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Plant Extracts, Spices, and Essential Oils Inactivate *Escherichia coli* O157:H7 and Reduce Formation of Potentially Carcinogenic Heterocyclic Amines in Cooked Beef Patties

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ABSTRACT: Meats need to be heated to inactivate foodborne pathogens such as *Escherichia coli* O157:H7. High-temperature treatment used to prepare well-done meats increases the formation of carcinogenic heterocyclic amines (HCAs). We evaluated the ability of plant extracts, spices, and essential oils to simultaneously inactivate *E. coli* O157:H7 and suppress HCA formation in heated hamburger patties. Ground beef with added antimicrobials was inoculated with *E. coli* O157:H7 (10^7 CFU/g). Patties were cooked to reach 45 °C at the geometric center, flipped, and cooked for 5 min. Samples were then taken for microbiological and mass spectrometry analysis of HCAs. Some compounds were inhibitory only against *E. coli* or HCA formation, while some others inhibited both. Addition of 5% olive or apple skin extracts reduced *E. coli* O157:H7 populations to below the detection limit and by 1.6 log CFU/g, respectively. Similarly, 1% lemongrass oil reduced *E. coli* O157:H7 to below detection limits, while clove bud oil reduced the pathogen by 1.6 log CFU/g. The major heterocyclic amines 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) were concurrently reduced with the addition of olive extract by 79.5% and 84.3% and with apple extract by 76.1% and 82.1%, respectively. Similar results were observed with clove bud oil: MeIQx and PhIP were reduced by 35% and 52.1%, respectively. Addition of onion powder decreased formation of PhIP by 94.3%. These results suggest that edible natural plant compounds have the potential to prevent foodborne infections as well as carcinogenesis in humans consuming heat-processed meat products.

KEYWORDS: *Escherichia coli O157:H7, heterocyclic amines, plant compounds, ground beef patties*

INTRODUCTION

Escherichia coli O157:H7 is the most common pathogen among the Shiga toxin-producing *E. coli* group.[1] The organism was first identified in 1982 when it was isolated from people who became sick after eating undercooked beef patties from a suspected contaminated lot of meat.[2] Since then, this foodborne pathogen has been isolated with increasing frequency from various food sources, including ground beef. Currently, *E. coli* O157:H7 causes about 73 000 illnesses in the United States annually. Ground beef products still remain the most common vehicle for foodborne illnesses resulting from a failure to cook meat products thoroughly.[3] Human infection by *E. coli* O157:H7 is associated with a wide range of clinical manifestations, including diarrhea, hemorrhagic colitis, hemolytic uremic syndrome (HUS), and death.[4]

Inactivation of *E. coli* O157:H7 in beef patties is usually carried out by cooking the meat at high temperatures to eliminate the risk of foodborne illness resulting from the ingestion of undercooked meats. Previous studies indicate that this practice also increases the risk of formation of heterocyclic amines (HCAs). These compounds might be associated with colon, rectal, breast, and other types of cancers in humans.[5−8]

Heterocyclic amines are formed in muscle tissues (beef, poultry, and fish) by a heat-induced third-order condensation of the following compounds: creatine/creatinine, amino acids, and glucose.[6,7,9] Animal-based protein-rich foods exposed to high temperatures might contain parts-per-billion (ppb) concentrations of potent mutagenic and carcinogenic HCAs, formed by frying or broiling meats until they are well done.[6,10−12] This conclusion is supported by the following recent studies. Cross et al.[13] found a positive association between red meat consumption and human esophageal squamous cell carcinoma as well as between 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline (DiMeIQx) intake and gastric cancer. John et al.[14] found that consumption of red meat processed at high temperature is associated with increased risk of advanced, but not localized, prostate cancer. An epidemiological study by Freedman et al.[15] revealed that consumption of red meat and saturated fat may be associated with increased chronic liver disease and risk of hepatocellular carcinoma and that white meat may be associated with reduced risk. Lauber and Gooderham[16] found that 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), which induces cancer of the colon, mammary gland, and prostate tissues in rats, exhibited estrogenic effects in human breast cancer cells at subnanogram levels.

