

Effect of Surface Dielectric Barrier Discharge on the Physiological Activities of Quercetin

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Abstract

In this study, using the surface dielectric barrier discharge (DBD) produced at atmospheric pressure to improve the physiological activities of quercetin was investigated. Quercetin (at 200 ppm) was treated using air DBD with an input power of 250 W. The tyrosinase inhibition effect and total phenolic content of quercetin increased from 38.96 to 91.58% and from 134.53 to 152.93 ppm, respectively, after 20 min of plasma treatment. The antioxidant activity of quercetin treated for 20 min in the lipid models was higher than that of quercetin treated for 0, 5, and 10 min. Furthermore, plasma-treated quercetin exhibited antimicrobial activity against *Listeria monocytogenes*, *Salmonella* Typhimurium, and *Staphylococcus aureus*, whereas activity was not shown in the control. Structural modifications of the quercetin molecule induced by plasma might be responsible for the improvements in its physiological activity. These results indicate that DBD plasma could be used to enhance the physiological activity of quercetin for various applications in food.

Key words: dielectric barrier discharge, atmospheric pressure plasma, quercetin, physiological activity

Introduction

Quercetin, one of the major dietary flavonoids, is widely distributed in plants and vegetables (Cook & Samman, 1996). Chemically, quercetin consists of two aromatic rings A and B linked by an oxygen-containing heterocycle (ring C). The multiple hydroxyl groups in the molecular structure of quercetin may play crucial roles in its widely demonstrated biological properties such as antioxidant, antiviral, and anticancer activities (Mendoza & Burd, 2011). However, the use of quercetin as a health-promoting agent has been limited by its poor bioavailability, which is caused by its low water solubility and high instability in neutral and alkaline media as well as in the small intestine, colon, liver, and kidney (Guo & Bruno, 2015). Many studies have proved that quercetin can bind with animal proteins such

as bovine serum albumin (Fang et al., 2011), collagen (Yang et al., 2009), and β -casein (Mehranfar et al., 2013), which may promote the solubility, stability, and bioavailability of quercetin. However, these techniques are inefficient and costly.

Plasma generates various species including photons, electrons, positive and negative ions, and neutral particles as well as reactive species with sufficient energy to break covalent bonds and induce numerous chemical reactions (Laroussi M, 2002; Yong et al., 2015). Low-temperature atmospheric pressure plasmas have attracted increasing interest in a variety of applications. The operative and configuration conditions of atmospheric plasma generator inactivation have been extensively reviewed (Moreau et al., 2008). Reactive oxygen and nitrogen species produced by plasmas can cause peroxidation of lipids and oxidation of protein and DNA (Montie et al., 2000).

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Recently, atmospheric pressure plasma has been continuously utilized and optimized for application in the food processing industries. The oxidative power of plasma was evaluated in order to preserve the qualitative characteristics of fresh-cut fruits and vegetables (Ramazzina et al., 2015), cheese (Yong et al., 2015), and raw meat (Jayasena et al., 2015). The technology also applied for meat curing process as nitrite source without external addition (Jung et al., 2015a; Jung et al., 2015b). In addition, dielectric barrier discharge (DBD) treatment improved the biological activity of naringin (Kim et al., 2014; Kim et al., 2015). In particular, preliminary data indicated that the water solubility index of quercetin was increased from 6.36 to 35.94% upon plasma treatment (data not shown). Therefore, the objective of this study was to evaluate the changes in the physiological activity of quercetin solution by encapsulated DBD at atmospheric pressure.

