

Processes to Preserve Spice and Herb Quality and Sensory Integrity During Pathogen Inactivation

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Abstract: Selected processing methods, demonstrated to be effective at reducing *Salmonella*, were assessed to determine if spice and herb quality was affected. Black peppercorn, cumin seed, oregano, and onion powder were irradiated to a target dose of 8 kGy. Two additional processes were examined for whole black peppercorns and cumin seeds: ethylene oxide (EtO) fumigation and vacuum assisted-steam (82.22 °C, 7.5 psia). Treated and untreated spices/herbs were compared (visual, odor) using sensory similarity testing protocols ($\alpha = 0.20$; $\beta = 0.05$; proportion of discriminators: 20%) to determine if processing altered sensory quality. Analytical assessment of quality (color, water activity, and volatile chemistry) was completed. Irradiation did not alter visual or odor sensory quality of black peppercorn, cumin seed, or oregano but created differences in onion powder, which was lighter (higher L^*) and more red (higher a^*) in color, and resulted in nearly complete loss of measured volatile compounds. EtO processing did not create detectable odor or appearance differences in black peppercorn; however visual and odor sensory quality differences, supported by changes in color (higher b^* ; lower L^*) and increased concentrations of most volatiles, were detected for cumin seeds. Steam processing of black peppercorn resulted in perceptible odor differences, supported by increased concentration of monoterpene volatiles and loss of all sesquiterpenes; only visual differences were noted for cumin seed. An important step in process validation is the verification that no effect is detectable from a sensory perspective.

Keywords: ethylene oxide, irradiation, sensory, spice, steam

Practical Application: Validated pathogen inactivation processes for herbs and spices influence color and odors in ways that can influence differences in sensory perception. This study demonstrated that some validated processes do not cause a detectable difference in sensory quality, thus providing additional evidence of potential value for application.

Introduction

Spices and herbs are ubiquitous in food systems, utilized for adding culinary interest, improving quality, preserving food, and extending shelf-life. Contamination of herbs and spices with pathogenic bacteria is well recognized, attributed in part to growing conditions and environment, sanitation and hygiene practices among harvest workers, and lack of good agricultural and manufacturing practices within some developing countries. Bacterial loads on untreated spices are high, readily within 10^4 to 10^7 cfu/g (Buckenhüskes 2001), introducing food safety and spoilage risks to fresh and processed prepared foods. Foodborne illness outbreaks ($n = 14$) linked to *Salmonella* and other bacterial pathogens has been attributed to spices (FDA 2013). Processing of herbs and spices prior to use in food systems provides a value for protecting food quality and safety, especially related to ready-to-eat foods, which do not receive additional processing (Little and others 2003). However, providing this additional food safety processing step can degrade herb and spice quality by altering volatile and flavor chemistry and color.

Processing methods for spices and herbs include fumigation with ethylene oxide (EtO), irradiation, and vacuum-assisted steam. While fumigation with EtO has been shown to significantly reduce microbial populations on spices (Leistritz 1997), some EtO-treated spices have been shown to undergo alterations in flavor and color (Vajdi and Pereira 1973). Volatile oil contents of black pepper and allspice were reduced by more than half when treated by EtO (Vajdi and Pereira 1973). Volatile compounds are lost due to the low pressure needed to remove EtO (Vajdi and Pereira 1973; Almela and others 2002).

Vacuum assisted-steam processing has been used with greater frequency due to greater consumer acceptance of this process than EtO or irradiation. However, there may be a decrease in quality from the high temperatures typically used in this process. This method of decontamination is associated with reduction of volatile oil content and some discoloration of spices (Modlich and Weber 1993; Almela and others 2002; Rico and others 2010).

Irradiation has been described as an effective, energy-efficient method for decontaminating spices (Schweiggert and others 2007). However, higher doses of ionizing radiation have been shown to change the physical and antioxidative properties of food products (Suhaj and others 2006; Chauhan and others 2009). Consumers have not been readily accepting of any product that has been treated with gamma rays, electron beam, and X-rays.

When selecting a treatment method, it is important to verify that the method successfully inactivates the targeted pathogen in order to deliver a safe product to the consumer. While safety is paramount, the validated process must also yield a product that matches in quality and is competitive in price. The

