Volatile Compounds in Cucumbers Fermented in Low-Salt Conditions

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Volatile compounds present in cucumbers fermented in 2% salt were analyzed using purge and trap concentration followed by GC-MS. Thirty-seven volatile compounds were identified from over 100 peaks detected using GC-MS. Most of the identified compounds did not change during fermentation. However, the ability of disrupted cucumber tissue to produce (*E*,*Z*)-2,6-nonadienal and 2-nonenal, the two most important volatiles in fresh cucumber odor, decreased during fermentation. In addition, linalool increased to levels several times its odor threshold during the first 10 days of fermentation.

Keywords: *Cucumis sativus; gas chromatography; mass spectrometry; Lactobacillus plantarum; Cucurbitaceae*

INTRODUCTION

Current commercial practice is to ferment and store cucumbers that are used in "processed" pickle products, such as hamburger dill chips, in large open tanks with 5−12% salt. These high salt levels are used to select for naturally occurring, homofermentative lactic acid bacteria to carry out the fermentation, and then to protect against spoilage after the active fermentation period (Fleming et al., 1995a). Before being used in products, these fermented cucumbers must be "desalted" with the generation of large volumes of salt solution that is too dilute to be economically recycled. However, the chloride level in the desalting solution is a serious disposal problem.

Procedures have been developed for brining cucumbers in closed anaerobic tanks at substantially lower salt concentrations (Fleming et al., 1988). This approach to fermentation has the potential to allow fermentation and storage of cucumbers at sufficiently low salt concentrations that desalting would no longer be required. A consequence of low salt storage would be that volatile compounds, both desirable and undesirable, which are removed during the desalting process, would remain in the final product. This investigation was undertaken to begin to characterize the volatile components in low-salt cucumber fermentations.

Recent studies of the volatile components of fresh cucumbers have resulted in identification of 30 compounds (Whitefield and Last, 1991). Schieberle et al. (1990) used aroma extract dilution analysis to determine the relative odor impact of volatile compounds from fresh cucumber. They found (*E*,*Z*)-2,6-nonadienal, which is rapidly formed when cucumber tissue is disrupted (Fleming et al., 1968), to have a much greater contribution to fresh cucumber odor than any other compound. The only previous published work on the volatiles of fermented cucumbers reported eight volatile compounds (Aurand et al., 1965).

The objectives of this investigation were to develop a sampling and analysis procedure for volatiles in fermented cucumbers, to identify volatile compounds present, and to characterize changes that occur during fermentation.

MATERIALS AND METHODS

Source of Reagents and Chemicals. All chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI), except isopropyl propionate from Lancaster Synthesis Inc. (Windham, NH).

Cucumber Fermentation. Disease-free size 2B cucumbers (32−38 mm diameter) were obtained from local processors. Lots of cucumbers were shipped to the procesors from various locations in the United States and Mexico in all seasons of the year. Cucumbers (680 g) were washed and packed into 1360 mL jars (46 oz). The jars were filled with 680 mL of cover solution so that after equilibration the concentrations of added components were 2% NaCl, 53 mM acetic acid (either glacial acetic acid or commercial vinegar containing 20% acetic acid was used) (Fleming et al., 1988). The jars were closed with lids with a single rubber septum so the jars could be inoculated and sampled with a syringe. Jars were inoculated with a 24 h culture of *Lactobacillus plantarum* MOP-3 grown in MRS broth with 5% NaCl to give 10^6 CFU/mL in the jars. Fermented cucumbers were analyzed for residual fermentable sugars, lactic acid, and acetic acid using HPLC (McFeeters, 1993).

Analysis of Volatiles. Samples were taken 21 days after inoculation to analyze volatiles present after completion of fermentation. For the analysis of volatiles during the time course of fermentation, duplicate slurries of the fresh cucumbers were analyzed and then duplicate jars were analyzed 3, 5, 7, 10, 14, and 21 days after inoculation. The slurry samples were prepared by homogenization of 400 g of fermented cucumbers or fresh cucumbers with 400 g of brine or deionized water, respectively, in a Waring blender for 20 s. The slurry

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S0021-8561(97)00472-X CCC: $15.00 © 1998 American Chemical Society
Published on Web 05/07/1998

(10 g) was spiked directly with 3 μL of 94.3 ppm toluene-d_8 as the internal standard just before it was transferred to a 25 mL fritted sparger (Angel Inc., Panorama City, CA) on a CDS 6000 purge and trap sampler (CDS Analytical Inc, Oxford, PA). After 10 min preheating at 50 °C, a helium purge of 40 mL min^{-1} was bubbled through the slurry for 30 min. Volatiles were adsorbed on a Tenax trap column (Supelco Inc, Bellefonte, PA) held at 40 °C during purging. The Tenax trap was dried for 3 min to remove trapped water and then heated at 180 °C for 6 min with a helium flow rate of 4.0 mL min^{-1} to desorb trapped volatiles.