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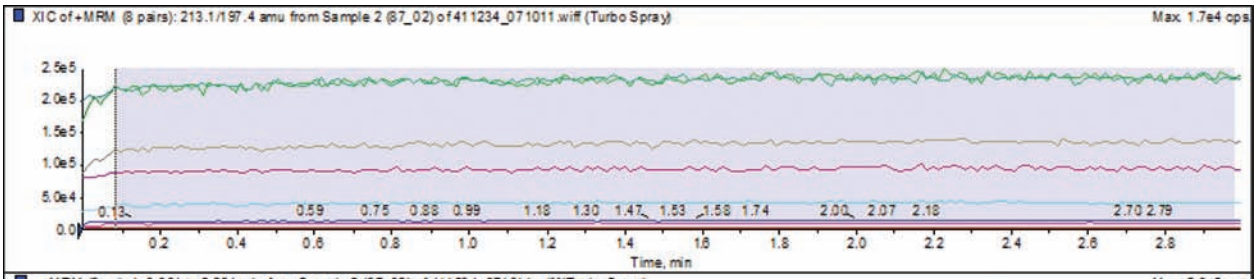

Data List for "+MRM(8 pairs): 0.084 to 2.984 min from Sample 2 (87_02) of 411234_071011.wiff (Turbo Spray)"

	Q1 Mass (amu)	Q3 Mass (amu)	Intensity (cps)	%Max. Intensity
1	213.0500	197.4000	1.5251e4	6.6406
2	213.0500	169.5000	3778.4135	1.6452
3	214.1230	198.6000	2.2967e5	100.0000
4	214.1230	130.3000	1.3210e5	57.5193
5	225.1200	209.4000	4.1964e4	18.2713
6	225.1200	139.2000	1.0437e4	4.5445
7	228.2130	209.6000	2.2918e5	99.7893

Figure 1. Extracted ion chromatogram of a beef extract (upper panel) and integrated signal intensity of MeIQx, PhIP, and trideuterated PhIP (middle and lower panels). The integrated signal intensity is presented as a graph in the middle panel and numerically in the lower panel.

The following four HCAs have been listed as potential human carcinogens: 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), and PhIP.[7,17] Human exposure to the HCAs MeIQx and PhIP occurs primarily through the consumption of cooked meats. Dietary exposure to total HCAs has been estimated to range from <1 to 17 ng/kg of body weight per day.[9,18,19]

Inactivating *E. coli* O157:H7 and concurrently decreasing the formation of carcinogenic HCAs in heated meats is a challenging problem. Thus, there is a need to find alternative methods that can serve the dual purpose of efficiently inactivating *E. coli* O157:H7 and preventing the formation of HCAs in heated meats.

In a previous study we showed that carvacrol, an active component of oregano essential oil, concurrently reduced *E. coli* O157:H7 and HCAs in cooked ground beef patties.[20] To extend our knowledge about the dual beneficial effects of plant compounds, the specific objective of the present study was to evaluate both antimicrobial and heterocyclic amine-inhibiting properties of a series of plant extracts, spice powders, and essential oils in grilled hamburger patties.

MATERIALS AND METHODS

Bacterial Cultures. The test organism used was *E. coli* O157:H7 strain 35150. Stock cultures of the organism were maintained in cryovials at −80 °C and activated by transferring 100 μL into tryptic soy broth (TSB; EMD Chemicals Inc., Gibson, NJ). The bacterial cultures were maintained in TSB at 4 °C with monthly transfers. For experimental use, an overnight culture of the organism was grown in TSB at 37 °C for 18−24 h.

Heterocyclic Amines. MeIQx, PhIP, and 2-amino-1-(trideuteromethyl)-6-phenylimidazo[4,5-*b*]pyridine (PhIP-3D) were purchased from Toronto Research Chemicals, Inc., North York, Ontario, Canada. PhIP-3D was used as the internal standard in all experiments.