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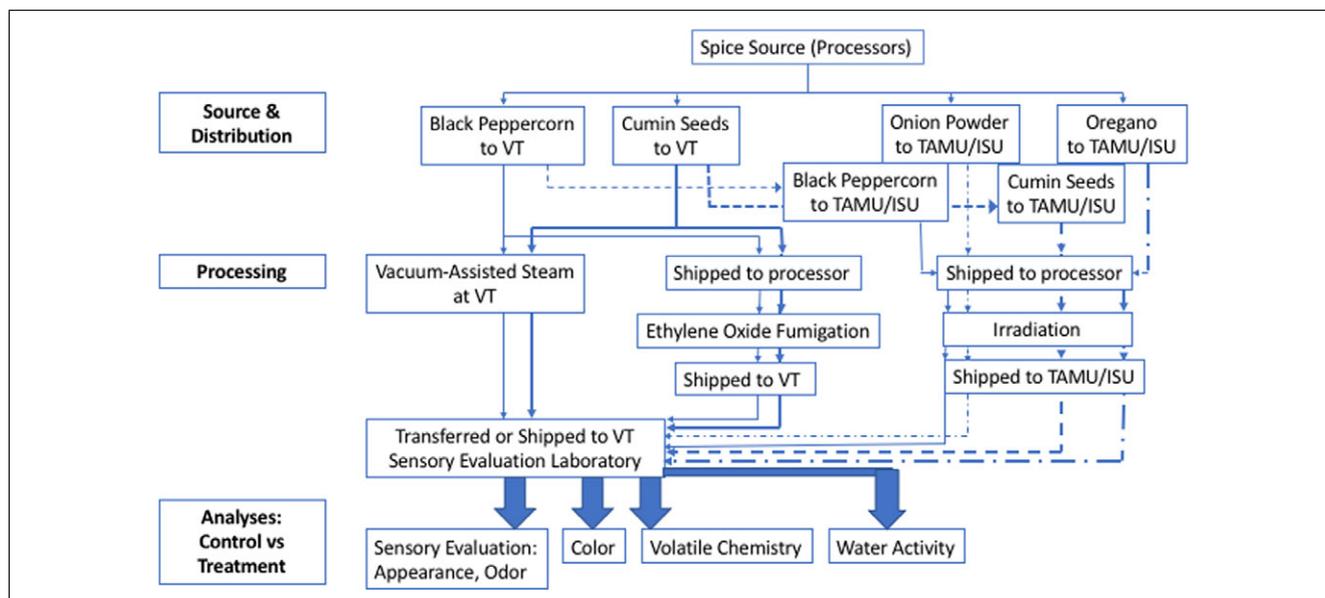


Figure 1—Handling, processing, storage, and assessment of spices for this study.

objective of the study was to determine if various processing methods, at conditions demonstrated to inactivate *Salmonella*, applied to selected spices and herbs yield a product that is not different from the untreated (control) product based on sensory parameters (color, odor), with supportive analytical assessment based on volatile chemistry and selected physical attributes (color, water activity (a_w)).

Materials and Methods

Spice and herb processing

Figure 1 illustrates the handling, processing, storage, and assessment methods for the experiment.

Materials and storage conditions. Bulk spices and herbs (whole black peppercorns (source location: India), cumin seeds (Madagascar), onion powder, oregano (Turkey)) were provided by national spice processors without further processing. Peppercorns and cumin seeds were shipped to the Virginia Tech Food Science and Technology Dept. (VT FST) food microbiology laboratory. Peppercorns and cumin seeds, from the same lot used at Virginia Tech, were shipped from VT FST to Animal Sciences Dept. of Iowa State Univ. (ISU) and Texas A&M University (TAMU). Onion powder and oregano were shipped to ISU and TAMU directly from spice processor as these spices were not evaluated by EtO or vacuum-assisted steam.

VT FST evaluated conditions associated with inactivation of *Salmonella enterica* on black peppercorns and cumin seeds using vacuum-assisted steam and EtO fumigation processes (Newkirk 2016). ISU and TAMU assessed irradiation process efficacy on *Salmonella* on black peppercorns, cumin seeds, onion powder and oregano. Based on these initial studies, efficacious processes were selected to determine if processes caused notable sensory or physical quality changes to the products.

Storage conditions prior to receipt of herbs and spices were comparable between each control and the treated spice/herb at the respective university microbiology laboratory locations; control spices/herbs did not go through shipping/storage conditions for EtO or irradiation processing as did the treated spices/herbs. One lot each of freshly processed products and comparable controls

were provided to the Virginia Tech Sensory Evaluation Laboratory (VT SEL) directly from the respective university microbiology lab in the Virginia Tech Dept. of Food Science and Technology within a few days after processing. Upon receipt by VT SEL, products were stored in a dry, dark environment at room temperature (21 °C) until testing. Control (untreated spice or herb samples) and treated products were stored in sealed double layer zip lock plastic storage bags (Kroger brand, Cincinnati, Ohio, U.S.A.).

Ethylene oxide. Polywoven polypropylene bags of black peppercorn and cumin seeds (2.27 kg (5lbs) per spice) were processed with ethylene oxide by Steris Corporation (South Plainfield, N.J., U.S.A.) per U.S. EPA label specifications using their proprietary process. U.S. EPA label specifications are as follows: spices were placed in the chamber and a mixture of ethylene oxide and air compatible with the chamber design was introduced into the chamber at a concentration of ethylene oxide not to exceed 500 mg/L, with a dwell time not to exceed 6 h. The gas was then evacuated from the chamber using a sequence of not less than 21 steam washes (injections and evacuations) between 1.5 PSIA and 2.0 PSIA while maintaining a minimum chamber temperature of 46.11 °C (Honeywell 2008).