Materials and Methods

1. Sample and plasma treatment and visible emission spectrum

Quercetin was purchased from Sigma Co. (St. Louis, MO, USA). A 200 ppm quercetin solution in methanol was prepared for each experiment and diluted before use. The plasma apparatus was used by Kim et al. (2014). The DBD actuator, which consisted of strip electrodes and a 280 μm -thick polytetrafluoroethylene sheet, was installed in the inner wall of the airtight plastic container ($137 \times 104 \times 53 \text{ mm}^3$). One electrode, which was buried as seen in Fig. 1, was connected to the power supply (bipolar square-waveform voltage at 15 kHz) while the 10 mm-wide electrode, spaced 5 mm apart, was grounded. The air surface discharge was generated inside the container with an input power of 250 W. Quercetin samples were placed in a glass dish

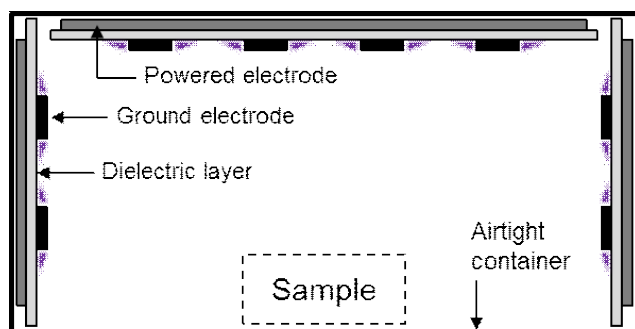


Fig. 1. Schematic cross-section of the DBD system for quercetin treatment.

at the bottom of the container and treated for 0, 5, 10, and 20 min. The visible emission spectrum of the discharge was obtained through spectrometers (Acton SP-750i) with the relevant optical setups.

2. Lipid oxidation

Oil emulsion was prepared for the 2-thiobarbituric acid reactive substances (TBARS) measurement. Soybean oil (1%) was homogenized (T10 basic, Ika Works, Germany) in double-distilled water (DDW) at 23,000 rpm for 2 min with 200 μL of quercetin solution. Tween 20 (100 μL) was added as an emulsifier. Lipid oxidation was measured by TBARS using a spectrophotometer (X-ma 1000, Human Corp., Korea) (Jo et al., 2002). The TBARS values were expressed as micrograms of malondialdehyde per gram of sample.

3. Total phenolic content

The total phenolic content of the samples was estimated colorimetrically using the Folin-Ciocalteu method (Subramanian et al., 1965). Samples (0.1 mL) were added to the Folin-Ciocalteu reagent (0.2 mL), and 3 mL of 5% sodium carbonate solution was added. The reaction samples were mixed thoroughly by vortexing and after incubating the mixtures for 2 h at room temperature, the absorbance at 765 nm was measured using a spectrophotometer (X-ma 1000, Human Corp., Korea). The results were quantified based on a standard curve generated using gallic acid.

4. Tyrosinase inhibition effect

The tyrosinase inhibition effect was observed spectrophotometrically by monitoring the increase in the absorbance caused by the production of dopachrome from L-3,4-dihydroxyphenylalanine (L-DOPA) (Kim et al., 2014). Samples (0.2 mL) were added to 0.4 mL of the reaction mixture containing 10 mM L-DOPA (Sigma), 0.1 M potassium phosphate buffer (pH 6.8), and mushroom tyrosinase (100 U/mL, Sigma). Methanol was used as the control. The reaction mixture was incubated at 25°C for 15 min. The amount of dopachrome produced in the reaction mixture was measured at 475 nm using a spectrophotometer (X-ma 1000, Human Corp., Korea). The tyrosinase inhibition effect (%) was calculated as follows:

$$\text{Tyrosinase inhibition effect (\%)} = [1 - (A_1/A_0)] \times 100$$

where A_0 is the absorbance of the control solution and A_1 is the absorbance of the sample.

5. Antimicrobial activity

1) Test microorganisms

Listeria monocytogenes (KCTC 3569), *Salmonella* Typhimurium (KCTC 1925), and *Staphylococcus aureus* (KCTC 1916) were obtained from the Korean Collection for Type Culture (KCTC, Daejeon, Korea). *S. aureus* was cultivated in tryptic soy broth. *L. monocytogenes* and *S. Typhimurium* were cultivated in tryptic soy broth containing 0.6% yeast extract and nutrient broth, respectively. Sterilized broths were inoculated using agar slant cultures of the microorganisms, and after incubating for 24 h at 37°C, 0.1 mL of culture was transferred to new broth and cultivated for 17 h. The cultures were centrifuged at 3,000 rpm for 15 min at 4°C in a refrigerated centrifuge (UNION 32R, Hanil Science Industrial, Co., Ltd., Korea). Culture pellets were washed twice with sterile saline solution and the pellets were re-suspended in sterile saline solution at a final cell density of $10^7 - 10^8$ CFU/mL.