Plant Compounds. The following test plant compounds were evaluated: (a) plant extracts—olive (CreAgri Inc., Hayward, CA), apple skin (Apple Poly LLC, Morrill, NE), green and black teas (LKT Laboratories, St. Paul, MN), and grape seed (Mega Natural, Madera, CA); (b) spice powders obtained from local markets—onion, mustard, paprika, garlic, oregano, cumin, and turmeric; (c) essential oils obtained from Lhasa Karnak Herb Co., Berkeley, CA—allspice, clove bud, and lemongrass.

Sample Preparation and Thermal Treatment of Ground Beef. Ground beef (93% lean) was purchased from a local grocery store, placed in a stomacher bag, and stored at −20 °C. To allow thawing, samples were removed from the freezer and transferred to a 4 °C refrigerator 24 h before the experiments. Samples were left at room temperature for about 30 min to equilibrate before use. The test antimicrobial compound was added to the ground beef to obtain a concentration of 1% (w/w) in the case of essential oils and 5% (w/w) in the case of plant extracts and spices. Samples with added antimicrobial were mixed manually and then inoculated with 350 μL of *E. coli* O157:H7 overnight culture and stomached (AES Laboratories Smasher, Chemunex, France) at a fast speed (550 strokes/min) for 1 min. The final concentration of *E. coli* O157:H7 in the meat was ~10^7 CFU/g.

The inoculated meat samples were made into patties of 6 cm diameter. Control patties without antimicrobials were included in all experiments. A cookie cutter was used to achieve uniformity of the patties. Ground beef patties were cooked on a griddle (Presto 07050

Black Cool-Touch Foldaway Griddle, National Presto Industries, Eau Claire, WI) set to 400 °F and preheated for 15 min. The surface temperature of the heating element was estimated to be ~200 °C. The internal temperature of each patty was monitored with a type K thermocouple (Traceable total-range thermometer, VWR International, Brisbane, CA) that was inserted into the geometric center of each ground beef patty. The patty was turned over when the internal temperature reached 45 °C. The patty was then left on the heating element for 5 min, removed, and immediately cooled in ice-cold water. Separate samples were taken and used for microbial analysis and for solid-phase extraction to separate the HCAs from the meat matrix.

Microbial Sampling after Treatment. After thermal treatment and cooling, each patty was weighed and added to 9 parts of buffered peptone water (BPW; EMD Chemicals Inc., Darmstadt, Germany). Samples were stomached at fast speed (550 strokes/min) for 1 min. Serial dilutions of samples were made in 0.1% peptone water (Difco, Becton Dickinson, Sparks, MD) as needed. Plating was done on sorbitol MacConkey agar (SMAC; Difco). The plates were incubated at 37 °C for 24 h and then enumerated for survivors. Negative controls were also evaluated for each sample.

Solid-Phase Extraction of Meat Samples. The method used for extraction of HCAs was modified from the original method described by Gross and Grüter.[21] After cooling, the cooked patty was weighed, and 3 parts by volume of 1 M NaOH (EMD Chemicals Inc., Darmstadt, Germany) was added. The mixture was blended (Oster, Boca Raton, FL) for 5 min. A 16 g sample was obtained and spiked with 75 μL of PhIP-3D standard solution (0.5 ng/μL). The homogenate was mixed with 14 g of Extrelut NT 20 material (Chem Tube Hydromatrix, Varian, Lake Forest, CA) and transferred to a 50 mL Extrelut extraction column (Extrelut NT 20, Merck KGaA, Darmstadt, Germany) followed by elution with ethyl acetate (HPLC grade, Fisher Scientific, Fair Lawn, NJ). A Bond Elute PRS cartridge (500 mg, Varian, Palo Alto, CA) was coupled to the Extrelut column immediately as the solvent reached the bottom of the column. After collection of 40−50 mL of the flow through, the PRS cartridge was uncoupled and transferred to the vacuum manifold (Bio-Rad Laboratories, Hercules, CA) for 10 min with full vacuum. The cartridge was attached to a peristaltic pump (Masterflex 7523-00, Bernant Co., Cole-Parmer Instrument Co., Barrington, IL) to be washed with 6 mL of 0.1 M HCl (EMD Chemicals Inc., Darmstadt, Germany) followed by 15 mL of 40:60 methanol (Honeywell International Inc., Burdick & Jackson, Muskegon, MI), 0.1 M HCl, and 2 mL of distilled water. A Bond Elute C_{18} cartridge (100 mg, Varian) preconditioned with 2 mL of distilled water followed by 2 mL of methanol was coupled to the PRS cartridge, and the retained compound was eluted with 20 mL of 0.5 M ammonium acetate, HPLC grade (Fisher Scientific), pH 8.0, over a period of 10−15 min. The C_{18} cartridge was dried on the vacuum manifold for 20 min, and the retained compound was eluted with 0.8 mL of 9:1 methanol and NH_4OH (Ricca Chemical Co., Arlington, TX) into plastic tubes (wide hinge cap PP microtube, 1.5 mL, Sarstedt, Newton, NC). The extracts were stored at −20 °C until they could be analyzed using mass spectrometry (MS).