Vacuum assisted-steam. Black peppercorn and cumin seeds (50 g and 40 g, respectively) in muslin sachets were placed in a lab-scale kettle wrapped with heat tape to stabilize temperature and fitted with a steam generator. Temperature was recorded using T-type thermocouples and logged using a data logger (Omega, model RDXL6SD, Stamford, Conn., U.S.A.) while pressure was measured using the pressure gauge. Preconditioning in absence of spices was performed to increase air temperature. Processing was achieved by applying a vacuum to 4.7 psia followed by using steam injection until a measured temperature of the air achieved a temperature of 85 °C and 7.5 psia. The dwell time then occurred for 2 min for black peppercorns and 1 min for cumin seeds followed by a vacuum pulled to 4.7 psia to prevent any condensation from forming in the kettle. Pressure was slowly released with an air inbleed. These conditions were previously demonstrated to result in a 5-log reduction of *Salmonella* (Newkirk 2016). No inoculated spices were processed at the same time as samples used for sensory analysis.

Irradiation. Oregano, onion powder, black peppercorn, and cumin seeds were irradiated (Steris Isomedix Services Radiation Technology Center, Libertyville, Ill., U.S.A.) to a target dose of 8 kGy in paper bags. Dose uniformity ratio (DUR) is the ratio of the maximum dose to the minimum dose. A commercially irradiated shipment of spices might have a DUR in the range of 1.2 to 1.5, depending on product density and configuration. The minimum dose was 8.22 and maximum was 8.42 kGy for black peppercorn, with a DUR of 1.02. For the remainder of the spices/herbs, the minimum was 8.08 and the maximum was 8.41, with a DUR of 1.04 irradiated spices and herbs were delivered to VT SEL in paper bags, then transferred to double layer plastic bags for storage.

Sensory assessment of spice quality retention

Spices and herbs were evaluated for sensory and physical parameters within 2 wk of processing unless otherwise stated. This window of time accounted for the time from processing, receipt by the respective university laboratory, and delivery to the VT SEL. Spices go through a variety of steps over several months, from harvest through processing and packaging, with additional storage and transportation time needed for products to reach the consumer (Schweiggert and others 2007). Therefore, while changes in sensory quality may have occurred between processing and sensory assessment, the changes would not reflect any practical concern for spice processors or for those purchasing the spice.

Sample preparation. A 1% spice or herb infusion was prepared. Spice/herb (40 g) was added to hot water (4000 mL; 87.7 °C) and maintained at temperature for 5 minutes, then strained and discarded. Infusion (20 mL samples) was filled into amber glass bottles (30 mL; Wheaton, Millville, N.J., U.S.A.) and sealed. Samples were stored in a dark refrigerator for 12 to 18 h. Prepared samples were removed from refrigerator approximately 2h before testing to temper to room temperature (20.5 ± 1 °C). Spices/herbs (2 g) used for visual appearance evaluation were measured and placed in clear glass bottles (40 mL clear vial, screw top; Supelco, Bellefonte, Pa., U.S.A.), capped, and stored in the dark at room temperature until sensory testing. The control samples for each comparison were the untreated products provided to the VT Sensory Evaluation Laboratory by the respective microbiology laboratories.

Similarity testing. Sensory analysis on spice/herb appearance and odor was performed by triangle testing for similarity (Meilgaard and others 2007). Similarity testing was used to provide confidence that no discernible difference was evident between the unprocessed and the processed spice. Each processed spice was compared to the control spice from the same lot. Samples were identified by 3-digit codes.

Two spices (cumin seed, black peppercorn), processed by the same method (EtO, vacuum assisted-steam, or irradiation), were evaluated on the same day; participants, therefore, received 4 triangle sets, with appearance followed by odor sets for a given spice. Sensory testing for irradiated onion powder and irradiated oregano were completed on the same day. On a given day of evaluation, the order of spice presentation was balanced across participants so there was no fatigue or bias created by sequence of spices. Time between sample sets was approximately 30 to 45 s. Order of samples within a set was randomized.

Pre-established statistical parameters for similarity testing included $\alpha = 0.20$, $\beta = 0.05$, and a proportion of discriminators (p_d) estimated at 20%. The minimum number of panelists needed for the targeted power was 84. The number of observations achieved

for each comparison varied, with 88 to 90 observations for EtO- and vacuum-assisted steam-treated spices and 74 to 82 responses for irradiated spices (Table 1). Significant differences were determined as described in Meilgaard and others (2007).

This study was approved by the Virginia Tech Institutional Review Board (IRB # 13-757). All participants provided consent on each day prior to participating in any sensory testing. Participants were from the university and local community, which influenced our selection of a p_d of 20%. There was no training provided, other than basic directions for completing the test. In general, we assume that the tested participants reflected the abilities of a general population although no demographics were collected and no prescreening acuity were assessed.

Panelists were seated in partitioned booths with white LED light (Gotham Architectural Downlighting, Conyers, Ga., U.S.A.) and equipped with touch screen monitors. Sensory Information Management Systems (SIMS 2000, Sensory Computer Management, Morristown, N.J., U.S.A.) recorded individual responses and provided a statistical summary of outcomes for each test.

