Development of Oxidized Odor and Volatile Aldehydes in Fermented Cucumber Tissue Exposed to Oxygen

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Changes in volatile compounds in fermented cucumber tissue during exposure to oxygen were investigated by purge and trap sampling, followed by GC-MS. Hexanal and a series of *trans* unsaturated aldehydes, (*E*)-2-pentenal, (*E*)-2-hexenal, (*E*)-2-heptenal, and (*E*)-2-octenal, increased in fermented cucumber slurries exposed to oxygen. Sensory evaluation of oxidized odor was correlated with the increase in aldehyde concentrations. Other identified volatile components present after fermentation did not show major changes during exposure to oxygen. There was no decrease in the formation of aldehydes in fermented cucumber samples that were heated to inactivate enzymes before exposure to oxygen. These results indicated that the formation of aldehydes in oxygen was due to nonenzymatic reactions.

Keywords: *Cucumis sativus; pickle; Lactobacillus plantarum; lipoxygenase; lipid oxidation*

INTRODUCTION

Up to 40% of pickling cucumbers are fermented in salt brine for bulk storage prior to processing into pickle products (Fleming et al., 1995). Cucumbers are fermented in 5−8% salt. After the active fermentation period, additional salt may be added to give a final salt concentration during storage of up to 12% (Fleming et al., 1995). This laboratory has been involved in the development of low-salt fermentation methods to reduce the amount of waste generated with high levels of both chloride and biological oxygen demand (BOD) (Fleming et al., 1988). Recently, we have done an initial investigation of the volatile components present in cucumbers fermented in only 2% NaCl under anaerobic conditions (Zhou and McFeeters, 1998).

When fermentation tanks are emptied, cucumbers are exposed to air for a considerable period of time as they are moved through final processing operations. A problem from this exposure can be development of off-flavors. If cucumbers are fermented in low salt, they will not require desalting. This may reduce the time of exposure to air during the final processing steps. However, the volume of water used to remove salt during a desalting operation also dilutes volatile components that are somewhat water-soluble. If off-flavors were to form in a low-salt fermentation, there would be no postfermentation desalting step to reduce the level of off-flavor components.

The approach used to determine changes in volatile compounds that occur when fermented cucumbers are exposed to oxygen was to flush cucumber slurries with oxygen. Following exposure to oxygen, volatile components were sampled using a purge and trap procedure and analyzed by GC-MS. Zhou and McFeeters (1998) found the standard deviation for sampling and analysis of cucumber volatile components from fermented cucumber slurries to be ∼±6%. Use of purge and trap sampling of volatiles from plant tissues has previously been done for bell peppers (Luning et al., 1994), broccoli (Forney et al., 1991), tomatoes (Buttery and Ling, 1993), and muskmelons (Yabumoto et al., 1978). Flushing samples with oxygen or air to induce oxidative changes has been reported for rapeseed oil by Erkilla et al. (1978) and for soybean oil by Warner and Mounts (1984).

The objective of this work was to determine the changes in volatile components in fermented cucumbers that might be related to off-flavor development when they are exposed to oxygen.

MATERIALS AND METHODS

Disease-free, washed, size 2B cucumbers (35−38 mm diameter) from a local processing plant were brined in 1360 mL jars. A pack-out ratio of 50% cucumbers/50% cover solution was used. All cover solutions contained 18 mM $Ca(OH)_2$, 53 mM acetic acid, and 2% NaCl (upon equilibration of the brine with the fruit) (Fleming et al., 1988). The brines were inoculated to contain 10^6 CFU mL^{-1} of a 24 h culture of *Lactobacillus plantarum* MOP-3 from this laboratory's culture collection. This organism was grown in MRS broth with 5% NaCl (Difco Laboratories, Detroit, MI) overnight at 30 °C immediately prior to brining. The cells were centrifuged and suspended in saline before injection into the jars. During fermentation, jars were stored at ambient temperature (23−27 °C). Brine samples were analyzed for the concentration of fermentation end-products by HPLC (McFeeters, 1993).

Effect of Air or Anaerobic Atmosphere on Cucumber Slurry Preparation. An initial experiment was conducted to determine whether major changes in volatile components would occur during sample preparation if cucumber slurries were prepared in air. Slurries were prepared from jars of fermented cucumbers either in air or in an anaerobic glovebox (Coy Laboratory Products, Grass Lake, MI). The jars of fermented cucumbers and the blender were placed in the glovebox before the jars were opened. The glovebox was filled with a 5% H_2/10% CO_2/85% N_2 anaerobic gas mixture and contained a palladium catalyst for scrubbing residual oxygen so the oxygen concentration was maintained at <1 ppm during sample blending.