Sample Preparation for Mass Spectrometry of Heterocyclic Amines. The volume of the extracted sample was reduced to 20−40 μL via a Savant SpeedVac Plus SC110A centrifuge (Savant Instruments, Inc., Hicksville, NY). Half of the sample was diluted with 0.1% (w/w) formic acid (98%, EMD Chemicals, Inc., Darmstadt, Germany) in methanol to a total volume of 250 μL. The theoretical concentration of the internal standard in the reconstituted sample was 75 pg/μL on the basis of 100% recovery. This concentration provided an MS signal intensity comparable to the signal intensities of the compounds of interest.

Mass Spectrometric Analysis. MS analysis of the samples was performed on an ABI/Sciex 4000 QTRAP hybrid triple-quadrupole linear ion trap mass spectrometer (Applied Biosystems, Foster City, CA) with a TurboIonSpray source and Analyst 1.4.1 software. The analytes were introduced into the instrument via infusion at 7 μL/min by syringe infusion pump model 22 (Harvard Apparatus, Holliston, MA). HCA signal intensities were measured using multiple reaction monitoring (MRM) in the positive mode. As the compound of interest is being fragmented, the instrument detects m/z values of the parent ion paired with the m/z values of the predetermined fragment ion (Figure 1).

MRM transitions in this experiment were as follows: MeIQx, m/z 214.123 → 198.60; PhIP, m/z 225.12 → 209.40; trideuterated PhIP, m/z 228.213 → 209.60. The following instrument parameters were used: source temperature, 150 °C; source voltage, 4800 V; dwell time for all transitions, 100 ms; acquisition duration, 3 min. The collision energy (CE), declustering potential (DP), and cell exit potential were optimized using automated optimization, and they were, respectively, as follows: 96, 39, and 4 V for MeIQx, 75, 43, and 12 V for PHIP, and 111, 45, and 6 V for trideuterated PhIP.

Statistical Analysis. Treatments were repeated three times with one control for each set of repeats. The average and standard deviation (±SD) were calculated for each treatment for both *E. coli* O157:H7 population and HCA levels. For the microbiological data, one-way analysis of variance (ANOVA; Stata-ease, version 10.0, StataCorp LP, College Station, TX) was used to determine statistical significance ($p \leq 0.05$) between the control and treatments.

RESULTS AND DISCUSSION

Antimicrobial Effects against *E. coli* O157:H7 in Heated Hamburger Patties. *Plant Extracts.* Different extracts showed varying antimicrobial effects on *E. coli* O157:H7 (Figure 2). Olive extract was the most active

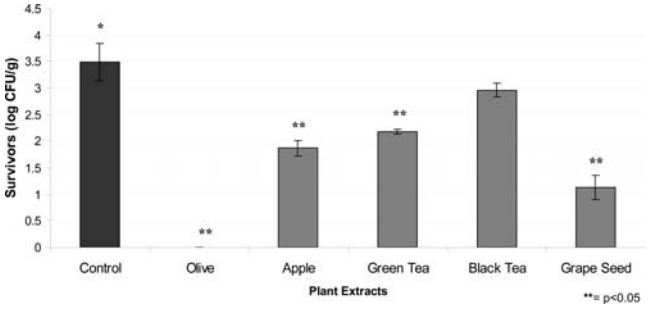

Figure 2. Effect of plant extracts on *E. coli* O157:H7 in heated ground beef patties.