Gas Chromatography/Mass Spectrometry (GC-MS). The system consisted of an HP 5890 II GC with an HP 5972 mass selective detector (MSD) (Hewlett-Packard, Palo Alto, CA). Desorbed volatile compounds were delivered to an HP-5 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; Hewlett-Packard) without splitting. The oven temperature was held at −20 °C during the 6 min desorption period with a 4.0 mL min^{-1} helium flow rate. The oven temperature was then programmed to increase from −20 °C to +220 °C at 15 °C min^{-1} with a final 1 min hold at 220 °C. Helium carrier gas was programmed to give a constant linear velocity of 42.4 cm s^{-1} (1.5 mL min^{-1}). MSD settings were as follows: MS interface and ionization source temperature, 280 °C; electronic ionization voltage, 70 eV; scanning mass range, 35−350 amu. The electron multiplier voltage was set 200 V above the voltage selected by the "maximum autotuning" procedure.

Compound Identification and Relative Abundance. NIST75K mass spectrum library with HP G1034C MS ChemStation software (version 03.04) (Hewlett-Packard) was used for preliminary identification. Kovats indices for identified peaks were calculated on the basis of retention time relative to normal alkanes (Kovats, 1965). The calculated Kovats index and MS fragmentation pattern of each unknown were compared with those of authentic reference standards under identical experimental conditions. Only identified compounds which were present in all fermentations from 10 lots of cucumbers were reported. Relative concentration of each peak was estimated by the ratio of total ion peak area to that of the internal standard. The concentration of selected compounds was calculated using regression equations determined using standard solutions injected at three different concentrations into a sample brine solution.

RESULTS

The procedure for sampling and analysis of volatiles from fresh and fermented cucumber slurries and fermented cucumber brines was generally based upon the method used by Luning et al. (1994) for volatile analysis of bell peppers. It was found that total ion chromatogram peak areas obtained were near maximum after 30 min purging with helium at 40 mL min^{-1} flow rate. The total areas of the total ion chromatogram and one selected compound, linalool, are shown in Figure 1. The change in the peak area of linalool was typical of that observed for a number of compounds.

A faster increase in the oven temperature (15 °C/min) was used than has been usual for separation of volatile compounds from foods. Approximately 100 peaks were detected on the total ion chromatogram, whether a 5 °C/min temperature ramp or 15 °C/min ramp was used for chromatography, suggesting that peak resolution was not substantially degraded with a faster temperature program. The ability to identify chromatographic peaks with the MS detector was somewhat improved with the 15 °C/min temperature program because peak heights were moderately greater. For smaller peaks, this tended to give a better quality mass spectrum at the center of the peaks. Using the standard operating parameters, the mean standard deviation of the internal standard (toluene-d_8) peak area was 5.8% for 18 chromatographic runs over a 3 day period.

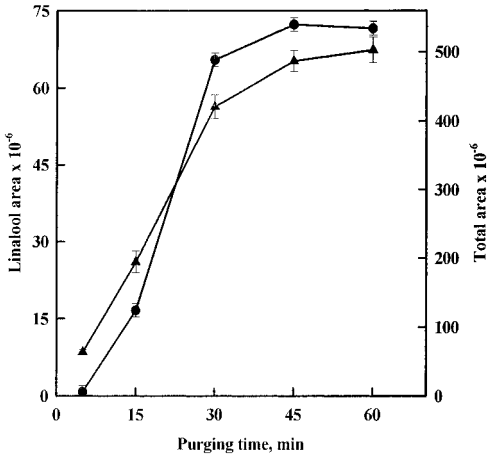

Figure 1. Effect of purging time on peak area of linalool (●) and total area (▲) of the total ion chromatogram excluding the area of the ethyl acetate, acetic acid, and internal standard peaks.

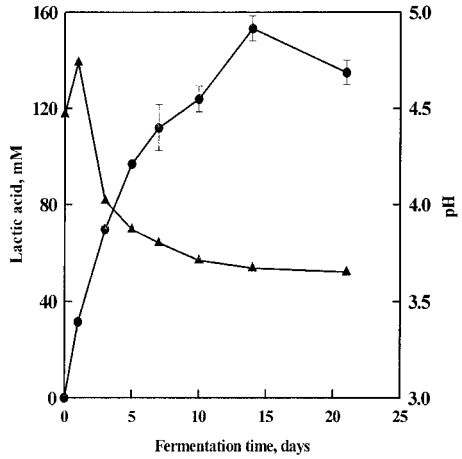

Figure 2. Changes in lactic acid concentration (●) and pH (▲) in a cucumber fermentation with a 2% salt concentration.

