DEVELOPMENT OF NATURAL NITRITE SOURCE BY ATMOSPHERIC PRESSURE PLASMA

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Abstract − **This study investigated the effect of atmospheric pressure plasma (APP) treatment on the nitrite content and functionality of plant extracts. Ethanolic extracts of *Perilla frutescens* (EEP) were prepared and treated with APP for 60 min. Lyophilized EEP (LEEP) treated with APP for 60 min contained 3.74 mg/g nitrite. The control (LEEP without APP) contained no nitrite. The minimum inhibitory concentration (MIC) of LEEP for *C. perfringens* was 200 μg/mL. The control did not inhibit *C. perfringens* growth between 25 and 1000 μg/mL. MICs of LEEP and the control against *S.* Typhimurium were 25 and 50 μg/mL, respectively. New nitrite sources with increased antimicrobial activity can be produced from natural plants by APP treatment.**

Key Words – antimicrobial activity, nitrite, plant extract

I. INTRODUCTION

Nitrite has multi-functional roles in meat products. Recently, an interest of natural nitrite source is increased because an increase in consumer concern regarding the use of chemically synthetic compounds. Natural nitrite sources can be derived from natural plants that contain nitrate (NO_3^-). However, natural plants that do not contain nitrate are not candidate natural nitrite sources, even though they have strong antimicrobial and antioxidative activities. Plasma is an ionized gas that can be generated by supplying energy to gas. Discharges in the gas phase include reactive oxygen and nitrogen species [1]. In the gas-liquid environment, discharges in the gas phase can penetrate into liquid and cause various chemical processes. A previous study reported that water treated with plasma showed antimicrobial activity with the generation of various nitrogen compounds including nitrite as well as other species in water [2]. In addition, the increase of antioxidative and antimicrobial activity of flavonoid with atmospheric pressure plasma (APP) treatment was reported [3]. We hypothesized that novel sources of nitrite derived from a natural plant can be produced. Through such a process, an increase in the nitrite level of plant extracts can be expected following plasma treatment regardless of their inherent nitrate level, which may be accompanied by an increase in the antioxidative and antimicrobial activity of plant extracts. Therefore, the aim of this study was to investigate the nitrite level and antimicrobial activity of plant extracts following treatment with atmospheric pressure dielectric barrier discharge (DBD) plasma.

II. MATERIALS AND METHODS

Ethanolic extracts of red perilla leaves (EEP) were prepared and then subjected to atmospheric pressure plasma treatment (APP). EEP treated with APP for 60 min was lyophilized. The nitrite content in lyophilized EEP (LEEP) was measured by HPLC. The total phenolic content and DPPH radical scavenging activity of LEEP were evaluated as antioxidative activity. The antimicrobial activity of LEEP against *S.* Typhimurium (SL 1344), and *C. perfringens* (NCTC 8239) was measured by a minimum inhibitory concentration (MIC) assay. All experiments in this study were performed in triplicate. A general linear model was generated using the raw data, and Tukey's multiple range test was used to compare significant differences between least square mean values ($p < 0.05$).

III. RESULTS AND DISCUSSION

LEEP contained as much as 3.74 mg/g nitrite, while the control contained no nitrite (Table 1). Various studies have reported an increase in the nitrite content in liquid following APP treatment under atmospheric air conditions [1, 4, 5]. Nitrite can be formed in liquid by post-discharge reactions with APP treatment. Nitrogen oxides from gas-phase discharges diffuse into liquid and react with water molecules, producing nitrites. There was no significant difference in the total phenolic content and EC_{50} value of DPPH radical scavenging between the control and LEEP. *C. perfringens*

growth was not significantly inhibited following control treatment from 25 to 1000 μg/mL. However, the MIC of LEEP for *C. perfringens* was 200 μg/mL. In addition, the MIC of LEEP for *S.* Typhimurium was 25 μg/mL, while that of the control was 50 μg/mL. Previous studies have shown increased antimicrobial activity of liquid by APP treatment [1, 5]. Plant extracts contain various compounds, including phenolic acid and flavonoids. Therefore, the synergistic effect of different compounds in plant extracts with substances generated by APP treatment could also be responsible for the increased antimicrobial activity.

Table 1. Properties of lyophilized ethanolic extracts from red perilla leaf (LEEP) following treatment with atmospheric pressure plasma

	Control[1]	LEEP[2]	SEM[3]
Nitrite content (mg/g)	-[b]	3.74[a]	0.019
Antioxidative activity			
Total phenolic content (mg/g)	160.6	157.3	4.86
EC_{50} value of DPPH radical scavenging (μg/mL)	275.3	278.3	4.20
Antimicrobial activity			
MIC[4] for *C. perfringens*	-[5]	200	
MIC for *S.* Typhimurium	50	25	

[1]Lyophilized ethanolic extract from red perilla leaf without treatment with atmospheric pressure plasma
[2]Lyophilized ethanolic extract from red perilla leaf following treatment with atmospheric pressure plasma for 60 min
[3]Standard error of the mean (n = 6)
[4]Minimum inhibitory concentration that significantly inhibited bacterial growth
[5]No inhibition was found at concentrations from 25 to 1000 μg/mL
[a,b]Different letters within the same column represent significant differences ($p < 0.05$)

IV. CONCLUSION

The APP treatment of plant extract resulted in the generation of nitrite and an increase in the antimicrobial activity of powdered plant extract. Therefore, the production of a new nitrite source from natural plants, regardless of their inherent nitrate level, is possible with APP treatment. In addition, natural plants have strong antioxidative and antimicrobial activity and may be good candidates as new nitrite sources following an increase in antimicrobial activity by APP treatment.

ACKNOWLEDGEMENTS

This study was financially supported by research project (PJ012254) for Development of Advanced Core Technology for Agriculture, Rural Development Administration, Republic of Korea.

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