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After a jar had been opened either in air or inside the glovebox, the cucumbers were immediately cut into pieces and blended in a Waring blender with an equal volume of cover liquid from the jar. Blending was done for 20 s. Immediately after slurry preparation, a 10 g sample of the slurry was analyzed for volatile components. Duplicate slurries prepared from different jars in air or under anaerobic conditions were compared for volatile components.

The fact that only 2 of 37 identified compounds changed when blending was done in air compared to anaerobic blending (see Results and Discussion) showed that blending cucumbers in air was an acceptable procedure for sample preparation. Thus, all subsequent sample preparation was done in air.

Oxidation and Volatile Component Sampling. Slurries of fermented cucumbers were prepared by homogenization of 400 g of fermented cucumbers with 400 g of brine in a Waring blender for 20 s. Two hundred grams of slurry was transferred to a 236 mL jar, which was hermetically sealed with a lid with two rubber septa. Needles (18G, 3.8 cm length) were placed in the jar lid septa, so the inlet needle was submerged in the slurry and the outlet needle was in the headspace of the jar. The jars of slurry were flushed with O_2 or N_2 at 40 mL min^{-1} for 10 min. Flushed samples were incubated at 30 °C in a water bath. Volatile components were analyzed by purge and trap GC-MS from identically prepared jars at four times during a 96 h incubation period.

Duplicate jars were analyzed at each sampling time and then discarded. From each jar, 10 g of slurry was transferred to a 25 mL fritted sparger tube (Angel Inc., Panorama City, CA). Toluene-d_8 (94.3 ng) was added in 1 μL of methanol solution as internal standard in the cucumber slurry. The sparger was attached to a CDS 6000 purge and trap (P&T) sampler (CDS Analytical Inc., Oxford, PA). Samples were preheated for 10 min at 50 °C, and then a helium flow of 40 mL min^{-1} was bubbled through the slurry for 30 min. Volatiles swept from the slurry passed through a 40 °C water elimination trap and then were adsorbed at 40 °C on a Tenax trap (Supelco Inc, Bellefonte, PA). After the purge period, the Tenax trap was sparged with dry helium for 3 min to remove the water. Volatile components were transferred directly to the GC column by heating the Tenax trap to 180 °C for 6 min with a carrier gas flow rate of 4.0 mL min^{-1} through the trap.

Gas Chromatography−Mass Spectrometry (GC-MS). The GC-MS system consisted of an HP 5890 II GC and model 5972 mass selective detector (Hewlett-Packard, Palo Alto, CA). Volatile compounds from the Tenax trap were transferred without splitting onto an HP-5 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness, Hewlett-Packard). The oven temperature was held at −20 °C during volatile desorption from the Tenax trap. The column was heated at 15 °C min^{-1} to a temperature of 220 °C. There was a 1 min hold time after the column reached 220 °C. Helium was delivered at a constant linear velocity of 42.4 cm s^{-1} (1.5 mL min^{-1}) during the temperature program. The MS detector was turned on at the end of the Tenax trap desorption period. MS interface and ionization source temperatures were set at 280 °C. The detector had an ionization voltage of 70 eV, with the electron multiplier voltage set 200 V higher than that determined by the maximum autotuning procedure. A mass range of 35−350 Da was scanned.

Compound Identification and Quantitation. Kovats indices (KI) of compounds were calculated on the basis of retention time of normal alkanes (Kovats, 1965). The calculated KI and MS fragmentation patterns of unknowns were compared with those of authentic reference standards under identical analytical conditions. The concentrations of hexanal, (*E*)-2-pentenal, (*E*)-2-hexenal, (*E*)-2-heptenal, and (*E*)-2-octenal were determined from calibration curves. Calibrations were done by adding three concentrations of these aldehydes to fermentation brine solutions.

Heating Fermented Cucumbers for Enzyme Inactivation. After fermentation, triplicate 1360 mL jars of cucumbers were heated to a center temperature of 74 °C and held for 15 min. The jars were cooled in cold tap water. Slurries were prepared from the heated jars, incubated in the presence of oxygen, and analyzed for volatile components. These were compared to the volatile components formed during oxidation of slurries from triplicate jars of nonheated, fermented cucumbers.