Statistical analyses. Sensory data was analyzed based upon statistical parameters mentioned above and equations outlined in Meilgaard and others (Meilgaard and others 2007). The critical number of correct responses (based on 84 responses) for significant difference was 35. The critical number for each test was dependent on the number of observations provided.

Analytical evaluation of spice quality

Water activity. Water activity readings, on two samples for each treatment, was determined using a Aqualab CX-2 Water Activity Meter (Decagon Devices, Pullman, Wash., U.S.A.). Readings from irradiated products were conducted several months after processing; initial readings conducted at time of sensory testing were inaccurate. We attribute this to aromatics condensing on the surface of the chilled mirror and altering the readings (<http://www.aqualab.com/assets/Manuals/AquaLabCX-2v3.pdf>). Polypropylene glycol, sometimes used in oleoresin of black pepper (Tainter and Grenis 2001), is known to interfere with the instrumentation.

Color evaluation. Color was measured with a hand-held Minolta CR-300 Chroma Meter (Osaka, Japan) in the CIE $L^*a^*b^*$ color scale, in triplicate for each sample ($n = 2$ samples per spice per process treatment). Color readings were taken on a sheet of white paper and through clear Plexiglas to ensure a flat surface for color readings and prevent spice dust from contaminating the lens of the chromameter.

Volatile chemistry. Solid phase microextraction (SPME) samples were analyzed on a Shimadzu GC-MS (Model QP-2010 Ultra, Shimadzu Co., Kyoto, Japan) using a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (50/30 μm , 2 cm length) (Supelco, Bellefonte, PA). An autosampler (Model AOC-5000, Shimadzu Co., Kyoto, Japan) was used to move samples through the extraction and injection processes. Samples were extracted for 30 min at 40 °C, then injected and desorbed for 5 min in a 240 °C injection port, with a split ratio of 1:20. The fiber was conditioned for 2 min after each sample injection/desorption. The compounds were separated on a Phenomenex Zebron Carbowax column (60 m \times 0.25 mm \times 0.25 μm ; Torrance, Calif., U.S.A.) using helium as a carrier gas (1 mL/min, constant flow). The oven temperature program began at 40 °C (isothermal for 0.5 min) and was ramped up to 240 °C at a rate of 8 °C/min. Samples were analyzed on two samples per spice per process treatment. Identification of peaks was done by

Table 1—Differences in visual appearance and odor between control and ethylene oxide, vacuum assisted-steam, or irradiation-treated spices (black peppercorn, cumin seed, onion powder, and oregano) based on sensory similarity testing.

Spice	Test	Process								
		Ethylene oxide (EtO)			Vacuum assisted-steam			Irradiation		
		# of responses		P-value	# of responses		P-value	# of responses		P-value
		Correct	Total		Correct	Total	Correct	Total		
Peppercorn	Appearance	25	89	0.878	35	90	0.157	24	81	0.794
	Odor	28	88	0.657	40	90	0.018*	34	83	0.089
Cumin Seeds	Appearance	42	90	0.008*	41	89	0.009*	34	82	0.076
	Odor	42	90	0.006*	35	90	0.157	31	82	0.227
Onion Powder	Appearance	NA						51	74	<0.0001*
	Odor							40	74	0.0002*
Oregano	Appearance							23	75	0.700
	Odor							25	75	0.543
# panelists		88-90			89-90			74-82		
Critical value		35			35					

Statistical parameters for similarity testing: proportion of discriminators = 20%; $\alpha = 0.20$; $\beta = 0.05$; indicates a *P*-value <0.05
NA, not applicable.

comparison to NIST 11 Spectral Library (<http://www.nist.gov>). Evaluation was completed within 3 mo after receiving the products.

Statistical analyses. Means and standard deviations were calculated for each spice (control, treatment) for each process. Comparison between control and treatment for each spice and process was completed for color values and for d_w by *t*-test with significance established at $\alpha = 0.05$.

Results and Discussion

Sensory assessment of spice and herb quality retention

The spices we evaluated had been implicated in *Salmonella* outbreaks or recalls and represented a variety of forms (whole, ground, leaf/flake) (FDA 2013). The processes and selected parameters had been validated for efficacy against *Salmonella* on the selected spices (Newkirk 2016). The optimum outcome from this project was identification of a processing method, designed to inactivate *Salmonella*, that did not cause distinctive differences in visual or odor sensory quality or analytical measures of quality for a variety of spices. The sensory design minimized type II error while retaining a relatively low type I error, thus providing an interpretation that the probability of a difference being discernible to the broader population was low.

