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Determination of Formaldehyde in Cigarette Smoke and Inhibitory Effect of Plant Volatile Extracts on the Formation of Formaldehyde

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Abstract Formaldehyde (FA) is a carcinogenic compound present in cigarette smoke. In this study, the amount of formaldehyde was analyzed in 5 kinds of cigarettes and the inhibitory effect of plant volatile extracts on the formation of FA was investigated. After extraction of the cigarette sample, FA was converted into its thiazolidine derivatives by reaction with cysteamine, and then measured using a gas chromatography-nitrogen phosphorus detector (GC-NPD). The concentrations of FA in cigarette smoke were found between 138.24 and 217.82 μmol/g cigarette smoke. Extracts isolated from Welsh onion (*Allium cepa* L.), garlic (*Allium sativum* L.), crown daisy (*Chrysanthemum coronarium* L.), green pepper (*Capsicum annuum* L.), and sesame dropwort (*Oenanthe javanica* DC) were used for analyzing their inhibitory effects on the formation of FA. The inhibitory effects of extracts of Welsh onion, garlic, crown daisy, green pepper, and sesame dropwort on the formation of FA were 64, 47, 38, 47, and 19%, respectively.

Keywords: formaldehyde, thiazolidine derivative, gas chromatography-nitrogen phosphorus detector, aroma extract

Introduction

Formaldehyde (FA) is highly irritating to the eye, dermatitis, asthma, respiratory, and pulmonary edema (1). It is a gaseous substance (boiling point=−19°C) with a pungent odor. FA has been classified as a human carcinogen (Group 1) by the International Agency for Research on Cancer (IARC) and as a probable human carcinogen by the U.S. Environmental Protection Agency (USEPA). Large amounts of FA are emitted from paper products, furniture, building material, permanent-press fabrics, and some cosmetics (2). FA can directly cross-link to different macromolecular substances, such as amino acids, proteins, and nucleic acids (3), and consequently cause biological complications including carcinogenesis (4).

Because of the increasing concerns on the levels of FA in the environments, efforts to develop simple and reliable methods of quantitating FA have been generated. Colorimetry is the most common method for FA determination. FA is derivatized to form colored products or chromophores and quantitation of these products is based on the absorption of the chromophores at specific wavelengths. A major drawback of colorimetry is that other compounds that absorb at the wavelength of interest can interfere with the quantitation process (1,5,6).

Chromatographic methods have been extensively used for FA analysis. These methods commonly involve derivatization of FA with reagents such as cysteamine, 2,4-dinitrophenyl-hydrazine (2,4-DNP), semicarbazide, hydroxylamine, or dansyl hydrazine. The resulting derivatives of these reagents and FA can be analyzed by gas chromatography (GC) or liquid chromatography (LC) (4,7). FA and most carbonyl compounds react readily with cysteamine under moderately basic conditions at pH 8 and 25°C to form thiazolidine as shown in Fig. 1. Thiazolidine derivatives are more stable and less volatile (boiling point=164°C) than FA but sufficiently volatile for GC (7). A highly sensitive and specific nitrogen-phosphorus detector (NPD) or flame photometric detector (FPD) in sulfur mode can be used due to the presence of nitrogen or sulfur in the compound. An advantage of the GC-NPD or GC-FPD method for thiazolidine analysis is the absence of interference by common solvent contaminants, which can occur with other detectors (4).

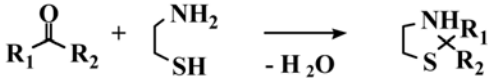

Fig. 1. Derivatization of carbonyl (FA, R_1=R_2=H, M_w=30) with cysteamine to form alkylthiazolidine (thiazolidine, R_1=R_2=H, M_w=89).

It has been suggested that diets rich in vegetables have therapeutic effects on anti-hepatotoxic, anti-inflammatory, antioxidant, and anti-carcinogenic activity. The inhibition of malonaldehyde (MA) formation by aroma extracts and aroma components isolated from clove and eucalyptus has previously been reported (8). The extracts of eucalyptus and clove effectively inhibited MA formation (8). Plant volatiles containing antioxidant compounds such as phenolic compounds, carotenoids, flavonoids, phenolic acid, tocopherols, and ascorbic acid decreased the level of carcinogenic substances such as aldehydes including MA and FA (8-11). Welsh onion, garlic, crown daisy, green pepper, and sesame dropwort have their own aroma (11) and are important sources of flavors in Asian cuisine (12).