compound, reducing the pathogen to below detection limits (<10 CFU/g) compared with that of the control (3.5 log CFU/g). Polyphenols represent 12% of the components in olive extract, with hydroxytyrosol contributing 6%.[22] In vitro studies have demonstrated the antimicrobial properties of hydroxytyrosol against pathogenic bacteria, including *Salmonella* Typhi, *Vibrio parahemolyticus*, and *Staphylococcus aureus*.[23,24]

Apple skin extract reduced the *E. coli* O157:H7 population by 1.6 log CFU/g. A similar effect was seen by Fratianni et al.[25] using ethanol extracts of apple peel. Juneja et al.[26] found that a 3% apple skin extract rendered *E. coli* O157:H7 more sensitive to heat (70.2% reduction in *D* values; time (min) to kill 90% of the bacteria) in sous-vide cooked ground beef at 62.5 °C. The addition of green tea extract also reduced *E. coli* O157:H7 populations by 1.3 log CFU/g. In vitro studies using brain heart infusion (BHI) with added green tea extract at a concentration of 10 mg/mL reduced *E. coli* O157:H7 by 2.5 log CFU/mL after 24 h.[27] Juneja et al.[26] also found that the addition of 3% green tea extract rendered *E. coli* O157:H7 more sensitive to heat (57.7% reduction in *D* values) in sous-vide cooked ground beef at 62.5 °C. These findings suggest that the green tea extract facilitates the thermal destruction of *E. coli*.

No antimicrobial effect was observed with the added black tea extract. Our findings are consistent with those of Kim et

al.[28] and Turkmen et al.,[29] who attributed the low activity of black tea extract to the presence of lipopolysaccharides on the outer membrane of Gram-negative bacteria. This observation may not be a general one because we previously reported large variations in both catechin and theaflavin content in commercial green and black teas as well as the susceptibility of tea flavonoids to degradation during storage.[30,31] It is possible that the black tea extract used in the present study had low levels of bioactive flavonoids. Grape seed extract, however, exhibited antimicrobial activity by reducing bacterial populations by 2.2 log CFU/g. A strong antimicrobial effect of grape seed extract against *E. coli* O157:H7 has been seen when used in combination with tartaric acid.[27]

Spice Powders. The addition of 5% spice powders showed no antimicrobial effect and in some cases enhanced growth of *E. coli* O157:H7 (Figure 3). Onion powder appeared to have

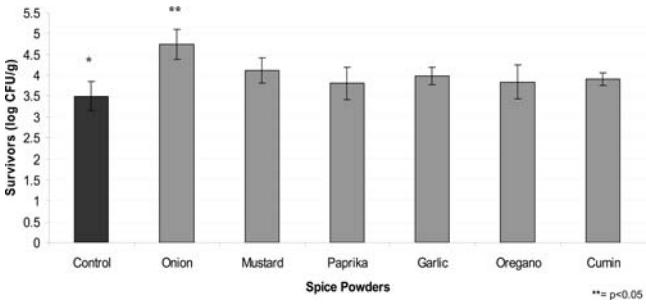

Figure 3. Effect of spice powders on *E. coli* O157:H7 in heated ground beef patties.

induced bacterial growth by 1.2 log CFU/g. Mustard powder also induced *E. coli* O157:H7 growth by 0.6 log CFU/g. The enhancement of bacterial growth suggests that these powders might provide added nutrients for the bacteria. By contrast, mustard flour or its active ingredient (allyl isothiocyanate) did exhibit antimicrobial activity against *E. coli* O157:H7 in unheated ground beef patties.[32,33]

Paprika, garlic, and oregano powders induced limited bacterial growth (≤0.5 log CFU/g) with no significant difference ($p > 0.05$) compared with control values. Sasaki et al.[34] found that the powder from freshly harvested garlic was more effective against *E. coli* O157:H7 than from a one-year-old garlic plant. Gupta and Ravishankar[35] observed no antimicrobial activity of fresh ground garlic or commercial garlic paste (5%) against *E. coli* O157:H7 in ground beef stored for two weeks at 4 °C. Essential-oil components are known to be more active than the powder form.[36] Oregano powder prepared from leaves contains only 2−3% essential oils and so is much less potent than oregano oil or carvacrol.

