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RESEARCH NOTE

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Utilization of Masking Techniques to Ameliorate Agricultural Odorants

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Abstract Different masking materials were evaluated for their ability to ameliorate odor of model poultry manure solution by assessing their effects on sensory pleasantness and odor intensity. Results indicated extracts from Eastern red cedar leaves, Loblolly pine needles, and commercial masking agents such as pine extract and odor neutralizer were effective ($p<0.05$) for masking odor of model poultry manure solution by increasing ($p<0.05$) pleasantness (82 and 86% increases in pleasantness using red cedar and pine needle extracts, respectively) and decreasing ($p<0.05$) odor intensity (odor intensity reduction by 66 and 76% using red cedar pine needle extract). The most odor-active compound in Loblolly pine needle extract was α-terpineol (1,573.8 ug/g) which is responsible for aroma of pine trees (piney) and effective for ameliorating agricultural odors.

Keywords: *odor masking agent, SPME-GC-O, sensory*

Introduction

Mississippi's poultry industry, the number one agricultural industry in the state, is the 4th largest producer of broilers in the United States (1). Increased poultry production has caused an increase in by-products including waste (e.g. manure) and offal (e.g. head, feet, blood, and feathers). In addition to disposal problems of by-products, odor problems associated with manure must be resolved to avoid conflict between farmers and the communities in which they have contact. Odors are produced primarily by anaerobic decomposition of organic matter and include nitrogen-based derivatives such as amines, skatole, and indole, sulfur-based compounds such as mercaptans and sulfide derivatives, and short-chained fatty acids such as acetic, butyric, isobutyric, valeric, *etc.* (2).

Numerous studies have focused on agricultural odor control methods (3-6). These methods are grouped into four categories: masking agents, counteractants, odor absorption chemical compounds, and biological treatments (7-9), among which masking agents are odorous compounds that are more pleasant than foul odors and thus effectively cover up or mask the offensive odors. Pierce *et al.* (10) investigated cross-adaptation, which is defined as the decreased or temporary loss of sensitivity to an odorant after exposure to a different odorant. Compounds with similar structures such as 3-methyl-2-hexanoic acid (sweaty odor) and its ethyl esters (fruity odor) may be responsible for this cross-adaptation phenomenon (10). Engen and Lindstrom (11) reported significant cross-adaptation among a homologous series of aliphatic alcohols that differ in carbon chain length. Cain (12) and Cain and Engen (13) studied the phenomenon of cross-adaptation between *n*-propanol and *n*-pentanol, two structurally similar odorants. Pierce *et al.* (10) demonstrated a significant reduction in the perceived intensity of androstanone (5-α-androstan-3-one) immediately following exposure to an odorless structural analog (3-methylidene-5-α-androstane).

Counteractants are mixtures of aromatic components that cancel or neutralize malodorous compounds so that the intensity of the mixture is less than that of the constituents. An example of the effectiveness of one counteractant is the 1,4-nucleophilic addition to the malodorant (14). In addition, other physico-chemical associations that occur between counteractants and foul smelling compounds are also involved in this odor control methodology. Faryniarz *et al.* (15) earned a patent for reducing malodors that occur in humans using counteractants such as cedar extract, cinnamic alcohol, diethyl phthalate, galaxolide and diethyl phthalate mixture, geranyl phenyl acetate, guaiac wood oil, linalyl benzoate, and phenyl ethyl phenyl acetate.

Porous products that have large surface areas can be used to adsorb odors prior to their release into the environment. In particular, the large surface area of the powdered activated carbon makes it an effective absorbent in environmental and agricultural areas (16). Generally, biological treatments can be conducted through dietary supplementation that may alter microbial populations or enzymatic degradation pathways and reduce the production of malodorous compounds. Armstrong *et al.* (17) investigated the effects of dietary copper materials such as cupric sulfate and cupric citrate on swine odor. Results indicated that swine waste from animals that had been fed cupric sulfate (225 μg/g) and cupric citrate (66 μg/g or 100 μg/g) had decreased malodor intensity. Arthur and Anker (18) investigated hydrogen sulfide odor control technology by reducing sulfates to sulfides with a chemical enzyme blocker found in sulfate-reducing bacteria. Giffard *et al.* (19) reported reducing malodorous compounds in dogs through feeding activated charcoal and zinc acetate. These deodorant materials decreased malodor of flatulence in dogs by altering the production of gas in the large intestine.