2) Paper disc diffusion assay

The antimicrobial activity was determined by the paper disc diffusion assay. Briefly, 0.1-mL aliquots of the test organisms were spread over the surface of agar plates. Sterilized filter-paper discs were saturated with 50 μ L of each of the samples (filtered using 0.22- μ m syringe filters) and incubated for 48 h, after which the diameters of the microbe-free clear zones around the disc on the culture plates were measured. The control sample was prepared using methanol.

6. High performance liquid chromatography (HPLC) analysis

An Agilent HPLC system 1100 equipped with a diode-array detector was used for the chromatographic separation of quercetin subjected to the plasma treatment. HPLC analysis was carried out on a YMC-Pack ODS A-302 column (4.6 mm i.d. \times 150 mm; YMC Co., Ltd.) and was developed at 40°C with 1% HCOOH/MeCN (82:18, flow rate: 1.0/min, detection: 254 nm).

7. Statistical analysis

Data were analyzed using SAS software (Release 8.01, SAS Institute, Inc., Cary, NC, USA). Statistical analysis was per-

formed using one-way analysis of variance. When significant differences were detected, the differences among the mean values were determined by Duncan's multiple-comparison test at a confidence level of $p < 0.05$. Mean values and standard errors of the mean are reported.

Results and Discussion

1. Visible emission spectrum from the plasma

Optical emission spectrum of the DBD plasma obtained by optimal spectroscopy (SP2750, Princeton Instruments) is shown in Fig. 2. Due to the air discharge, strong nitrogen lines, $N_2(C^3\Pi_u-B^3\Pi_g)$, $N_2^+(B^2\Sigma_u^+-X^2\Sigma_g^+)$ and $NO_v(A_2\Sigma^+-X_2\Pi)$, are observed. RONS such as N_2^+ and NO_v , are considered to be responsible for quercetin activation.

2. Lipid oxidation and total phenolic content

The TBARS value of oil emulsion containing 200 μ L of quercetin solution was higher than that of the control without quercetin added (Table 1). In particular, quercetin solution treated for 20 min has a significantly lower TBARS value than that of samples treated for 0, 5, and 10 min. Plasma treatment of quercetin decreased the lipid oxidation during storage. This phenomenon is explained by free radical production by molecules, which can be decreased by the treatment (Fornes et al., 1993). One of the most important components in natural substances is polyphenol. The content of total phenolic compounds in quercetin solution was 180.67 ppm. After quercetin was treated by DBD for 20 min, the concentration of phenolic contents increased to

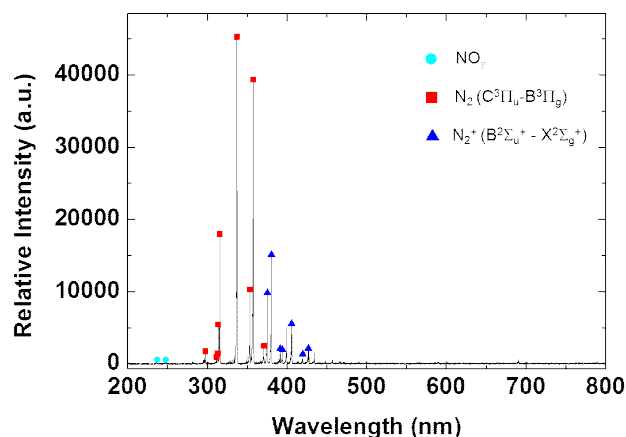


Fig. 2. A typical emission spectrum emitted by the air discharge plasma. NO, N_2 , N_2^+ molecular spectra were observed.

