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## Chemical Composition of the Volatile Extract and Antioxidant Activities of the Volatile and Nonvolatile Extracts of Egyptian Corn Silk (*Zea mays* L.)

Ahmed El-Ghorab,<sup>†</sup> Khaled F. El-Massry,<sup>†</sup> and Takayuki Shibamoto<sup>\*,‡</sup>

Flavor and Aroma Department, National Research Center, Dokki, Cairo, Egypt, and Department of Environmental Toxicology, University of California, Davis, California 95616

A total of 36 compounds, which comprised 99.4% of the extract, were identified by gas chromatography and mass spectrometry (GC–MS) in the volatile dichloromethane extract obtained from Egyptian corn silk. The main constituents of the volatile extract were *cis*- $\alpha$ -terpineol (24.22%), 6,11-oxidoacor-4-ene (18.06%), citronellol (16.18%), *trans*-pinocamphone (5.86%), eugenol (4.37%), neo-iso-3thujanol (2.59%), and *cis*-sabinene hydrate (2.28%). Dried Egyptian corn silk was also directly extracted with petroleum ether, ethanol, and water. All extracts from solvent extraction and the volatile extract described above exhibited clear antioxidant activities at levels of 50–400 µg/mL in the 2,2-diphenyl-1-picrylhydrazyl (DPPH)/linoleic acid assay. The ethanol extract inhibited DPPH activity by 84% at a level of 400 µg/mL. All samples tested via the  $\beta$ -carotene bleaching assay also exhibited satisfactory antioxidant activity with clear dose responses. This study indicates that corn silk could be used to produce novel natural antioxidants as well as a flavoring agent in various food products.

KEYWORDS: Corn silk; volatile extract; α-terpineol; antioxidant; 1,2-diphenyl-1-picrylhydrazyl; citronellol

### INTRODUCTION

Corn silk (Zea mays L.) refers to the stigmas from the female flowers of maize. Fresh corn silk resembles soft silk threads 10-20 cm long that are either light green or yellow-brown in color. Corn silk consists of various chemicals, including proteins, vitamins, alkaloids, tannins and mineral salts (1, 2), carbohydrates (3), steroids (4, 5), and flavonoids (6–10) as well as volatile chemicals (11–15). Corn silk has been used as a remedy for various diseases such as inflammation of the bladder and prostate as well as treatment for irritation in the urinary system. Therefore, numerous commercial products made from corn silk for medicinal use are available today (16).

There have been many reports on the biological activities of corn silk constituents, including antibiotic activity toward corn earworm by a flavone glycoside maysin (7), attractant activity toward corn earworm by volatiles (11), inhibition of IgE formation by glycoproteins (2), immune enhancement by nonstarch polysaccharides (3), resistance to insect attacks by flavones (8), anticoagulant activity by neutrosugar/aminosugar derivatives (17), and antioxidant activity by flavonoids (10).

In recent years, there has been growing interest in finding natural antioxidants, including volatile chemicals, in plants because they inhibit oxidative damage and consequently prevent diseases, such as cancer (18), atherosclerosis (19), aging (20), leukemia (21), and rheumatoid arthritis (22). Moreover, consumers' concern has come to focus on potential adverse effects of synthetic antioxidants (23).

Volatile chemicals present in natural plants have been widely used in aroma therapy since ancient times, suggesting that they have some beneficial health effects in addition to their pleasant odor. Antioxidant activities have been reported in volatile extracts obtained from various plant substances, including beans (24), clove bud (25), herbs and spices (26), bud and leaves of capers (27), and various essential oils (28).

In this study, volatile chemicals of Egyptian dried corn silk (*Z. mays* L. *Poaceae*) obtained from steam distillation followed by dichloromethane extraction were analyzed by both gas chromatography (GC) and gas chromatography and mass spectrometry (GC–MS). In addition, volatile extracts and the extracts obtained by direct extraction of the same plant with petroleum ether, ethanol, and water were tested for antioxidant activity using two testing systems.