No antimicrobial effect was observed with added cumin powder. Arici et al.[37] tested various Turkish black cumin oils in vitro at concentrations of 0.5%, 1.0%, and 2.0% against *E. coli* O157:H7 (10^6 to 10^7 CFU/mL). The most active oil showed inhibition zones of 15 and 19 mm around *E. coli* O157:H7 colonies at concentrations of 1.0% and 2.0%, respectively.

In this study, we have demonstrated that powdered spices, which are more commonly used in the kitchen than essential oils, are highly effective in inhibiting the formation of heterocyclic amines, but are not active against *E. coli* O157:H7. In general, plant antimicrobials when applied to foods are less effective against *E. coli* O157:H7 than in laboratory media. The difference might be due to the complexity of the food matrix, changes in pH and water activity, slow-release lipophilic antimicrobials from the fat component, and increased heat resistance.

Essential Oils. Essential oils are known to exhibit antimicrobial effects.[36] In this study, three essential oils were tested for their antimicrobial activity against *E. coli* O157:H7 (Figure 4). Lemongrass oil exhibited high antimicrobial activity.

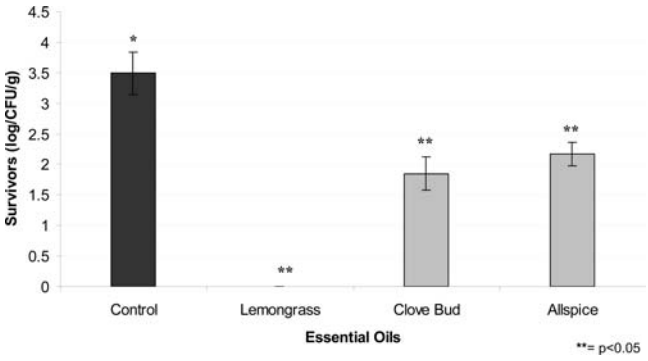

Figure 4. Effect of essential oils on *E. coli* O157:H7 in heated ground beef patties.

It reduced the bacterial population to below detection limits (3.5 log CFU/g). The addition of clove bud oil to ground beef patties decreased the *E. coli* O157:H7 population by 1.6 log CFU/g. In vitro studies by Barbosa et al.[38] showed that clove oil was the most effective essential oil for inactivating nonpathogenic *E. coli* and *Salmonella enterica*, followed by lemongrass oil. When tested on minced meat, however, the effectiveness of both oils was less than that observed in vitro. Added allspice oil decreased the *E. coli* population by 1.3 log CFU/g. Allspice oil and its active component eugenol were previously found to be effective in vitro against *E. coli* O157:H7.[39]

Inhibition of Heterocyclic Amines in Heated Hamburger Patties. Table 1 shows that there was a reduction in HCA levels with all treatments compared with those in untreated controls. HCA formation also varied across all heat-treated controls and treatments. Although MeIQx was formed in greater amounts than PhIP, the greatest inhibitory effect of the test compounds was on PhIP formation. The same effect was seen by Quelhas et al.[40] using green tea marinades on pan-fried beef (75% reduction in PhIP) and Melo et al.,[41] who observed a pronounced reduction in PhIP (83−88%) in pan-fried meat marinated with beer or white wine.

Plant Extracts. The addition of plant extracts at 5% significantly reduced the formation of HCAs in heated ground beef patties (Table 1). Rosemary extracts also significantly decreased the levels of MeIQx and PhIP in beef patties.[42]

Olive extract inhibited the formation of MeIQx and PhIP by 79.5% and 84.3%, respectively; and the corresponding reduction by apple extract was 76.1% and 82.1%. Compared with the control, green tea extract induced a decrease in MeIQx and PhIP by 31.4% and 86% and grape seed extract by 50.4% and 78.9%, respectively. Among all extracts tested, green tea treatment resulted in the greatest reduction in PhIP. Green tea contains up to 30% antioxidative catechins by dry weight.[43] Quelhas et al.[40] found that marinating beef in green tea for 6 h resulted in a significant decrease (75%) in the levels of PhIP, but no reduction in MeIQx content. Cheng et al.[44] found that a 0.1% (w/w) concentration of the green tea phenolic compound

