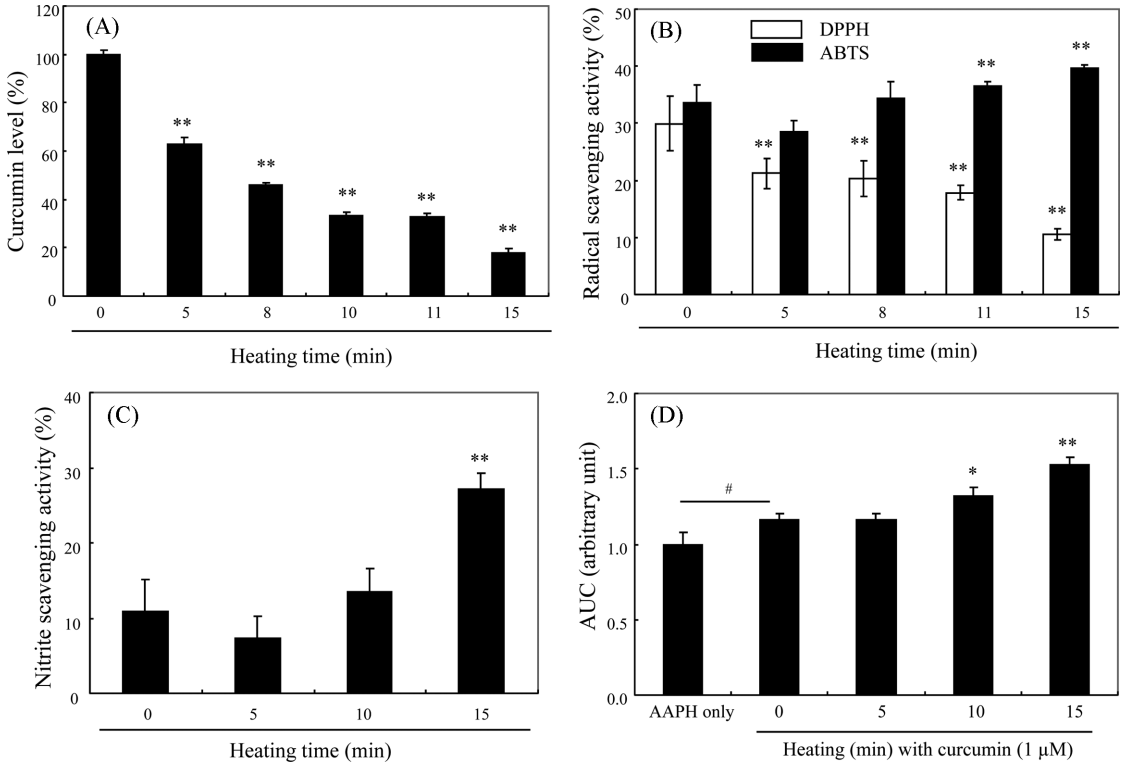

Fig. 5. Changes in stability and anti-oxidant activity of curcumin by the regular heating treatment. Curcumin in DW (40 μM) was heated at 95°C for indicated time periods. After treatment, residual curcumin level (A), DPPH and ABTS radicals (B), and nitrite (C) scavenging activities were analyzed. AUC of the fluorescence changes for evaluating AAPH radical scavenging activity was also analyzed (D). Each value represents the mean±SD (n=4-8). *,** Significantly different from control according to Student's t-test (*p<0.05, **p<0.01). #Significantly different from control without curcumin according to Student's t-test (#p<0.05 in D).

scavenging activity of curcumin against nitrite was enhanced according to the increase in radiation intensity; curcumin treated with 500 W increased the activity more than 100% as compared to that of untreated control (Fig. 4A). AAPH radical scavenging activity of curcumin also increased significantly as the radiation intensity increased. AAPH radical generated in a reaction mixture decreased the fluorescence intensity of fluorescein; the decrease of fluorescence was impeded in the presence of curcumin and microwave-treated curcumin (Fig. 4B). Curcumin treated with higher intensity of microwave radiation delayed the fluorescence decrease more effectively. AUC of the fluorescence change in the presence of curcumin were extended by 17%; the AUC was further increased by 51% with curcumin treated with microwave radiation at 500 W for 5 min (Fig. 4C).