#### MATERIALS AND METHODS

Chemicals and Plant Samples. *tert*-Butylhydroquinone (TBHQ) was purchased from Sigma Chemical Co. (St. Louis, MO). Dichloromethane and ethanol were obtained from Fisher Scientific Co., Ltd. (Fair Lawn, NJ). 1,2-Diphenylpicrylhydrazyl (DPPH) and  $\beta$ -carotene were bought from TCI AMERICA (Portland, OR). All solvents were obtained from VWR International (Brisbane, CA).

<sup>\*</sup> To whom correspondence should be addressed: Department of Environmental Toxicology, University of California, One Shields Avenue, Davis, CA 95616. Phone: (530) 752-4523. Fax: (530) 752-3394. E-mail: tshibamoto@ucdavis.edu.

<sup>&</sup>lt;sup>†</sup> National Research Center.

<sup>&</sup>lt;sup>‡</sup> University of California.

#### Antioxidants and the Composition of Corn Silk

The dried corn silk (*Z. mays* L. *Poaceae*) (yellow in color), with the Arabic name Shawashi El- Dora, was collected from a cornfield in Tersa Tukh Kalubia, Delta Middle Egypt. The collected corn silk was placed in a polyethylene bag and stored at -20 °C until it was used for the experiments.

**Preparation of the Volatile Extract.** Dried corn silk (200 g) was placed in a 3 L round-bottom flask with 1 L of deionized water. The solution was steam-distilled for 4 h. The distillate (900 mL) was extracted with 100 mL of dichloromethane using a liquid–liquid continuous extractor for 6 h. After the extract was dried over anhydrous sodium sulfate, the solvent was removed by a rotary flash evaporator. The distillation was stopped when the volume of extract was reduced to approximately 1 mL. Then the solvent was further removed under a purified nitrogen stream until the volume was reduced to 0.5 mL (1.04 g) of volatile extract. The experiment was replicated three times.

Preparations of Extracts with Petroleum Ether, Ethanol, and Water. Corn silk (200 g each) was soaked in 100 mL each of petroleum ether (bp, 40–60 °C), ethanol (95%), and purified water in three different 500 mL Erlenmeyer flasks at room temperature for 7 h. After the solvents were filtered, the petroleum ether and ethanol extracts were condensed to 3.00 and 6.80 g, respectively, by a rotary flash evaporator. The water was further removed from the water extract using a freezedryer (1.96 g). The condensed extracts were stored in the dark at -5 °C until further experiments could be conducted.

**DPPH Radical Scavenging Assay.** The radical scavenging activity of corn silk samples was measured by a previously reported method (29). The solution of DPPH in methanol ( $6 \times 10^{-5}$  M) was prepared daily, immediately before the UV measurements were taken. Various concentrations of each sample (100, 200, and 400 µg/mL) were added to a 1 mL DPPH solution. The reaction mixtures were shaken vigorously and allowed to stand for 30 min at room temperature. The absorbance of the samples was measured with a spectrophotometer at 517 nm. In this assay, TBHQ was tested for antioxidant activity by this method to validate the assay. The experiment was replicated three times.

**Determination of Antioxidant Activity by the** *β*-**Carotene Bleaching Assay.** Antioxidant activity of the samples was also examined by a *β*-carotene/linoleic acid system reported previously (*30*). Briefly, 1 mL of a *β*-carotene solution (1 mg/mL in chloroform), 40 µL of linoleic acid, and 400 µL of Tween 80 (water soluble vitamin E) were transferred to a round-bottom flask. Chloroform in the samples was evaporated off under a nitrogen stream. Then, 100 mL of distilled water was added slowly to the residue and the mixture vigorously agitated to give a stable emulsion. To an aliquot of 4.5 mL of this emulsion was added 500 µL (50–400 µg/mL) of appropriately diluted samples in a 10 mL test tube. The tubes were placed in a water bath at 50 °C, and the absorbance was measured after 120 min at 470 nm. A blank sample was prepared by adding 500 µL of distilled water to the control reaction mixtures, and the absorbance was measured immediately after preparation at 470 nm.