Table 1. Reduction of Heterocyclic Amines in Ground Beef Patties Cooked for 5 min by Plant Extracts, Spice Powders, and Essential Oils

compound	heterocyclic amine	control ratio[a]	treatment ratio[b] (±SD)	reduction (%)
		Plant Extracts		
olive	MeIQx	155.8	31.9 ± 0.3	79.5
	PhIP	84.9	13.3 ± 1.0	84.3
apple skin	MeIQx	197.5	47.2 ± 15	76.1
	PhIP	92.1	16.4 ± 8.8	82.1
green tea	MeIQx	114.3	78.4 ± 8.4	31.4
	PhIP	119.4	16.7 ± 4.4	86.0
grape seed	MeIQx	125.3	62.1 ± 6	50.4
	PhIP	118.2	24.9 ± 1.8	78.9
		Spice Powders		
onion	MeIQx	124.6	27.3 ± 8.3	78.0
	PhIP	122.5	6.9 ± 0.93	94.3
paprika	MeIQx	106.8	33.2 ± 6.5	68.9
	PhIP	78.2	10 ± 0.4	87.2
garlic	MeIQx	56	18.9 ± 0.6	66.2
	PhIP	36	5.4 ± 1.3	85.0
oregano	MeIQx	129	65.1 ± 32.6	49.5
	PhIP	109.9	16 ± 10.1	85.4
turmeric	MeIQx	78.5	40.3 ± 3.4	48.6
	PhIP	83.8	22.1 ± 3.1	73.6
cumin	MeIQx	122.4	92.7 ± 8	24.2
	PhIP	114.6	55.3 ± 6.8	51.7
		Essential Oils		
clove bud oil	MeIQx	99.5	64.6 ± 7.3	35.0
	PhIP	75.2	36 ± 7.7	52.1

[a]Ratio of the signal intensity of each heterocyclic amine to the signal intensity of the internal standard. [b]$n = 3$. The average and standard deviation were calculated on the basis of three replicates.

epigallocatechin lowered the formation of PhIP in pan-fried beef by 30%.

Spice Powders. In general, spice powders had a lower anti-HCA effect than the plant extracts (Table 1). Onion powder was the most effective treatment, reducing PhIP by 94.3% and MeIQx by 78%. Dong et al.[45] showed that the addition of fresh-cut onion (2 g) to beef patties fried at 230 °C for 8 min on each side inhibited the formation of MeIQ and PhIP by 88% and 79%, respectively.

Paprika and garlic also decreased the formation of MeIQx by 68.9% and 66.2% and PhIP by 87.2% and 85%, respectively. Gibis et al.[46] observed up to a 28.6% reduction in MeIQx in fried beef patties treated with garlic marinades. Inhibition of HCAs was also observed with added oregano and turmeric. Other investigators found oregano to be effective at inhibiting PhIP.[47] Cumin, among all spices tested, displayed the lowest inhibiting effect.

Clove Bud Oil. MeIQx and PhIP content in heated ground beef patties with 1% clove oil were reduced by 35% and 52.1%, respectively (Table 1).

Related Studies. In related studies, (a) Persson et al.[48] found that frying beefburgers in virgin olive oil reduced the formation of HCAs compared to refined olive oil, presumably because the former has a higher content of phenolic compounds,[49] (b) Monti et al.[50] found that fresh-made olive oil which contained a high amount of phenolic compounds inhibited HCA formation in a model system to a greater extent than did a one-year-old olive oil, (c) Cheng et al.[51] found that apple, elderberry, and grape seed extracts reduced HCA formation by up to 70% in fried beef patties, (d) Smith et al.[52] found that commercial marinades reduced the formation of HCAs in grilled beef steaks by up to 88%, (e) Puangsombat et al.[53] reported that fingerroot, rosemary, and turmeric significantly decreased HCA formation in cooked beef patties and that the HCA inhibition was correlated with the total phenolic content and free radical scavenging activities of the spices, and (f) Shaughnessy et al.[54] found that chlorophyllin and yogurt reduced fried-meat-induced colorectal DNA damage associated with carcinogenesis.