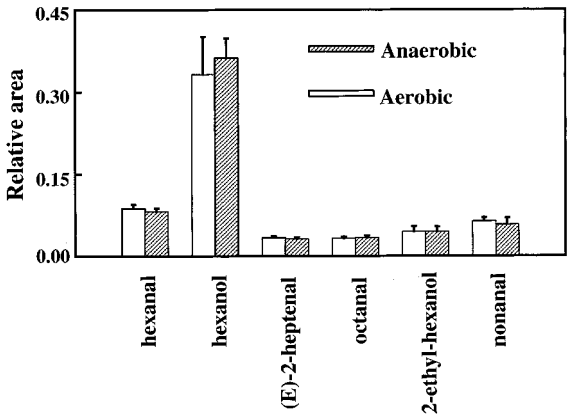

Figure 1. Comparison of the relative peak areas of selected volatile compounds in fermented cucumbers after preparation of slurries in air or in an anaerobic hood. The relative area is the ratio of the peak area to that of the internal standard.

Sensory Evaluation of Oxidized Aroma in Fermented Cucumbers. A trained sensory panel of 20 people was used for the evaluation of oxidized aroma in fermented cucumbers. Panelists were trained in three sessions to detect the oxidized aroma and to score the intensity of this aroma. Fermented cucumber slurries that had been oxidized for 0, 24, 48, and 96 h were prepared. Slurry samples (15 mL) were placed in 60 mL, wide-mouth, snap-cap jars to present to the panelists. A rank-rating method (Kim and O'Mahony, 1997, 1998) was used. Panelists were given a cardboard strip labeled "weakest" at one end, "strongest" at the other, and divided into nine sections, numbered 1−9. The four randomly numbered samples in closed snap-cap jars were given to the panelists. In addition, each panelist was given a nonoxidized reference sample and a highly oxidized reference sample, which were assigned scores of 1 and 9, respectively. The panelists were asked to evaluate the unknowns one at a time for oxidized aroma only and then to place them at the appropriate location on the cardboard strip scale. Panelists were allowed to re-evaluate the samples after the initial placement and change the ratings until they were satisfied with their ratings. Each panelist evaluated two sets of samples presented on consecutive days. Intensity scores were related to the concentrations of aldehydes formed during oxygen exposure by linear regression analysis.

Statistical Analysis. Differences among treatments were determined by analysis of variance using SAS version 6.12 software (SAS Inc., Cary, NC).

RESULTS AND DISCUSSION

Initially, we were concerned that oxidative changes might occur during the preparation of fermented cucumber slurries. Comparison of volatile components in slurries made in air and in an anaerobic glovebox in the presence of <1 ppm of oxygen showed a significant difference in peak areas for only 2 of 37 compounds previously identified in fermented cucumbers by Zhou and McFeeters (1998). Linalool was higher by 29% and geraniol by 18% in slurries prepared anaerobically. However, no significant differences were observed for some aldehydes and alcohols (Figure 1), which were thought to be likely to change in the presence of oxygen and might be related to lipid oxidation. These results showed that, in contrast to fresh cucumbers, in which (*E*,*Z*)-2,6-nonadienal is formed within seconds when the tissue is blended in air (Fleming et al., 1968), or the rapid formation of (*Z*,*Z*)-3,6-nonadienal in blended wa-

Table 1. Effect of 96-h Incubation with Oxygen on the Relative Peak Areas of Volatile Compounds Present in Fermented Cucumbers[a]