Analysis of Chemicals in the Volatile Extract. Volatile compounds in the volatile extracts obtained by three replicate experiments were identified by comparison with the Kovats gas chromatographic retention index I(31) and by the mass spectral fragmentation pattern of each GC component compared with those of authentic compounds and/or NIST/EPA/NIH Mass Spectral Library (NIST 05) ASCII Version. An Agilent model 6890 gas chromatograph equipped with a 30 m  $\times$  0.25 mm (inside diameter) ( $d_f = 0.25 \,\mu$ m) bonded phase DB-5 fused silica capillary column (Agilent, Folsom, CA) and a flame ionization detector (FID) was used to obtain the Kovats index, which was also compared with published data (32, 33). The oven temperature was increased from 35 to 220 °C at a rate of 3 °C/min and held for 40 min. The linear helium carrier gas flow rate was 29 cm/s. The injector temperature was 200 °C, and the detector temperature was 250 °C. An Agilent model 6890 gas chromatograph interfaced with an Agilent 5791A mass selective detector (GC-MS) was used for mass spectral analysis of the GC components at a MS ionization voltage of 70 eV. A 30 m  $\times$  0.25 mm (inside diameter) ( $d_f = 0.25 \,\mu$ m) DB-5 bonded phase fused silica capillary column (Agilent) was used for GC. The linear velocity of the

Table 1. Compounds Identified in the Volatile Extract

| compound                     | I <sub>DB-5</sub> <sup>a</sup> | I <sub>DB-5</sub> <sup>b</sup> | GC peak area (%) <sup>c</sup>   |
|------------------------------|--------------------------------|--------------------------------|---------------------------------|
| isovaleric acid              | 831                            | 834                            | $\textbf{0.35}\pm\textbf{0.01}$ |
| ( <i>E</i> )-hexen-2-al      | 842                            | 854                            | $0.27\pm0.01$                   |
| tricyclene                   | 931                            | 926                            | $1.02\pm0.09$                   |
| α-pinene                     | 936                            | 939                            | $0.76\pm0.03$                   |
| verbenene                    | 976                            | 967                            | $0.67\pm0.02$                   |
| sabinene                     | 984                            | 976                            | $1.59\pm0.11$                   |
| carene                       | 1008                           | 1011                           | $0.50\pm0.00$                   |
| o-cymene                     | 1020                           | 1022                           | $0.40\pm0.01$                   |
| cis-sabinene hydrate         | 1093                           | 1097                           | $2.28\pm0.12$                   |
| camphor                      | 1145                           | 1143                           | $0.60\pm0.03$                   |
| trans-pinocamphone           | 1161                           | 1160                           | $5.86\pm0.43$                   |
| cis-chrysantheol             | 1164                           | 1162                           | $1.16\pm0.10$                   |
| <i>cis</i> -α-terpineol      | 1198                           | 1195                           | $24.22\pm2.12$                  |
| 3-neoisothujanol             | 1214                           | 1215                           | $2.59\pm0.20$                   |
| trans-carveol                | 1225                           | 1217                           | $0.54\pm0.02$                   |
| citronellol                  | 1234                           | 1228                           | $16.18\pm1.01$                  |
| <i>cis</i> -carveol          | 1236                           | 1229                           | $0.51\pm0.02$                   |
| <i>p</i> -cymen-7-ol         | 1289                           | 1287                           | $0.41\pm0.01$                   |
| thymol                       | 1294                           | 1290                           | $1.33\pm0.07$                   |
| carvacrol                    | 1297                           | 1298                           | $0.34\pm0.00$                   |
| citronellyl acetate          | 1342                           | 1354                           | $0.55\pm0.02$                   |
| neoisodihydrocarveol acetate | 1350                           | 1356                           | $0.54\pm0.00$                   |
| eugenol                      | 1361                           | 1356                           | $4.37\pm0.21$                   |
| aromadenderene               | 1442                           | 1439                           | $0.66\pm0.03$                   |
| thujopsadiene                | 1464                           | 1462                           | $1.14\pm0.06$                   |
| 7-epi-α-selinene             | 1517                           | 1517                           | $1.13\pm0.08$                   |
| acor-4-ene (6,11-oxido)      | 1529                           | 1531                           | $18.06\pm1.12$                  |
| selina-3,7(11)-diene         | 1544                           | 1542                           | $0.41\pm0.01$                   |
| germacrene (B)               | 1554                           | 1556                           | $0.36\pm0.01$                   |
| ledol                        | 1564                           | 1565                           | $3.87\pm0.05$                   |
| globulol                     | 1579                           | 1583                           | $3.27\pm0.07$                   |
| viridiflorol                 | 1590                           | 1590                           | $0.50\pm0.01$                   |
| $\beta$ -himachalene oxide   | 1613                           | 1610                           | $0.36\pm0.00$                   |
| 1-epi-cubenol                | 1627                           | 1627                           | $0.44\pm0.01$                   |
| cubenol                      | 1644                           | 1642                           | $0.65\pm0.01$                   |
| farnesol                     | 1690                           | 1697                           | $1.55\pm0.02$                   |