Analytical Aspects. Here, we will briefly mention available information on the content of bioactive compounds in apple skin, olive, green tea, and grape seed extracts, onion powder, and clove bud oil which we found to inhibit both *E. coli* and HCAs (Table 1, Figures 1−4).

According to the manufacturer (private communication), the commercial olive powder used in the present study, Hidrox-12, was prepared by freeze-drying an organic olive water extract. The powder consists of 98−99% olive pulp and 1−2% citric acid and contains a 12% mixture of bioactive phenolic compounds. We previously reported that the same batch of the extract inhibited *S. enterica* on organic leafy greens[55] and *Staphylococcus aureus* and the *Staphylococcus* enterotoxin A (SEA) in laboratory media.[56] According to the manufacturer (private communication), the apple skin extract used in the present study contains an 82% concentration of a mixture of phenolic compounds. In previous studies, we reported that this extract inhibited *S. enterica* on organic leafy greens[55] and *Listeria monocytogenes* after incorporation into edible apple films[57] and also facilitated thermal destruction of *E. coli* O157:H7 in cooked ground beef.[26] Unlike fresh onions, 16 dehydrated commercial onion powders contained no or very low levels of flavonoids,[58] suggesting that the inhibition of *E. coli* and HCAs by an onion powder observed in the present study may not be due to flavonoids. It would be of interest to compare the antimicrobial and anti-HCA effects of high-flavonoid fresh onions and low-flavonoid onion powders. We also reported that the content of seven polyphenolic compounds (catechins) of a large number of green teas varies widely,[30] suggesting that it would also be of interest to compare the antimicrobial and anti-HCA activities of low- and high-catechin green teas. Grape seed extracts are reported to be a rich source of bioactive flavonols and proanthocyanidins.[59,60] The clove bud oil evaluated in the present study which contains up to 90% eugenol[61,62] inactivated *E. coli* O157:H7 and *S. enterica* in apple juice and inhibited *E. coli* O157:H7, *S. enterica*, and *L. monocytogenes* after incorporation into edible apple films.[61,63] The activity of eugenol against the same pathogens in apple juice was identical to that of clove bud oil.[61] The following references offer an entry into the literature on the composition of spices evaluated in the present study: allspice,[64] cumin,[65] garlic,[66] mustard,[67] paprika,[68] and turmeric.[69]

It is also relevant to note that the content of bioactive compounds of plant extracts such as apples, olives, and onions is influenced by several factors, including variety, environment, soil fertility, and geographic origin.[70,71] Therefore, the antimicrobial and antiheterocyclic amine effects of a specific extract may not always be the same when compared to those of other extracts prepared from plants harvested at different locations and environments. Moreover, knowledge of the composition of multiple components of an extract does not allow relating the bioactivity to a specific compound in the

extract since the effect may be due to additive or synergistic effects of two or more components. This aspect merits further study.

In conclusion, overall, olive and apple skin extracts and clove bud essential oil exhibited both antimicrobial and anti-HCA activities against *E. coli* O157:H7 and HCAs (MeIQx and PhIP), respectively. Among all treatments, olive extract and lemongrass oil were the most effective antimicrobials against *E. coli* O157:H7, reducing the bacterial population to undetectable levels, whereas olive extract and onion powder had the greatest inhibitory effects on HCA formation. Indeed, olive extract treatment resulted in the greatest reduction in the levels of MeIQx (79.5%) and onion powder was the most successful treatment in reducing PhIP levels (94.3%). Our results demonstrate that the addition of plant compounds to uncooked ground beef has the potential to improve the microbial food safety of heated ground beef patties and to contribute to cancer prevention in humans. Additional studies using animal models are needed to confirm the dual antimicrobial and anti-HCA beneficial effects observed in the present study. Studies of sensory (organoleptic) properties of plant-compound-containing hamburgers would also benefit consumers.

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