The purpose of this study was to quantify FA in 5 kinds of cigarette smoke by applying derivatization, extraction, and gas chromatographic analysis of FA. In addition, we examined the inhibitory effects of plant volatile extracts on the formation of FA.

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Materials and Methods

Materials Welsh onion (*Allium cepa* L.), garlic (*Allium sativum* L.), crown daisy (*Chrylsanthemum coronarium* L.), green pepper (*Capsicum annuum* L.), and sesame dropwort (*Oenanthe javanica* DC) were grown and harvested at Dongguk University Farm located in Goyang, Gyeonggi, Korea (2007). Three kinds of domestic cigarettes and 2 imported cigarettes were purchased from a local retail shop.

Reagents Cysteamine hydrochloride, thiazolidine, and 2,4,5-trimethylthiazole were purchased from Sigma-Aldrich (St. Louis, MO, USA). Dichloromethane and sodium sulfate were purchased from Junsei Co. (Tokyo, Japan). A standard stock solution of 2,4,5-trimethylthiazole was prepared by adding 10 mg of 2,4,5-trimethylthiazole to 1 mL of chloroform (4).

Sample preparations for the analysis of FA Cysteamine hydrochloride (0.7 g) was dissolved in 200 mL of deionized water and the pH of the solution was adjusted to 8 with 6 N NaOH solution. The cysteamine solution (approximately 200 mL) was placed in a separatory funnel whose headspace was evacuated at 25 mm Hg for 10-15 min. Immediately after about 1 mm of the unlit end of the cigarette was inserted into the tip of the separatory funnel, a cigarette was lit. The weight of cigarette was measured before the sample preparation and that of ash with cigarette butt was also measured to determine the weight of smoke. Most cigarettes were fitted into the tip of the funnel. Cigarettes with a diameter smaller than the inside diameter of the tip of the funnel were wrapped with masking tape in order to fit. The cock of the separatory funnel was opened gradually to draw the smoke into the separatory funnel. It took 20 sec to completely smoke one cigarette. After the smoke was sucked into the separatory funnel, the funnel was shaken for 5 min in order to let the carbonyl compounds in the smoke react thoroughly with the cysteamine. After the pH of the reaction mixture was reconstituted to 7 with a 1 M HCI solution, it was extracted with 50 mL of chloroform using a liquid-liquid continuous extractor (ACE Glassware Co., Seoul, Korea) for 3 hr. The extract was dried over anhydrous sodium sulfate for 12 hr. After removal of the sodium sulfate, the volume of the extract was adjusted to exactly 50 mL with chloroform. An 100 μL of a standard solution of 2,3,5-trimethylthiazole was added as an internal standard prior to GC analysis. The concentration of this solution was 80 μmol/mL. Sample preparation and analysis of all samples were done in triplicate (n=3). A statistical analysis was processed using Microsoft Excel T-test program.

Control blanks An aqueous solution (200 mL) containing 0.7 g of cysteamine was extracted with 50 mL chloroform at pH 8, as done for the smoke samples. This extract was used as a blank sample for the control experiment.

Preparation of volatile extracts from plants To isolate aroma compounds of plants, the 5 plants (100 g) were homogenized (Macro homogenizer, Omni International, Marietta, GA, USA) in liquid nitrogen to prevent the loss of volatiles. The ground plants were placed in a 3-L round-bottomed flask with 1 L deionized water. The solution was steam-distillate at 55°C for 4 hr under reduced pressure (95 mmHg) and we could obtain steam-distillate (about 800 mL) of each plant. Two-hundred mL of total steam distillate was used to investigate the inhibitory effects on FA formation.

Measurement of inhibitory effect of volatile extracts on the formation of FA To determine the inhibitory effect of aroma extracts on FA, cysteamine hydrochloride (0.7 g) was dissolved in 200 mL of aroma steam distillate and the pH of the solution was adjusted to 8 with 6 N NaOH solution. The cysteamine solution (approximately 200 mL) was placed in a separatory funnel whose headspace was evacuated at 25 mm Hg for 10-15 min. The method to determine FA in the solution was same as the part of 'sample preparations for the analysis of FA'. Sample preparation and analysis of all samples were done in triplicate (n=3).