The objective of this study was to measure the masking effects of Eastern red cedar extract, Loblolly pine needle extract, and two different commercial substances (pine

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extract and odor neutralizer) on the odor of a model solution containing odor-active compounds identified in poultry manure. In addition, the major odorant in the Loblolly pine extract was identified and quantified through instrumental analysis.

Materials and Methods

Materials The following compounds were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA): butyric acid, isobutyric acid, *p*-cresol, indole, skatole, dimethyl trisulfide, ethyl butyrate, methyl alcohol, and chlorobenzene (internal standard). Ethyl alcohol was purchased from Fisher Scientific (Pittsburgh, PA, USA), and α-terpineol and propylene glycol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Commercial masking substances (pine extract and odor neutralizer) were purchased from a local food market. Natural masking materials (Eastern red cedar and Loblolly pine needles) were collected from five different trees in Starkville, Mississippi, USA, immediately prior to extraction. A 50/30-μm DVB/CAR/PDMS StableFlex solid phase microextraction (SPME) fiber for a manual holder was purchased from Supelco (Bellefonte, PA, USA).

Extraction of natural masking materials Eastern red cedar and Loblolly pine needles (250 g each) were separately extracted using ethanol (333 mL) and odor-free water (667 mL) in a Soxhlet extraction apparatus at 160°C for 6 hr (20). Each extract was concentrated using a Rotavapor (Büchi Model RE111 Laboriums-Technik AG, Flawil, Switzerland) at room temperature until panelists detected almost no ethanol odor. The concentrated extracts were stored at 4°C until the analysis was performed.

Preparation of a model poultry manure odor solution The most important compounds in poultry manure are butyric acid, isobutyric acid, *p*-cresol, indole, skatole, and dimethyl trisulfide (2). Subsequent model poultry manure odor solutions were prepared according to the method of Yasuhara (2) to reflect gas chromatography olfactometry results and included the important odor compounds (Table 1) along with odor-free water. Odor-free water was prepared by boiling deionized water until 1/3 of the original volume remained in the flask. Indole and skatole were dissolved in propylene glycol, and odor-free water was added to reach the desired concentrations. Among the nine solutions, sample 6 was chosen by four trained members to possess odor that most closely resembled fresh poultry manure (Table 1) and was utilized in the masking study.

Preparation of ethyl butyrate Ethyl butyrate (10 μg/g) solution was prepared using odor-free water in a 100-mL amber glass bottle with a screw cap, and was mixed with red cedar extract to formulate a masking agent solution.

Sensory evaluation Six different masking agents were utilized: Eastern red cedar extract, Loblolly pine needles extract, ethyl butyrate, a mixture of ethyl butyrate and Eastern red cedar extract (1:1 v/v), commercial pine extract, and commercial odor neutralizer (absorbing and masking materials). The samples were prepared by combining 2 mL model solution with 1 mL masking agent in 7 mL solvent-saver scintillation vials with white polyethylene caps (Kimble Glass Inc., Vineland, NJ, USA). All samples were prepared a day in advance and individually provided to panelists. Ten panelists were recruited from the Department of Food Science and Technology, Mississippi State University, and trained (three sessions) to evaluate odor intensity and pleasantness using both a control (fresh poultry manure) and a model poultry manure odor solution. The purpose of training panelists was to familiarize them with odors and to provide practice in distinguishing the manure odors and odor intensities in order to decrease the variation of sensory data. Panelists were provided duplicate sets (A and B) of samples that had been assigned random three digit numerical codes in sensory booths located at the Department of Food Science and Technology. Odor intensity was evaluated with a 15-point line score, which was arranged horizontally and marked from: no odor = 0, moderate = 7.5, and extremely strong = 15. This odor intensity scale was modified from a 15-point category scale described by Meilgaard *et al.* (21). Odor pleasantness was evaluated by trained panelists utilizing a 22-point horizontal line scale (21) that ranged from negative 11 (extremely unpleasant) to positive 11 (extremely pleasant) with 0 as neither pleasant nor unpleasant. The data was transformed from the original scale to a 0-22 scale, where 0 was extremely unpleasant, 11 represented neither pleasant nor unpleasant, and 22 represented extremely pleasant.