Table 1. 2-Thiobarbituric acid reactive substances (TBARS) values (μg malondialdehyde/g) of oil emulsion containing plasma-treated quercetin during storage at 37°C

| Treatment time (min) | Storage time (hr) | | | SEM ¹⁾ |
|----------------------|-------------------|---------------------|----------------------|-------------------|
| | 0 | 1.5 | 3 | |
| Methanol | 0.59 ^b | 0.85 ^{bx} | 1.34 ^{ax} | 0.086 |
| 0 | 0.59 ^b | 0.71 ^{bxy} | 1.30 ^{axy} | 0.058 |
| 5 | 0.58 ^c | 0.72 ^{bxy} | 1.19 ^{axyz} | 0.026 |
| 10 | 0.58 ^b | 0.63 ^{by} | 1.03 ^{ayz} | 0.041 |
| 20 | 0.60 ^b | 0.58 ^p | 0.94 ^{az} | 0.058 |
| SEM ²⁾ | 0.010 | 0.053 | 0.083 | |

¹⁾ Standard error of the mean (n=9), ²⁾(n=15)

^{a-c} Values with different letters within the same row differ significantly ($p<0.05$)

^{x-z} Values with different letters within the same column differ significantly ($p<0.05$)

195.17 ppm (Table 2). The increase in phenolic content is consistent with the result reported by previous study (Kim et al., 2014).

Various studies have reported a correlation between phenolic content and antioxidant activity. Liu et al. (2012) determined that UV-C irradiation tended to increase the antioxidant activity and phenolics of quercetin in tomato fruits. Fan et al. (2003) showed that the phenolic content and antioxidant activity in lettuce exhibited a high response to irradiation.

The increased total phenolic content could be attributed to the release of phenolic compounds from the glycosidic components and the degradation of larger phenolic compounds into smaller ones by irradiation, with a consequent improvement in the extraction yield of the phenolic compounds as a result of the

Table 2. Total phenolic content and tyrosinase inhibition effect of quercetin treated by plasma

| Treatment time (min) | Total phenolic content (ppm) | Tyrosinase inhibition effect (%) |
|----------------------|------------------------------|----------------------------------|
| 0 | 180.67 ^a | 12.49 ^a |
| 5 | 182.33 ^{ab} | 29.91 ^b |
| 10 | 185.00 ^b | 53.18 ^c |
| 20 | 195.17 ^c | 78.34 ^d |
| SEM ¹⁾ | 0.050 | 0.104 |

¹⁾ Standard error of the mean (n=12)

^{a-d} Values with different letters within the same column differ significantly ($p<0.05$)

change in the tissue structure of samples by irradiation (Choi et al., 2009; Yang et al., 2012). Another reason for the increase by plasma treatment might be the breakage of multimeric polyphenols. It had been reported that water molecules were dissociated into hydroxyl radicals and peroxides by plasma treatment. These radicals easily broke the glycosidic bonds and covalent linkage in the large molecules and made smaller ones (Choi et al., 2010). Recently, Kim et al. (2015) showed that the new flavanone glycoside, naringin A, containing a methoxyalkyl group exhibited significantly improved antioxidant properties of naringin with DBD.

3. Tyrosinase inhibition effect

Because tyrosinase is responsible for the biosynthesis of pigment melanin in human skin, tyrosinase inhibition effect is an important standard for the application in the cosmetics industry due to its skin-whitening effect (Wu & Park, 2003). The original tyrosinase inhibition effect of quercetin was 12.49%, and increased to 78.34% after DBD plasma treatment for 20 min (Table 2). Kim et al. (2015) also demonstrated that the tyrosinase inhibition effect of naringin increased from 6.12% to 83.30% upon plasma treatment.

Other results for the tyrosinase inhibition effect were reported in Japanese honeysuckle and green tea byproducts treated by irradiation (Byun et al., 2004; Lee et al., 2006). Phenolic compounds, such as flavonoids, tannins and lignin, are mainly responsible for the tyrosinase inhibition (Kim et al., 2009). Based on our earlier results of flavonoid content, increased tyrosinase inhibition could be due to increased levels of flavonoids derived from the breakdown of Cya-3-O-xylrut that particularly affects tyrosinase inhibition levels (Lee et al., 2011).