Instrumental analysis An Agilent Model 6890 GC system equipped with an NPD system and a 30 m×0.35 mm i.d. DB-WAX column (J&W Scientific, Folsom, CA, USA) was used for the quantitative analysis of thiazolidines. The oven temperature was programmed from 60 to 180°C at 4°C/min and held for 2 min. The injector and detector temperatures were 250°C. The linear velocity of the helium carrier gas was 1.5 mL/min. The split ratio was 20:1 and injection volume was 1 μL. The quantitative analysis was conducted according to the internal standard method. The concentration of FA in the unknown sample was determined by the mass of thiazolidine in the sample (1 mol of FA corresponds to 1 mol thiazolidine).

Recovery test Thiazolidine (50 nmol/mL) and 2,3,5-trimethylthiazole (80 μmol/mL) were added to sample without thiazolidine according to the procedure described above. The sample was prepared and analyzed by GC-NPD as described above. Then, the recovery rate was calculated based on the following equation.

$$\% \text{ Recovery} = \frac{(\text{Conc. spiked sample} - \text{Conc. unspiked sample})}{(\text{Conc. added thiazolidine})} \times 100$$

Results and Discussion

Standard calibration curve and recovery rate The GC-NPD chromatogram of a thiazolidine standard using 2,4,5-trimethylthiazole as an internal standard is shown in Fig. 2. The retention time of thiazolidine and 2,4,5-trimethylthiazole were 10.17 and 12.83 min, respectively. A standard calibration curve was generated as shown in Fig. 3. The range of thiazolidine standards was between 25 and 100 nmol/mL. The correlation coefficient (R^2) of the standard calibration curve was 0.9936. The recovery rate of thiazolidine was 93±7.2%.

Concentration of for FA in cigarette smoke The GC-NPD chromatogram of cigarette sample is shown in Fig. 2. The concentrations of thiazolidine, a derivative of FA, are presented in Table 1. The concentrations of FA in cigarette

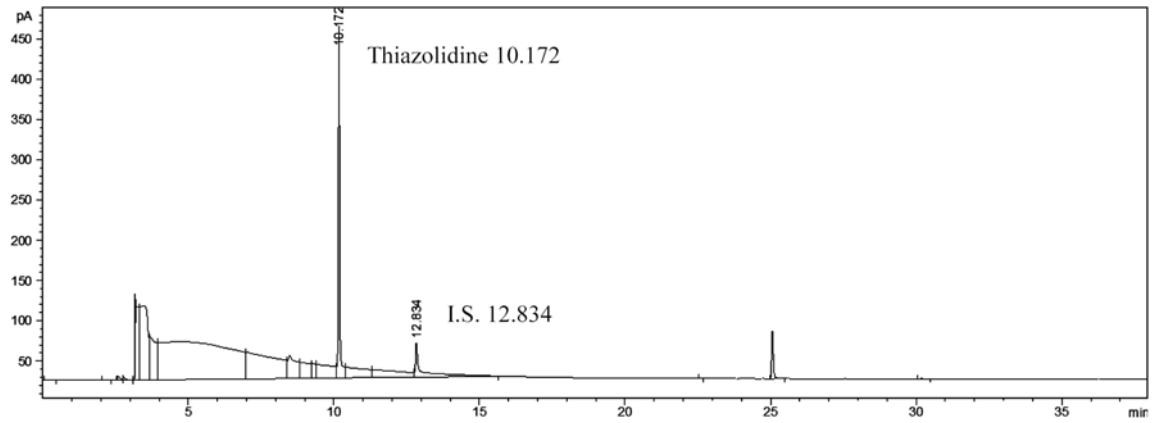

Fig. 2. A GC-NPD chromatogram of a thiazolidine (derivative of FA) standard using 2,4,5-trimethylthiazole as the internal standard.