When more participants than the calculated critical value (Meilgaard and others 2007) could discern a difference between the control and processed samples within a comparison ($P < 0.05$), then the process was not effective at preserving sensory quality. An effective process for a specific spice or herb would not create an obvious difference in either visual appearance or odor quality. Irradiation processing met these criteria for three spices (black peppercorn, cumin seed, and oregano); irradiated onion powder was different in both appearance and odor from the control (Table 1). Black peppercorn processed by EtO retained both visual and odor quality similar to the control; both visual and odor quality were different in EtO-processed cumin seed. Quality differences were noted in both black peppercorn (similar in appearance; difference in odor) and cumin seed (similar in odor; difference in appearance) subjected to the vacuum assisted-steam process.

The sensory profile of processed spices may change due to the influence of chemical and enzymatic conditions promoted by the process. The breakdown of plant or seed tissue, change in pressure, oxygen, moisture in an environment, or elevated temperature or

light can increase the potential for oxidation (Schweiggert and others 2007). The lexicons for various spices have been published, illustrating the sensory contributions of low and high quality spices and herbs for foods (Lawless and others 2012). Black pepper, for example, is very complex, with a primary basic taste (bitter) contribution, a feeling factor (heat), woody character (cedar/woody, pine), earthy/dirty/musty notes (cardboard, musty), soapy and terpene characteristics noted in the spice (Lawless and others 2012). Onion powder has more sulfur character (fruity sulfur, hydrogen sulfide, rubbery sulfur, old sulfur/vegetative) and with both sweet and bitter basic tastes, metallic and pungent feeling factors, cooked (toasted) and earthy/dirty/musty (musty/cardboard) notes (Lawless and others 2012). Changes in process that influence sensory profile can influence culinary application in a food system. Changes in chemical and enzymatic function may also alter potential bioactive contributions of antioxidants.

As the purpose of our study was to validate that the processed product matched the control (preprocessed) product, we chose a difference test in similarity mode. This approach, which is easy and can utilize untrained or experienced panelists, is targeted when establishing that two products are so similar that no discernible differences are noted (Meilgaard and others 2016). We assumed that the control product had the high-quality characteristics desired of the final processed product. Therefore, by establishing that no discernible difference was evident, we can interpret that the treatment process did not change the visual appearance or odor quality of the tested spices. This is a very effective first step in the process of making decisions for selection of appropriate processes, recognizing that this assessment did not verify if any changes in flavor resulted in the spice and how it influenced any flavor effects during application. A limitation is that, when sensory differences are noted, then this method does not provide sufficient information to describe the differences. We cannot ascertain if differences affected consumer perception of quality in a way that would diminish acceptability for the pure spice or for its applications. For example, further evaluation is needed to determine if changes in visual appearance would influence consumer decision to purchase or use the spice. We also cannot interpret how the products differed based on our sensory methods alone. In such a situation, further testing, such as a descriptive sensory methods and analytical testing, is warranted. Descriptive testing, using established lexicons and reference standards for training panelists (Lawless and others 2012), is time intensive and expensive but has value in

Table 2—Mean (\pm SE) $L^*a^*b^*$ with T-test for control and ethylene oxide, vacuum assisted steam, or irradiation-treated spices (black peppercorn, cumin seed, onion powder, oregano).

Spice	Test	Process					
		ethylene oxide					
		L	t-Test	a*	t-Test	b*	t-Test
Peppercorn	Control	19.71 \pm 0.05	0.0819	2.90 \pm 0.63	0.144	5.68 \pm 1.23	0.0142*
	Treatment	19.02 \pm 1.00		3.73 \pm 1.40		6.90 \pm 0.17	
Cumin Seeds	Control	47.32 \pm 0.01	0.0006*	3.44 \pm 0.12	0.451	10.53 \pm 1.03	0.0004*
	Treatment	45.10 \pm 1.43		3.61 \pm 0.62		20.14 \pm 0.53	

Spice	Test	Process					
		Vacuum Assisted-Steam					
		L	t-Test	a*	t-Test	b*	t-Test
Peppercorn	Control	22.6 \pm 0.48	>0.0001*	4.15 \pm 0.00	0.0037*	9.16 \pm 0.08	0.0003*
	Treatment	18.63 \pm 0.34		3.64 \pm 0.05		7.17 \pm 0.41	
Cumin Seeds	Control	45.24 \pm 1.31	0.0136*	3.91 \pm 0.18	0.0829*	17.93 \pm 0/91	>0.0001*
	Treatment	46.54 \pm 0.31		4.21 \pm 0.45		21.22 \pm 0.08	

Spice	Test	Process					
		Irradiation					
		L	t-Test	a*	t-Test	b*	t-Test
Peppercorn	Control	20.96 \pm 3.80	0.568	2.90 \pm 0.92	0.084	5.69 \pm 2.81	0.88
	Treatment	19.96 \pm 1.18		3.88 \pm 0.88		5.85 \pm 1.24	
Cumin Seeds	Control	45.21 \pm 0.85	0.024*	4.36 \pm 0.15	0.529	17.35 \pm 0.35	0.46
	Treatment	44.05 \pm 1.01		4.29 \pm 0.30		17.17 \pm 0.62	
Onion Powder	Control	79.89 \pm 4.28	0.004*	-1.22 \pm 0.09	8.41722E-11*	11.81 \pm 0.47	0.30
	Treatment	86.19 \pm 0.00		0.71 \pm 0.18		12.99 \pm 0.44	
Oregano	Control	51.66 \pm 1.94	0.812	0.15 \pm 0.22	0.44	24.74 \pm 0.08	0.30
	Treatment	51.50 \pm 0.71		-0.06 \pm 0.78		25.01 \pm 0.76	

*Indicates a P -value <0.05 ; $n = 2$ samples, each measured in triplicate.

providing further elucidation of specific changes in qualitative and quantitative aspects, order of appearance, and the overall impression delivered by the spice or herb (Meilgaard and others 2016). We chose to use analytical assessment to provide further detail of differences.