<sup>*a*</sup> Kovats index on the DB-5 column. <sup>*b*</sup> Kovats index on the DB-5 column from refs 32 and 33. <sup>*c*</sup> Values are means  $\pm$  the standard deviation (n = 3).

helium carrier gas was 30 cm/s. The injector and the detector temperatures were 250 °C. The oven temperature was increased from 35 to 220 °C at a rate of 3 °C/min and held for 40 min.

**Statistical Analysis.** Results were expressed as means  $\pm$  standard deviation of triplicate measurements (n = 3). One-way analysis of variance (ANOVA) was carried out to test any differences between the solvents used. Statistical comparisons between variables (*e.g.*, yields and antioxidant activity) were performed with Student's *t*-test using SPSS (Version 11.0).

#### **RESULTS AND DISCUSSION**

**Volatile Chemicals Identified.** Yields of the volatile extract from dried corn silk were  $0.52 \pm 0.03\%$  (w/w). Yields of petroleum and ethanol extracts were  $1.50 \pm 0.12$  and  $3.40 \pm 0.21\%$ , respectively. The values are means  $\pm$  the standard deviation (n = 3;  $P \le 0.05$ ).

**Table 1** shows the chemicals identified in volatile extracts. The total amount of oxygenated compounds, such as  $\alpha$ -terpineol and citronellol, comprised 47.51% of the extract. Monoterpenes and sesquiterpenes comprised 2.57 and 1.92%, respectively. The major compounds were *cis*- $\alpha$ -terpineol (24.22 ± 2.12%), 6,11-oxidoacor-4-ene (18.06 ± 1.12%), citronellol (16.18 ± 1.01%), *trans*-pinocamphone (5.86 ± 0.43%), eugenol (4.37 ± 0.21%), neo-iso-3-thujanol (2.59 ± 0.20%), *cis*-sabinene hydrate (2.28 ± 0.12%), sabinene (1.59 ± 0.11%), and thymol (1.33 ± 0.07%).

The volatile extract from corn silk contained high levels of  $\alpha$ -terpineol and citronellol.  $\alpha$ -Terpineol, isolated from American pine oil, has a sweet floral lilac-like odor commercially used



Figure 1. Results of the DPPH assay for radical scavenging activity of the extracts obtained from Egyptian corn silk.

for perfumery purposes (34). Citronellol, present in citronella essential oil, possesses a fresh rosy odor and has been used extensively in perfume compositions for various products such as soap, household products, and cosmetics (34). Sabinene (1.59  $\pm$  0.11%), which is one of the chemical compounds that contributes to the spiciness of black pepper (34), and thujop-sadiene (1.14  $\pm$  0.06%) are the major oxygenated compounds.

In this study, the main chemical class in the volatile extract of Egyptian corn silk was the terpenoid class, with compounds such as  $\alpha$ -terpineol and citronellol. On the other hand, a previous study on volatile constituents of maize silk reported that nonterpenoids such 2,3-dihydro-3,5-dihydro-4*H*-pyran-4-one and furfural were the major chemicals of five different maize genotypes (*15*). The individual components of the volatile oil of Egyptian corn silk clearly differ depending on its habitat as well. However, these results suggest that an essential oil from corn silk is a good source for fragrance ingredients.