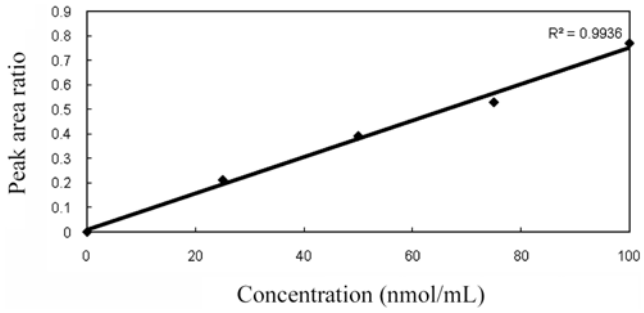

Fig. 3. Standard curve of thiazolidine (derivative of FA) through peak area ration of thiazolidine and 2,4,5-trimethylthiazole as the internal standard.

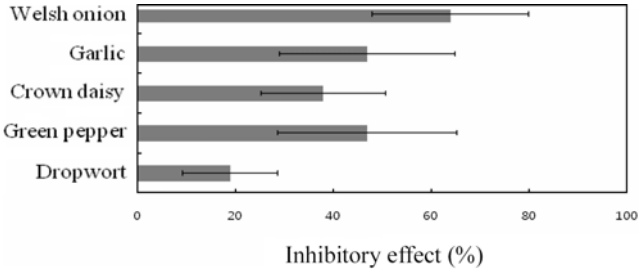

Fig. 4. Inhibitory effects (%) of various volatile extracts on the formation of formaldehyde generated from cigarette smoke.

Table 1. Levels of FA (formaldehyde) in cigarette smoke

Cigarette		Concentration (μmol/g)
Korean cigarette	A	217.8±14.0
	B	194.0±12.5
	C	141.2±3.9
Foreign cigarette	D	208.4±8.5
	E	138.2±24.1

smoke were between 217.82 and 138.24 μmol/g cigarette smoke. The concentration of FA revealed no significant difference between Korean cigarettes and imported cigarettes ($p>0.05$).

Inhibitory effect of plant volatile extracts on the formation of FA The volatile extracts of plants showed a strong antioxidant activity in the aldehyde/carboxylic acid assay and lipid/malonaldehyde (MA) assay (11,12).

The inhibitory effects of Welsh onion, garlic, crown daisy, green pepper, and sesame dropwort on the formation of FA in the smoke of Korean brand A cigarette were measured. The aroma extracts of Welsh onion inhibited the formation of FA by 64.5%, while that of garlic inhibited the formation of FA by 47%. Crown daisy, green pepper, and sesame dropwort aroma extracts inhibited the formation of FA by 38.3, 47.4, and 19.5%, respectively (Fig. 4). The inhibitory effect of Welsh onion aroma extract was comparatively higher than that of the others.

The inhibitory effect of each different volatile extracts is correlated with bioactivity of volatile chemicals in the extracts. The bioactivity could be antioxidant or antimicrobial activity. Among the identified volatile compounds of green pepper, 2-acetyl pyrrole was reported to have significant antioxidant activity (11). Yanagimoto *et al.* (13) reported that the antioxidant activities of pyrroles substituted with electron-withdrawing groups, such as acetyl, were higher than that of pyrroles substituted with electron-donating groups. Thymol was found in Welsh onion, but was also identified as one of major volatile antioxidants in eucalyptus (14). Heterocyclic compounds such as 2,4-dimethyl thiophene, 5-hydroxy methlyfurfural, and 5-methyl-2-octyl-(2H)-furan-3-one were demonstrated to have substantial antioxidant activity (13). Eugenol, which was identified in garlic in our study, has also been reported to have strong antioxidant activity (8).

In conclusion, FA in cigarette smoke was analyzed in 5 kinds of cigarettes and the inhibitory effect of several plant volatile extracts on the formation of FA was investigated. The plants commonly used in Korean food preparations, such as Welsh onion, garlic, crown daisy, green pepper, and sesame dropwort have their own aroma, and these aroma compounds inhibited varying amounts of formation of FA. Although their inhibitory effects were not very strong, their aroma extracts showed an obvious inhibitory effect on the formation of FA. Welsh onion, garlic, crown daisy, green pepper, and sesame dropwort aroma extracts inhibited the

formation of FA by 64.5, 47, 38.3, 47.4, and 19.5%, respectively. The possible inhibitory effects of more Korean plant extracts on the formation of harmful aldehydes including FA and acetaldehyde formed by cigarette smoke will be investigated as part of future studies.

Acknowledgment

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