The present results indicate that microwave radiation caused chemical degradation of curcumin, but certain anti-oxidant activities were rather enhanced. Scavenging activities of ABTS and AAPH radicals and nitrite were enhanced as radiation time and the power intensity increased, whereas that of DPPH radical was reduced by microwave treatment. Curcumin is poorly soluble in an aqueous system. It was, however, reported that heating treatment caused thermal degradation of curcumin and increased curcumin solubility in water (29). Accordingly, the increased anti-oxidant activity observed in the present study might be attributable to the improved curcumin solubility in aqueous system by microwave heating. On the contrary, DPPH radical scavenging assay was performed in an organic solvent system that could be a more favorable condition for lipophilic anti-oxidants; microwave radiation might degrade curcumin to more hydrophilic products and result in decreased potency in the scavenging activity of DPPH radical.

Comparison with a regular heating process As microwave radiation resulted in thermal degradation of curcumin and enhancement of certain bioactivities, it was investigated whether a regular heating process caused a similar phenomenon. Curcumin in an aqueous solution was heated at 95°C on a block heater, and its decomposition was analyzed at different time points. The regular heating process through conduction and convection also caused time-dependent reduction of curcumin stability (Fig. 5A); heating for 11 and 15 min at 95°C resulted in 67 and 82% of curcumin degradation, respectively; these were comparable with 3 and 5 min microwave heating in our experimental system. Curcumin treated under the regular heating process also showed a similar pattern of anti-oxidant activity changes; scavenging activity against DPPH radical turned out to become less potent but that of ABTS radical was enhanced with 11 min heating (Fig. 5B). The heating process also significantly