identified compound	CAS Registry No.[c]	relative area[b] O_2	relative area[b] N_2	ratio O_2/N_2
ethyl acetate	141-78-6	2.496	1.915	1.303
acetic acid	64-19-7	26.172	19.004	1.377
2-pentanol	6032-29-7	0.067	0.042	1.595
propyl acetate	109-60-4	0.280	0.259	1.081
dimethyl disulfide	624-92-0	0.076	0.079	0.962
3-methylbutanol	123-51-3	0.625	0.350	1.786
2-methylbutanol	137-32-6	0.257	0.246	1.045
ethyl butyrate	105-54-4	0.114	0.089	1.281
ethylbenzene	100-41-4	0.072	0.057	1.263
hexanol	111-27-3	0.427	0.361	1.183
2-methyl-1-butanol acetate	123-92-2	0.096	0.075	1.280
o-xylene	95-47-6	0.056	0.052	1.077
2-heptanol	543-49-7	0.128	0.068	1.882
benzaldehyde	100-52-7	0.035	0.039	0.897
dimethyl trisulfide	3658-80-8	0.089	0.072	1.236
octanal	124-13-0	0.053	0.043	1.233
hexyl acetate	142-92-7	0.039	0.028	1.393
2-ethyl-1-hexanol	104-76-7	0.036	0.027	1.333
eucalyptol	470-82-6	0.022	0.017	1.294
3,7-dimethyl-(*E*,*E*,*E*)-1,3,6-octatriene	3779-61-1	0.034	0.037	0.919
octanol	111-87-5	0.043	0.038	1.132
2-nonanone	821-55-6	0.058	0.042	1.381
2-methoxy-3-(1-methylethyl)pyrazine	25773-40-4	0.009	0.009	1.000
linalool	78-70-6	0.492	0.411	1.197
nonanal	124-19-6	0.080	0.063	1.270
tetrahydro-4-methyl-2-(2-methyl-1-propenyl)-2*H*-pyran	16409-43-1	0.139	0.126	1.103
(*E*,*Z*)-2,6-nonadienal	557-48-2	0.036	0.027	1.333
decanal	112-31-2	0.070	0.054	1.296
geraniol	106-24-1	0.013	0.010	1.300
2-undecanone	112-12-9	0.030	0.029	1.034
3,7-dimethyl-(*E*,*E*)-2,6-octadienate	1189-09-9	0.158	0.124	1.274
1-[2,6,6-trimethyl-(*E*,*E*)-1,3-cyclohexadien-1-yl]-2-buten-1-one	23726-93-4	0.022	0.015	1.467
tetradecane	629-59-4	0.029	0.023	1.261
α-caryophyllene	6753-98-6	0.012	0.016	0.750
hexadecane	544-76-3	0.020	0.021	0.952

[a] Compounds are listed in order of elution from GC. Hexanal and unsaturated aldehydes that showed major increases during oxidation are not included. [b] Peak areas were calculated relative to the peak area of internal standard (toluene-d_8). Results are the average of two replicates. [c] CAS Registry No. were supplied by the author.

termelon tissue (Cai and Cadwallader, 1997), rapid oxidative changes which affected the volatile components did not occur upon blending of fermented cucumbers. Because there was little difference in volatile components between slurry preparation in air or in the absence of oxygen, sample slurries were prepared in air in all subsequent experiments.

To evaluate the effect of incubating fermented cucumber slurries in the presence of oxygen, jars were flushed with oxygen and then sampled over a 96-h incubation period. Thirty-five of 37 volatile compounds that were previously identified in fermented cucumbers (Zhou and McFeeters, 1998) changed by <2-fold during 96 h at 30 °C in the presence of oxygen (Table 1). Among the components that showed little change during incubation in oxygen were the aldehydes, octanal, nonanal, decanal, and (*E*,*Z*)-2,6-nonadienal, which is the major odor impact compound in fresh cucumbers (Schieberle et al., 1990).

The two identified compounds present in fermented cucumbers, which showed major changes during incubation in oxygen, were hexanal and (*E*)-2-heptenal.

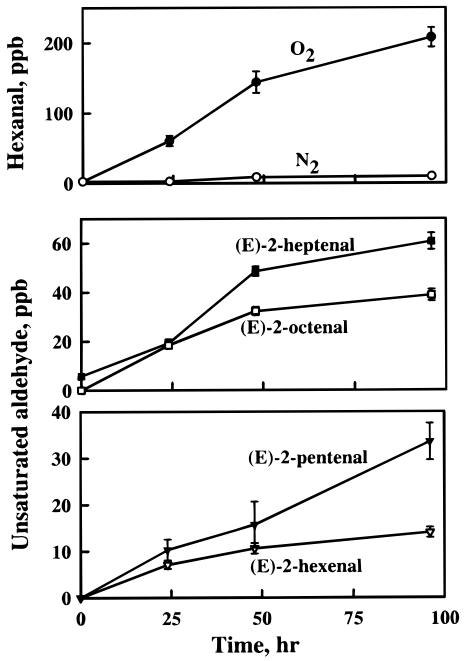

Figure 2. Changes in concentrations of hexanal in cucumber slurries flushed with oxygen (●) or nitrogen (○) and changes in (*E*)-2-heptenal (■), (*E*)-2-octenal (□), (*E*)-2-pentenal (▼), and (*E*)-2-hexenal (▽) in O_2-flushed cucumber slurries.