Antioxidant Activity of Corn Silk Samples. Figure 1 shows the results of the antioxidant test conducted via the DPPH assay. The standard compound TBHQ exhibited  $91.00 \pm 1.00\%$  radical scavenging activity at a level of 200  $\mu$ g/mL, indicating that this method is valid. All extracts exhibited satisfactory antioxidant activities at the tested levels (100, 200, and 400  $\mu$ g/mL). In decreasing order, the antioxidant activity of the samples was as follows: ethanol extract > water extract > volatile extract > petroleum ether extract. The highest activity  $(86.00 \pm 4.00\%)$ was obtained with the ethanol extract at a level of 400  $\mu$ g/mL. Clear dose-dependent activity was also observed in the case of ethanol and volatile extracts. The water extract inhibited DPPH activity by 81.00  $\pm$  6.00% at a level of 200  $\mu$ g/mL, but it did not show a dose response. The volatile extract exhibited 59.92  $\pm$  3.00 and 76.00  $\pm$  1.00% antioxidant activities at levels 100 and 400  $\mu$ g, respectively. The petroleum ether extract exhibited moderate inhibitions (~50%) at all three tested levels ( $P \leq$ 0.05). One recent report indicated that the scavenging abilities of the various essential oils for the DPPH radical ranged from 39% for angelica seed oil to 90% for jasmine oil at a level of  $200 \,\mu \text{g/mL}$  (28). The radical scavenging activity of these extracts might be due to the presence of phenolic compounds such as eugenol, thymol, and carvacrol as well as some polyphenols and flavonoids (7, 35, 36).

The  $\beta$ -carotene bleaching mechanism is a free radicalmediated phenomenon, resulting from the hydroperoxides formed from linoleic acid. In this study,  $\beta$ -carotene undergoes rapid discoloration due to the attack of free radicals formed upon abstraction of a hydrogen atom from the diallylic methylene group of linoleic acid. The presence of an antioxidant in the reaction mixture hinders the rate of bleaching by neutralizing



Figure 2. Results of the  $\beta$ -carotene bleaching assay for antioxidant activity of the extracts obtained from Egyptian corn silk.

free radicals formed in the system during incubation at 50 °C (*37*). **Figure 2** shows the results of antioxidant activity tests conducted via the  $\beta$ -carotene bleaching assay. The standard compound, TBHQ, exhibited 82.00 ± 3.80% antioxidant activity at a level of 200  $\mu$ g, indicating that this method is valid. All samples that were tested exhibited satisfactory antioxidant activity with clear dose responses. The ethanol extract also showed the highest activity among the extracts ( $P \le 0.05$ ). It inhibited the bleaching by 75.00 ± 2.70% at a level of 400  $\mu$ g/mL. At the same level of 400  $\mu$ g/mL, water (62.00 ± 1.90%), volatile extract (56.00 ± 1.9%), and petroleum ether (54.00 ± 3.40%) possessed moderate activities.

The results of this study suggest that the volatile oxygenated chemicals, such as  $\alpha$ -terpineol, citronellol, and eugenol, contributed to the antioxidant activity of a volatile extract from corn silk. A previous report showed that eugenol inhibited hexanal oxidation by nearly 100% at a level of 50  $\mu$ g/mL over 40 days. Eugenol also inhibited the formation of malonaldehyde from cod liver oil upon oxidation by 90% at a level of 80  $\mu$ g/mL (25).  $\alpha$ -Terpineol and citronellol reportedly possessed moderate antioxidant activities (38). The presence of phenolic compounds, including flavonoids, may also play a significant role in antioxidant activity of the ethanol and water extracts because most flavonoids have been isolated from natural plants by extraction with polar solvents such as methanol, ethanol, and water (39–42).

In this study, more than 99% of the volatile extract obtained from Egyptian corn silk (obtained via steam distillation followed by dichloromethane extraction) was found to be terpenoids. These terpenoids are well-known chemicals used in flavor and fragrance ingredients (34). The volatile samples (the volatile extract and petroleum ether extract) exhibited moderate antioxidant activities in the two kinds of testing systems. In addition, nonvolatile extracts obtained by ethanol and water extraction exhibited strong antioxidant activities. Analysis of nonvolatile extracts was not conducted in this study. However, the presence of the well-known nonvolatile antioxidants, such as flavonoids and polyphenol, has been reported in corn silks (4, 8, 10, 43). These results suggest that corn silk is a flavor ingredient source and a natural antioxidant supplement for various food products.

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