4. Antimicrobial effect

The growth inhibition zones of quercetin treated by plasma against foodborne pathogens were evaluated, as shown in Table 3. Results were obtained from the disc diffusion method. Quercetin did not initially exhibit any antimicrobial activity; however, antimicrobial activity was increased with increasing plasma treatment time. The diameters of clear zones were 24, 21, and 15 mm against *S. Typhimurium*, *S. aureus*, and *L. monocytogenes*, respectively, after 20 min of plasma treatment of quercetin. Kim et al. (2015) showed that naringin treated with DBD exhibited antimicrobial activity against foodborne pathogens, especially *S. Typhimurium*. Phenolics were the most important active compounds against bacteria control (Shoko et al., 1999). The chemi-

Table 3. Antimicrobial activity of quercetin of treated by plasma, measured using the paper diffusion method

| Pathogens | Diameter (mm) | | | | |
|---|-----------------|-------|-------|------------------|--------|
| | Methanol | 0 min | 5 min | 10 min | 20 min |
| <i>Salmonella</i> Typhimurium | - ¹⁾ | - | - | 16 ²⁾ | 24 |
| <i>Listeria</i> <i>monocytogenes</i> | - | - | - | 12 | 15 |
| <i>Staphylococcus</i> <i>aureus</i> | - | - | - | - | 21 |

¹⁾ No inhibition.

²⁾ Clear-zone diameter (disc diameter=8 mm)

cal structure of irradiated phenolics might not have changed, but the activity of the functional groups, such as -OH or -COOH, may change, resulting in higher antimicrobial activity (An et al., 2004).

5. HPLC analysis

The HPLC chromatograms of quercetin before and after plasma treatment (Fig. 3) showed that plasma treatment degraded quercetin, and that the degradation rate and extent increased when the treatment time was extended to 20 min. Considering the peak area of non-treated quercetin to be 100%, only 33.37% of quercetin was present after plasma treatment for 20 min, where as 5 new peaks were detected except for peak area of < 1% (Table 4). This phenomenon implies that quercetin is physically/chemically trapped by other components.

Bactericidal agents in plasmas are known to contain reactive oxygen species (ROS), UV photons, energetic ions, and electrons. The ROS generated by air-based plasmas have been identified as ozone, atomic oxygen, superoxide, peroxide, and hydroxyl radicals (Gaunt et al., 2006). Yang et al. (2012) showed that the ROS and molecules produced by irradiation are obviously capa-

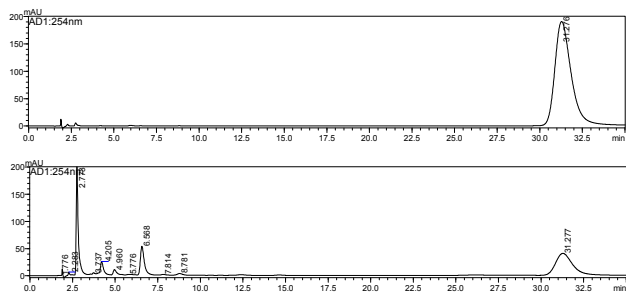


Fig. 3. HPLC chromatograms of quercetin treated by plasma. (a) control, (b) sample treated for 20 min.

Table 4. Relative peak area percentage of compounds generated from quercetin after plasma treatment.

| Retention time (min) | Peak area (%) |
|----------------------|---------------|
| 2.778 | 29.03 |
| 4.205 | 4.52 |
| 4.960 | 2.36 |
| 6.568 | 14.35 |
| 8.781 | 1.2 |
| 31.277 | 48.54 |
| Total | 100 |

ble of producing soluble quercetin, resulting in a high extraction efficiency of all phenolic compounds in onion.

Conclusion

Form the results, the physiological activities of quercetin including its antioxidant activity, tyrosinase inhibition effect, and antimicrobial activity can be improved by air DBD. Recent studies revealed that plasma-treated chicken breast and plasma-cured sausage did not show genotoxicity (Lee et al., 2016) and immune toxicity (Kim et al., 2016), respectively. These results indicate that quercetin activated by atmospheric pressure plasmas could be used as a functional material for various applications in food.

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