Table 1. Compositions of model poultry manure odor solutions that were utilized to determine which sample had the most similar odor to fresh poultry manure

Odorous	Sample Number								
Compound*	1	2	3	4	5	6	7	8	9
Butyric acid	500	500	500	500	500	1000	500	500	500
Isobutyric acid	500	500	500	500	500	500	1000	1000	500
Indole	1000	1000	1000	1000	1000	1000	1000	1000	1500
Skatole	1000	1000	1000	1000	1000	1000	1000	1000	1000
p-Cresol	0	1000	1000	0	0	500	0	500	500
DMTS	0	0	5	5	50	50	50	50	50

*The stock solution concentration for each odorous compound (Butyric acid, Isobutyric acid, Indole, Skatole, p-Cresol, DMTS) was 10 μg/g. The amount of stock solution utilized in each sample is listed in μL (5, 50, 500, 1,000, and 1,500) for each compound, and each sample's final volume was finished to 4,050 uL with distilled, deodorized water.

Analytical analysis of pine extract A Hewlett Packard (HP) 5890 plus series II GC equipped with a HP 5972 mass selective detector (Palo Alto, CA, USA) was used to analyze odor-active compounds in the pine needle extract. Compounds were extracted with a 50/30-μm DVB/CAR/PDMS StableFlex SPME fiber. The gas chromatograph was equipped with a 30 m × 0.25 mm i.d. × 1.0 μm film thickness DB-5 capillary column (J&W Scientific, Folsom, CA, USA) and equipped with a 0.75-mm i.d. glass SPME injection sleeve (Supelco, Bellefonte, PA, USA). The carrier gas (helium) was set at a constant flow rate of 1.0 mL/min. GC oven temperature was programmed as follows: initial column temperature, 40°C for 5 min; rate of temperature increase, 8°C/min; final column temperature, 250°C for 5 min; injector temperature, 225°C; detector temperature, 250°C; splitless mode capillary inlet; MSD capillary direct interface temperature, 280°C; ionization energy, 70 eV; mass range from 33 to 350 a.m.u.; and scan rate of 2.2 scans/s. The internal standard (IS; 5 μg/g) was prepared using chlorobenzene along with odor-free water to quantify compounds present in the pine needles. An internal standard response factor was calculated to test for linearity and sensitivity at four different concentrations (1, 4, 16, and 64 μg/g) of α-terpineol. The internal standard response factor (ISRF) was calculated utilizing the following equation:

$$ISRF = \frac{(\text{mass of } \alpha\text{-terpineol})\ (\text{peak area of IS})}{(\text{mass of IS})\ (\text{peak area of } \alpha\text{-terpineol})}$$

For quantification of α-terpineol, an internal standard (1.5 mL) was added into the pine needles extract (3.0 mL) in a 22-mL vial. The conditions were the same as those used for instrumental analysis. After obtaining peak areas for α-terpineol and the internal standard, the amount of α-terpineol was calculated using the following equation:

$$\text{Mass of } \alpha\text{-terpineol} = \frac{(\text{mass of IS})(\text{peak area of } \alpha\text{-terpineol})\ (\text{ISRF})}{(\text{peak area of IS})}$$

Triplicate runs were conducted. Relative standard deviation of peak areas was calculated for the internal standard and α-terpineol.