Table 2. Linear Correlation of Sensory Panel Scores for Oxidized Odor with the Concentration of Aldehydes Formed during Exposure of Fermented Cucumber Slurries to Oxygen[a]

compound	*F* value	PR > *F*	R^2
hexanal	391.15	0.0001	0.768
(*E*)-2-heptenal	373.29	0.0001	0.760
(*E*)-2-octenal	327.04	0.0001	0.735
(*E*)-2-hexenal	321.09	0.0001	0.731
(*E*)-2-pentenal	319.05	0.0001	0.730

[a] Twenty panelists rated two sets of samples prepared on different days.

Hexanal increased from <10 to >200 ppb, and (*E*)-2-heptenal increased from ~6 to 60 ppb (Figure 2). Neither hexanal (Figure 2) nor the unsaturated aldehydes (data not shown) increased in a nitrogen atmosphere. In addition, the unsaturated aldehydes, (*E*)-2-pentenal, (*E*)-2-hexenal, and (*E*)-2-octenal, which were not detected in fermented cucumbers or nitrogen-flushed control slurries, formed during exposure to oxygen but to a smaller extent than hexanal. These fermented cucumber slurries had an odor similar to that of pickle products exposed to air.

Sensory analysis of oxidized odor intensity of fermented cucumber slurries was conducted for different times of exposure to oxygen. The rank-rating method of Kim and O'Mahony (1997, 1998) was used. Linear regression analysis between sensory scores and the concentrations of the five aldehydes that formed during oxidation (Table 2) gave R^2 values between 0.73 and 0.76. The *F* ratio of these regressions was highly significant ($P \leq 0.0001$), indicating strong correlation between the odor intensity and the concentration of aldehydes. A graph of mean odor intensity scores as a function of hexanal concentration is shown in Figure 3.

GC-MS analysis showed changes only in hexanal, (*E*)-2-pentenal, (*E*)-2-hexenal, (*E*)-2-heptenal, and (*E*)-2-octenal when cucumber slurries were exposed to oxygen (Figure 2). Compounds previously identified (Table 1)

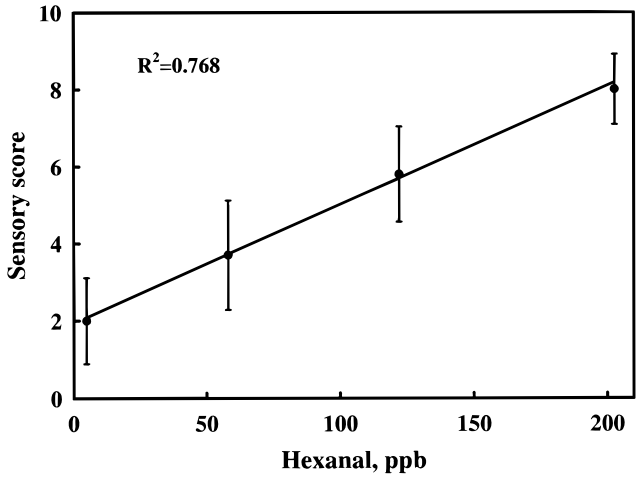

Figure 3. Correlation between sensory score for the intensity of oxidized odor and hexanal concentrations in fermented cucumber slurries exposed to oxygen. The points (●) show the mean of 40 sensory scores on two oxidized samples prepared on different days. The line is the linear regression line for all sensory scores.

did not change substantially. Among the unidentified peaks, none were observed to disappear during exposure to oxygen, nor did any new unidentified peaks appear. These results indicate that these aldehydes are the likely cause of oxidative odor in fermented cucumbers. However, the possibility that other compounds formed at levels too low to be detected by GC-MS may contribute to the development of oxidized odor cannot be eliminated.

Hexanal, (*E*)-2-heptenal, and (*E*)-2-octenal have been found to form from the break down of hydroperoxides of linoleic acid (Grosch and Schwarz, 1971; Galliard et al., 1976; Tressl et al., 1981; Luning et al., 1995). (*E*)-2-Pentenal and (*E*)-2-hexenal can be formed from linolenic acid (Grosch and Schwarz, 1971; Grosch and Laskawy, 1975). Peng and Geisman (1976) have shown that linoleic acid and linolenic acid are the two most abundant fatty acids present in cucumber lipids.