It is valuable to note that all questions cannot be answered by a single sensory test method. The decision about appropriate selection of sensory testing methods is contingent on the purpose of the study (Meilgaard and others 2016). Guidance by a trained sensory professional improves the potential of meeting the study goals; several test methods may be required to meet all of the specific goals for research or for industry application. Future research on sensory description of differences may be warranted but was not critical for this project. Future research also could establish the importance of differences to overall quality and culinary use and applications. Discerning the importance of the quality attribute, as supported by consumer perception in real application context, helps establish if the differences are reducing the value of the spice or are not of importance. Integration of descriptive information with consumer perception using advanced statistical methods is helpful for creating insight (Meilgaard and others 2016).

Analytical evaluation of spice and herb quality

Visual differences. Spices and herbs contribute visual effects to foods through natural pigments and the size of the seed, flake, or leaf. Retaining color characteristics in spices and herbs is important so as to reduce discoloration of the final food product. Appearance differences were noted for EtO and vacuum assisted-steam processed cumin seeds as well as for irradiated onion powder (Table 2). EtO resulted in darker (lower L^*), more yellow (increase

in b^*) cumin seeds. Cumin seeds processed by vacuum assisted-steam had a significant increase ($P < 0.05$) in L^* , a^* , and b^* . Irradiated onion powder was lighter (higher L^*), and more red (higher a^*) than the control.

The brown/black color of black pepper is attributed to the phenol structures due to enzymatic oxidation of (3,4-dihydroxy phenyl) ethanol glycoside (Zachariah and Parthasarathy 2008)/ While no visual sensory differences were observed for any other sensory comparisons, there were some color measures that were different. Peppercorn had a significant increase in b^* after processing with EtO ($P < 0.05$). Vacuum assisted-steam processed peppercorns had lower L^* , a^* , and b^* values compared to control ($P < 0.05$). Significant differences were found in irradiated cumin seeds, which had a lower L^* . While differences in color measurement may be relatively small, the combination of differences may be observed when comparing to a control. However, if no comparison were made, the differences may not be of great importance.

Differences in color as an effect of growing, drying, and storage conditions is expected. Variation within a lot is undoubtedly lower but may also exist. In this study, comparison of control products as provided for sensory testing illustrates that variation in color exists. We managed variation between control and treated spice or herb by using the same lot of product stored under similar conditions.

Volatile chemistry. The application of various processes is recognized to alter color and aroma by affecting the volatile composition (Schweiggert and others 2007). Wide variation in chemical composition of spices also can be attributed to cultivar, variation in the agricultural climate, maturity of raw material, and methodology of extraction and analyses (Zachariah and

Table 3—Changes in volatile compounds between controls and ethylene oxide, vacuum assisted steam, or irradiation-treated spices based on GC/MS analysis (n = 2).

Compound	Peppercorn			Cumin			Onion Powder Irradiation	Oregano Irradiation
	Steam	Ethylene oxide	Irradiation	Steam	Ethylene oxide	Irradiation		
α -Pinene	○	○	○	↑	↑	○		
Camphene								↑
β -Pinene	○	○	○	○	↑	○		
β -Phellandrene	○	○	○	↑	↑	○		
3-Carene	○	○	○					
β -Myrcene	○	○	↑	↑	↑	○		
α -Phellandrene	○	○	○	○	○	○		
D-limonene	○	○	○	○	○	○		
γ -Terpinene				○	↑	○		
o-Cymene	○	○	↑	○	○	○		
Terpinolene	○	○	↑					
α -Cubebene	○	○	○					
δ -Elemene	○	○	○					
α -Copaene	○	○	○					
Linalool								↑
Caryophyllene	○	○	○	○	○	○	○	
α -Caryophyllene	○	○	○					
β -Bisabolene								○
Cuminaldehyde				○	○	○		
2-Carene-10-al				○	○	○		
Anethole				○	○	○		
Caryophyllene oxide	○	○	○					
Carotol				○	○	○		
Thymol								○
Carvacrol								○

○ = presence of the compound in treated sample with less than 2-fold difference relative to control sample.

↑ = greater than 2-fold increase in compound over control sample.

↓ = greater than 2-fold decrease in compound over control sample.

Grey cell = compound not present in control or treated sample.