SPME-gas chromatography-olfactometry Three trained panelists evaluated pine needle extract utilizing a gas chromatography olfactometry system consisting of a Varian 3400 GC (Varian Instrument Group, Walnut Creek, CA, USA) equipped with a sniff port. GC was outfitted with a 30 m × 0.25 mm i.d. × 1.0 μm film thickness DB-5 capillary column (J&W Scientific, Folsom, CA, USA) and equipped with a 1078 glass SPME injector (54 mm × 5 mm o.d. × 0.8 mm i.d.) insert (Varian, Walnut Creek, CA, USA). The conditions of the GC were the same as those utilized for the GC-MS. The capillary column present in the GC oven extended through the heated and stainless steel sniff port. A glass funnel was fitted to the end of the sniff port and heated, and humidified air (air flow rate: 30 mL/min) was introduced into the sniff port upstream near the port where the column first entered. Air engulfed the capillary column effluent and carried it into the glass funnel, where the odor intensity was evaluated using a 15-point scale similar to the scale that was used for sensory analysis. Peaks (Osmegrams) representing odor intensity were developed utilizing Osme software (22).

Statistical analysis A completely randomized design with three replications was utilized to evaluate the effects ($p<0.05$) of masking agents on odor intensity and pleasantness in the poultry manure solution. When significant differences occurred ($p<0.05$), means were separated using Fisher's protected least significant difference (23). The statistical analysis was conducted using version 8.1 of SAS (24).

Results and Discussion

Sensory analysis of masking materials The results of sensory analysis for the model poultry manure odor solution treated with masking materials are shown in Figs. 1 and 2. When compared to the control, the commercial pine extract was most effective at increasing pleasantness

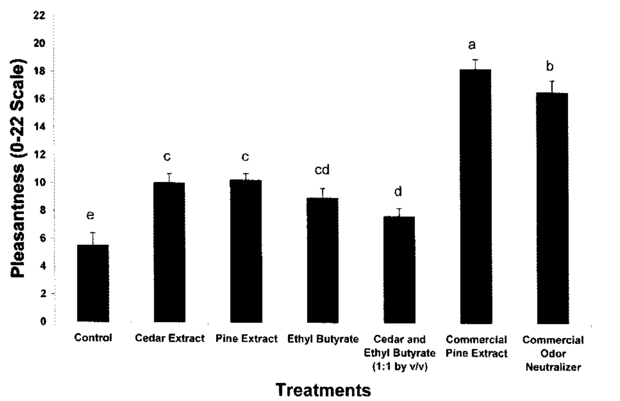

Fig. 1. Sensory pleasantness of a model poultry manure odor solution that was treated with several masking materials and rated on a 22 point horizontal line scale that ranged from 0 to 22 with 11 as neither pleasant nor unpleasant. Unlike superscripts indicate significant differences ($p<0.05$) among treatments. Standard error bars are included for each treatment.

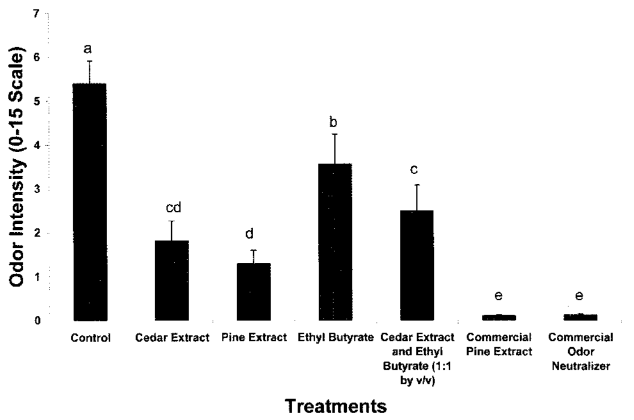

Fig. 2. Sensory odor intensity of a model poultry manure solution treated with several masking materials and rated on a 15 point line scale in which 0 = no odor and 15 = extremely strong. Unlike superscripts indicate significant differences ($p<0.05$) among treatments. Standard error bars are included for each treatment.