Previous studies related to aldehyde production by macerated, fresh cucumber tissue or tissue extracts (Gallaird and Phillips, 1976; Grosch and Schwarz, 1971) reported the involvement of lipoxygenase in the formation of hydroperoxides. We wanted to determine if lipoxygenase was involved in the formation of aldehydes in the fermented cucumber slurries. Fermented cucumbers (pH 3.6) were heated in the fermentation jars to an internal temperature of 74 °C for 15 min to ensure the inactivation of any lipoxygenase activity that might be present. Wardale and Lambert (1980) found that all detectable lipoxygenase activity was lost within 2 min when cucumber lipoxygenase was heated to 70 °C at its optimum pH of 5.5. Jang et al. (1995) reported similar results for the heat stability of cucumber lipoxygenase and also found the enzyme was less stable at pH 3.5 than at pH 5.5.

If aldehydes in cucumber slurries were formed as a result of lipoxygenase-catalyzed reactions, heating the fermented cucumbers before exposure to oxygen should have reduced or eliminated aldehyde formation. Instead, Figure 4 shows that hexanal production increased moderately in cucumber slurries prepared from fermented cucumbers after they had been heated. The other aldehydes observed in nonheated slurries increased to the same extent or to an extent slightly

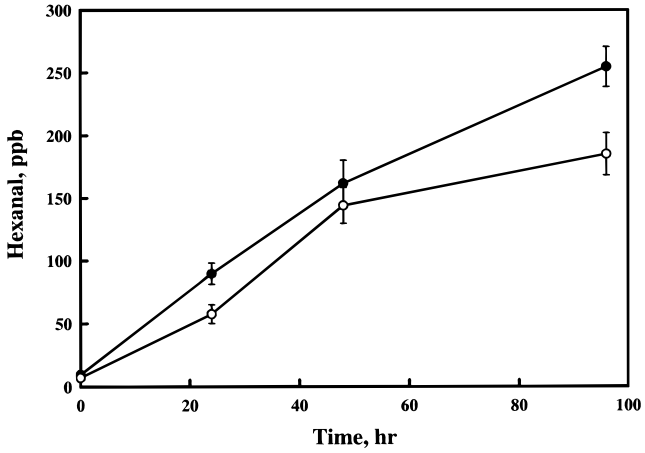

Figure 4. Changes of hexanal concentration in heated (●) and nonheated (○) fermented cucumbers during incubation with oxygen.

Table 3. Comparison of Aldehyde Formation after Oxidation of Heated and Nonheated Fermented Cucumber Slurry for 48 and 96 h

	48 h		96 h	
compound	heated, ppb	nonheated, ppb	heated, ppb	nonheated, ppb
(*E*)-2-pentenal	25.8 ± 0.85	22.7 ± 0.19	40.6 ± 0.85	35.6 ± 0.19
(*E*)-2-hexenal	12.4 ± 1.56	8.8 ± 0.19	17.3 ± 1.84	14.6 ± 0.19
(*E*)-2-heptenal	38.5 ± 5.80	31.5 ± 2.40	53.2 ± 1.84	49.9 ± 1.56
(*E*)-2-octenal	40.3 ± 2.26	39.7 ± 0.19	53.3 ± 2.40	48.1 ± 0.19

greater than hexanal in slurries prepared from the heated cucumbers (Table 3). These results showed that the aldehydes were not formed as a result of enzymatic acidity. The increases in aldehydes in slurries prepared from heated, compared to nonheated, fermented cucumbers may have been a result of tissue disruption caused by the heating.

CONCLUSIONS

Changes in volatile compounds were determined when fermented cucumber slurries were exposed to oxygen using purge and trap sampling, followed by GC-MS analysis. Most of the identified volatile components in fermented cucumbers did not change significantly. However, hexanal and four *trans* isomers of unsaturated aldehydes, including (*E*)-2-pentenal, (*E*)-2-hexenal, (*E*)-2-heptenal, and (*E*)-2-octenal, increased during oxidation. Statistical analysis indicated that formation of these aldehydes was strongly correlated with increased oxidized aroma scores. These aldehydes were the probable cause of the oxidative aroma in the cucumber slurries. There was no decrease in the formation of oxidative aldehydes when fermented cucumbers were heated to inactivate lipoxygenase. These results indicated that the aldehydes were formed due to nonenzymatic lipid oxidation.

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