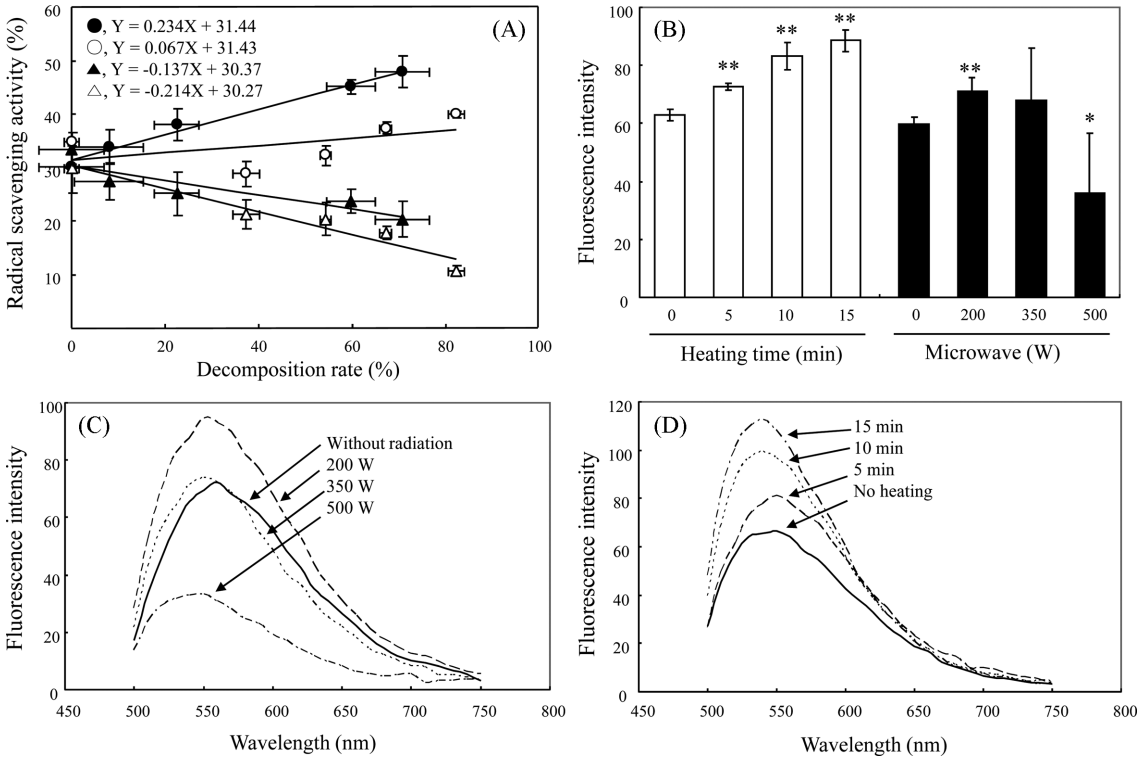

Fig. 6. Correlation between anti-oxidant activities and decomposition rates of curcumin, and changes in curcumin fluorescence properties by two heating methods. Linear correlation between degree of curcumin decomposition by microwave radiation (filled) or regular heating (hollow) and DPPH (triangle) or ABTS (circle) radical scavenging activities at the corresponding stages was performed (A). Heating for a different time at 95°C or microwave radiation (5 min) with different radiation intensity was performed on curcumin (40 μM), and changes in fluorescence intensity (B), fluorescence spectrum of microwave (C), and regular heating (D) samples were analyzed. Each value represents the mean±SD (n=5-10). *,** Significantly different from fresh control according to Student's t-test (*p<0.05, **p<0.01).

augmented scavenging activities of nitrite at 15 min and AAPH radical at 10 and 15 min heating (p<0.05) (Fig. 5C and 5D). These results caused by the regular heating process were consistent with those observed in microwave heating. It was, however, noticed that the degree of bioactivity changes was somewhat different between two heating methods even at a similar decomposition status. The enhancement of anti-oxidant activities of curcumin by microwave radiation appeared to be more pronounced than that by regular heating.

To compare the patterns of bioactivity changes by the two different heating processes, a correlation between curcumin decomposition rate (%) and anti-oxidant activities at the decomposition status (%) was performed (Fig. 6A). The scavenging activity of ABTS radical of curcumin showed a linear correlation with the decomposition rate by microwave radiation (R^2=0.98), whereas the scavenging activity of DPPH radical was inversely related with the rate of curcumin degradation (R^2=0.80). The enhancement of ABTS radical scavenging activity of curcumin treated by the regular heating was not exactly correlated to its decomposition rate (R^2=0.24), but inverse correlation between DPPH radical scavenging activity and curcumin decomposition was observed (R^2=0.94) (Fig. 6A). Interestingly, augmentation of ABTS radical scavenging activity by microwave heating was much more prominent than that by the regular heating; it was represented by a markedly higher slope (0.234 for microwave vs. 0.067 for regular heating). Yet, the decrease in the scavenging activity of DPPH radical of microwave-treated curcumin was less prominent as compared with curcumin treated with regular heating (0.137 vs. 0.214). Additionally, absorbance and fluorescence spectrums of curcumin treated with the two different heating processes were also compared. Changes in absorbance spectrum pattern of curcumin by the regular heating treatment were similar to those by microwave heating shown in Fig. 1C (data not shown). Changes in fluorescence intensity of curcumin treated with different heating methods, however, showed a quite different aspect (Fig. 6B). Fluorescence intensity of curcumin increased time-dependently when treated by the regular heating method; fluorescence intensity of microwave-treated curcumin showed a biphasic pattern that increased at lower microwave intensity but decreased at a higher intensity. Changes in fluorescence spectrum of curcumin also showed a gradually-increased pattern by the regular heating but irregular changes by microwave radiation; no substantial change was observed in the overall shape and peak wavelength (Fig. 6C and 6D).

The results suggest that the two heating processes produce different thermal degradation products and result in different

bioactivities. It is reported that major decomposition products of curcumin include ferulic acid, feruloyl methane, vanillin, vanillic acid, *p*-hydroxybenzaldehyde, and *p*-hydroxybenzoic acid that are generally more hydrophilic compounds with lower molecular weight (30,31). These products might exhibit more active anti-oxidant action in aqueous system showing the stronger scavenging activities against ABTS and AAPH radicals and nitrite but a decreased potency in DPPH radical scavenging action. The present results indicate that microwave heating enhanced these anti-oxidant activities of curcumin with higher potency as compared to the regular heating, suggesting that different profiles of thermal products were produced from curcumin under microwave radiation. It was reported that poor water solubility is one of the major limiting factors for bioavailability and bioactivity of curcumin in body, and a heat treatment could improve solubility and pharmacological efficacy of curcumin (29). Our results also suggest that microwave radiation might be a more favorable heating method for curcumin than other conventional heating methods. The precise chemical nature of thermal degradation products of curcumin by microwave radiation and the optimum microwaving conditions need to be explored further.

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Disclosure The authors declare no conflict of interest.

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