(232% increase), followed by commercial odor neutralizer (202%), pine needles extract (86%), red cedar extract (82 %), mixture of red cedar extract and ethyl butyrate (63%), and ethyl butyrate (39%) (p<0.05). The results of odor intensity indicated that the commercial pine extract and odor neutralizer were the most effective at reducing odor intensity (98% reduction), followed by pine needles extract (76%), red cedar extract (66%), mixture of red cedar extract and ethyl butyrate (54%), and ethyl butyrate (34%) (p<0.05). Results showed that the commercial materials may contain several additives that possess strong fragrances. For example, in commercial pine extract, a combination of pine and floral odors was detected; these floral odors increased the pleasantness of the poultry manure solution.

The extraction procedures for red cedar and pine needles were relatively simple and inexpensive. Belanger *et al.* (25) reported that the major compounds of Canadian Western red cedar oil were 1, 4-cineole, 3-terpinen-1-ol, and γ-eudesmol. Major volatile compounds from twigs, needles, and sprouts of pine were identified as α-pinene, β-pinene, limonene, and β-myrcene (26). In this study, the major volatile compounds from extracts (red cedar and pine needles) masked malodors of the model poultry manure odor solution and increased the pleasantness of the model solution. Ethyl butyrate was more pleasant (p<0.05) than the control, possibly due to its strong fruity odor (27). According to the cross-adaptation hypothesis, ethyl butyrate has the potential to increase pleasantness and decrease odor intensity of the model poultry manure odor solution. However, ethyl butyrate was the least effective masking agent among those tested. This may be due to the fact that its fruity odor was not as intense as the odors of the other masking agents, and the combination of the manure solution and fruity odor did not produce a desirable odor. The mixture of ethyl butyrate and red cedar extract gave slightly better results (p<0.05) (20% decrease in odor intensity) than ethyl butyrate due to the effect of the combination of odor-active compounds from both masking agents.

Analytical results of pine needles extract The major volatile compounds from pine needles extract, as determined through SPME-GC-MS, are acetic acid, 4-methyl-2-pentanone, styrene, linalool, ethyl ester benzoic acid, and α-terpineol, among which α-terpineol has a piney odor that is the major odor compound in the sample as determined by SPME-gas chromatography-olfactometry (SPME-GC-O). This compound was confirmed as α-terpineol by injecting an α-terpineol chemical standard into the GC-O and comparing its odor and retention time to the pine needle sample.

Internal standard response factor and linearity for α-terpineol The linear relationship (R^2) between α-terpineol and the internal standard was 0.9998, which indicates that the mass selective detector was sensitive for α-terpineol and the internal standard. The peak area for each sample was proportional to each concentration of α-terpineol, and the average internal standard response factor was 227.07. The relative standard deviation of the internal standard (chlorobenzene) response factor was 8.04%.

Quantification of α-terpineol The relative standard deviations for the peak area of the internal standard and of α-terpineol from the pine extract were 6.84 and 4.34%, respectively. Using the internal standard response factor, the concentration of α-terpineol present in the pine extract headspace was quantified as 1,573.8 μg/g; the threshold for α-terpineol is 330 ng/g in water (28). The pine extract contains 4,769 times the odor intensity (an odor-active value of 4,769) of the threshold of á-terpineol. Therefore, the high odor intensity of α-terpineol in Loblolly pine extract is highly responsible for its masking effect in the model poultry manure odor solution.

In conclusion, results from sensory analysis indicated that extracts from Eastern red cedar, Loblolly pine, and commercial masking agents (pine extract and odor neutralizer) were effective at reducing unpleasantness and odor intensity in the model poultry manure odor solution, with commercial samples being highly effective. In commercial pine extract, this effectiveness was attributed to the presence of a combination of odor active compounds with pine and floral characters. Through GC-MS and GC-O analyses, α-terpineol was identified as the major aroma-active compound in Loblolly pine needle extract. Utilization of α-terpineol in conjunction with other odor-active compounds that can be determined through similar GC-O analyses may have a significant impact in decreasing the agricultural odors in the industry. Commercial products that can increase pleasantness and decrease odor intensity of agricultural by-products are also available. It also may be feasible to feed animals these odor-active compounds to decrease the odor of their waste.

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