Parthasarathy 2008). Pressure used to remove EtO may increase the loss of volatiles. There is evidence that irradiation processes can modify the antioxidative properties of spices, which may contribute to increased oxidation of volatile oils. Application of steam requires that moisture must be removed; discoloration and reduction of volatile oils can occur, especially when applied to ground spices (Schweiggert and others 2007). Changes in volatile composition, whether by introduction of breakdown products, increase of oxidation end products, or loss of aroma-active volatiles, can reduce odor and flavor impact. Subtle changes can influence the odor and flavor quality, thus altering the value as a flavor ingredient.

Black peppercorn. The volatile chemistry of black pepper is complex. Kapoor and others (2009) reported 54 components extracted from black pepper essential oil with B-caryophyllene (29.9%) as the major component and limonene (13.2%), β -pinene (7.9%), sabinene (5.9%) as other volatiles found at high concentration. Mono- and sesquiterpenes are important contributors to the fine, pleasant pepper aroma; pinene and limonene side notes are also important (Jirovetz and others 2002). Jirovetz and others (2002) detailed the volatile profile of pepper (*P. nigrum*, *P. guineense*) essential oils using analytical volatile methods and further differentiated the odor impact by olfactometry. Change in odor impact of volatiles can differentiate black pepper.

We detected a total of 15 compounds at measurable levels during analysis of the various controls and treatments of peppercorns (Table 3; Table S1). Library identification of the compounds was confirmed by comparison of retention indices to previous findings (Jeleń and Gracka 2015). Odor descriptors are provided parenthetically for most identified volatiles, as reported by Jirovetz and

others (2002). Important monoterpene hydrocarbons included α -pinene (pine-like, sharp, woody, turpentine-like), β -pinene (dry-woody, pine-like, resinous-terpene-like, spicy), β -phellandrene (peppery, minty, refreshing, citrus-like), 3-carene (sweet, refined-limonene-like, spicy) and D-limonene (fresh, citrus-like, mild lemon, orange-notes). Caryophyllene (woody, spicy, terpene-like notes) was the most abundant sesquiterpene hydrocarbon as well as the largest volatile component overall at 24% relative to other identified compounds.

Pepper oils prepared by steam distillation are composed primarily of monoterpene hydrocarbons (70% to 80%) with sesquiterpene hydrocarbons composing most of the remaining volatiles (Zachariah and Parthasarathy 2008). Significant differences were found in odor between black peppercorn control and peppercorns processed by vacuum assisted steam. Vacuum assisted-steam treated samples showed concentration of most monoterpenes and loss of all sesquiterpenes compared to control samples, with the exception of δ -elemene. Samples treated with EtO showed concentration of most monoterpene compounds, with the exception of α -phellandrene (herbaceous, minty, peppery-woody, fresh, citrus) and terpinolene (sweet-piney, oily, petroleum-like). Some sesquiterpenes showed very little change (α -copaene (vegetable-like, hops-like, woody), caryophyllene and caryophyllene oxide (weak woody, warm, mild, weak spicy)), while others showed around 11% to 12% loss over the controls. Samples treated by irradiation showed significant concentration of all monoterpenes, ranging from 50.5% to 269.3% recovery as compared to control samples. Among sesquiterpenes, some showed minor concentration, some very little change and one a minor loss (α -caryophyllene).

Table 4—Mean¹ and standard deviation in water activity (a_w) control and ethylene oxide, vacuum assisted- steam, or irradiation-treated spices (black peppercorn, cumin seed, onion powder, oregano).

Spice	Process											
	Ethylene oxide				Vacuum assisted-Steam				Irradiation			
	Control	SD	Treated	SD	Control	SD	Treatment	SD	Control	SD	Treatment	SD
Peppercorn	0.596 ^a	± 0.000	0.327 ^b	± 0.004	0.563 ^a	± 0.001	0.436 ^b	± 0.006	0.619 ^a	± 0.000	0.448 ^b	± 0.001
Cumin Seeds	0.587 ^a	± 0.005	0.315 ^b	± 0.002	0.567 ^a	± 0.002	0.443 ^b	± 0.005	0.621 ^a	± 0.000	0.462 ^b	± 0.000
Onion Powder	NA		NA		NA		NA		0.396 ^a	± 0.004	0.430 ^b	± 0.001
Oregano	NA		NA		NA		NA		0.477	± 0.085	0.497	± 0.067

¹($n = 2$).^{a,b}Means within a process treatment for a given spice are significantly different at $P < 0.05$.

Overall, peppercorns treated with EtO showed less chemical changes in essential oil compound concentrations than vacuum assisted-steam treated or irradiated samples. A change in odor profile based on olfaction may be attributed to changes in odorant concentration such as a loss of or changes in proportional concentration of odor-active molecules. Highly odor-active compounds, even at low concentrations, can have a significant effect on the odor profile of a product. Compounds with lower odor impact, even if present in high concentration, may be less critical to the change in odor (Rice and Koziel 2015). Therefore, identifying changes in volatile concentration of the spice due to processing does not provide a complete interpretation of the quality effect. Elucidation of odor-impacting compounds, using gas chromatography-olfactometry, in tandem with human olfaction (sensory detection) of the product can provide validation. Further work is warranted for identifying which odor-active compounds were most critical to the changes in steam distilled black peppercorns and other spices that illustrated odor differences.

Cumin seeds. Cumin bouquet is strong, heavy and warm with a spicy-sweet aroma (Azeez 2008). The major volatile compounds in cumin are monoterpene hydrocarbons (30% to 50%); aldehydes and ketones are also major contributors (50% to 70%) (Weiss 2002), whereas sesquiterpenes are minor in concentration (Sowbhagya 2013). Ravi and others (2013) reported the compounds of highest concentration as cuminaldehyde (8% to 17%), β -pinene (22% to 27%), p -cymene (23% to 39%), γ -terpinene (11% to 27%), β -myrcene (1.3-1.75%), and p -mentha-1,4-diene-7-ol (1.0-5.5%) (Ravi and others 2013). We detected a total of 13 compounds at measurable levels during analysis of the various controls and treatments of cumin seeds (Table 3; Table S2). Major monoterpene hydrocarbons included β -pinene, γ -terpinene, and o -cymene. The most abundant sesquiterpene hydrocarbon and most abundant volatile compound overall, which also provides the distinctive odor of cumin, was cuminaldehyde. Identified compounds were similar to those characterized by Sahana and others (2011).

EtO-treated cumin seeds were significantly different in sensory odor as compared to control. Samples treated with EtO showed concentration of all compounds except anethole, which showed a 12.3% loss relative to the control sample. Some compounds were recovered as high as 200% to 600% of the control values. As with peppercorns, vacuum assisted steam treated samples tended to show concentration of all monoterpenes and some loss of all sesquiterpenes over control samples. In samples treated by irradiation, most monoterpenes showed a loss compared to the control, except D-limonene and o -cymene. Among sesquiterpenes, all showed concentration with the exception of anethole, which showed a loss of 44.4% relative to the control sample.

All processing treatments to which cumin seeds were exposed contributed to major changes in essential oil compound concentrations.

Onion powder. While a significant sensory odor difference was observed for irradiated onion powder, only caryophyllene was detected and monitored in the onion powder samples. Samples treated by irradiation had a concentration of the caryophyllene as compared to the control samples. The area counts for caryophyllene in onion powder were only 0.6% of those found in black pepper and caryophyllene is not known to be a volatile compound of interest in onion or onion powder, so the possibility of sample cross-contamination exists (Table 3; Table S3).

Oregano. No differences in sensory odor were detected for irradiated oregano. A total of 5 compounds were detected and monitored in the oregano samples (Table 3; Table S4). The most abundant compounds were carvacrol (93.2%) and β -bisabolene (4.0%), followed by camphene, linalool, and thymol. Figiel and others (2010) describes the major volatile components of fresh Polish oregano to be carvacrol and thymol while Díaz-Maroto and others (2002) report the major volatile component in commercial oregano to be linalool.

Irradiated samples showed extremely high concentration of camphene and linalool over the control samples, while the other compounds showed very slight losses. With carvacrol being the major volatile constituent and experiencing only about a 5% loss during treatment, the resulting quality and sensory should not have been significantly affected.

Water activity. Dried spices and herbs are typically classified as low moisture foods, with water activity within the range of 0.20 to 0.60 (Doyle and Buchanan 2013). Water activity of black pepper and onion powder has been reported as 0.409 and 0.351 (Peter 2001). Bowman and others (2015), in a study related to our reported project, reported a_w for black peppercorn and cumin seeds of 0.3 and 0.4, respectively. Control peppercorn and cumin seeds in our study had a_w of approximately 0.56 to 0.62, which is higher than expected; onion powder a_w was approximately 0.4 and a_w of oregano was about 0.49 (Table 4). After undergoing vacuum assisted steam, irradiation, or EtO processing, a_w for all spices decreased ($P < 0.013$) with the exception of irradiated oregano ($P > 0.05$) and fell within the expected a_w range. Differences in reported a_w for controls within a spice product suggest that a_w may be affected by storage conditions prior to processing or based on the product lot provided to each university lab. At Virginia Tech, products were stored in a walk-in cooler between studies. Changes in humidity over these storage conditions and the timeframe between studies could have contributed to these differences.

Conclusions

Processes effective for killing pathogens in herbs and spices affect sensory characteristics, often to the degree of differentiating spice quality from that of untreated spices. Volatile chemistry profiles and pigments are affected by a given process based on the individual spice or herb, suggesting that an important step in the process validation is the verification that no effect is detectable from a sensory perspective. The progression of the relationship of spices and herbs beyond culinary contributions and toward developing relationships to health and wellness further establishes the importance of identifying effective processes that protect spice and herb quality.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Table S1. Area Counts for Black Peppercorn

Table S2. Area Counts for Cumin Seed

Table S3. Area Counts for Oregano

Table S4. Area Counts for Onion Powder