Cucumbers were fermented in closed jars with 2% salt. The fermentable sugars, glucose and fructose, were not detected after 13 days of fermentation. This was considered to be the endpoint of the primary lactic acid fermentation (Fleming et al., 1988). In brine samples, lactic acid increased and pH decreased as expected for a homolactic acid fermentation (Passos et al., 1994; Figure 2). After 21 days, volatile compounds were determined in both the cover liquid and cucumber slurry samples. Figure 3 shows a representative total ion chromatogram of a fermented cucumber slurry sample. Thirty-seven compounds were preliminarily identified by a match of the mass spectrum with that in the NBS library. The identities of 30 of these compounds were confirmed on the basis of matches of both the mass spectra and Kovats index values obtained with authentic standards. The other seven compounds were identified on the basis of interpretation of the mass spectra (Table 1). Of the 37 compounds identified, there were 2 alkanes, 1 alkene, 9 alcohols, 5 aldehydes, 1 enal, 2 ketones, 1 carboxylic acid, 6 esters, 6 aromatics, 2 heteroaromatics, and 2 sulfur compounds.

In addition, six esters found in the fermented cucumbers came from the acetic acid used in the cover brine. This was determined by doing a purge and trap GC-MS analysis of the cover brine diluted with an equal volume of water. In Figure 3, these peaks are labeled

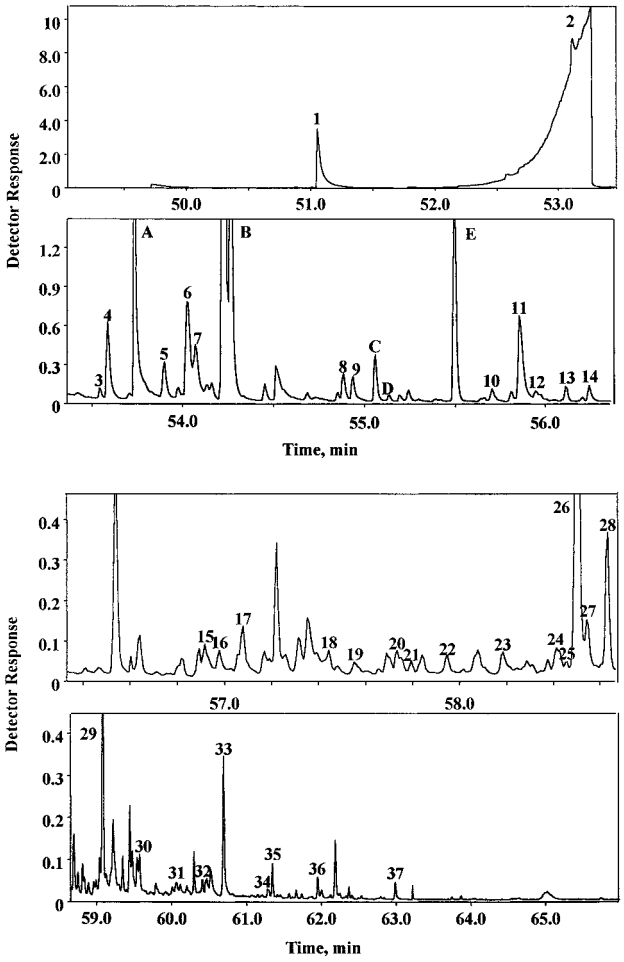

Figure 3. Typical total ion chromatogram of a 10 g slurry of fermented cucumbers. Peaks 1−37 were identified and are listed in Table 1. Peaks A−E were present in glacial acetic acid: (A) isopropyl acetate, (B) isopropyl propionate, (C) propyl propionate, (D) butyl acetate, and (E) isopropyl butyrate. Data collection on the GC-MS started at 49 min after purge and trap sampling and desorption.

1 and A−E. The esters were also identified on the basis of mass spectra and Kovats index matches with an authentic compound. They were (1) ethyl acetate, (A) isopropyl acetate, (B) isopropyl propionate, (C) propyl propionate, (D) butyl acetate, and (E) isopropyl butyrate. When vinegar was used as the source of acetic acid for fermentation cover brines, ethyl acetate was the only ester found, though the amount of ethyl acetate present was much greater than the amount present in glacial acetic acid.

To determine differences in volatile compounds in fermented cucumber slurry compared to fresh cucumber slurry, peak areas of the total ion chromatograms relative to the toluene-d_8 internal standard were compared (Table 1). Relative amounts of four compounds, hexanal, (*E*)-3,7-dimethyl-1,3,6-octatriene, (*E*,*Z*)-2,6-nondienal, and 2-undecanone, declined in fermented cucumber slurry compared to a slurry prepared from fresh cucumbers. Ethyl benzene, *o*-xylene, and benzaldehyde were not detected in fresh cucumber slurries but were present after fermentation. Acetic acid was added to the fermentation cover brine. Ethyl acetate was added as a component of glacial acetic acid, but it also appeared to form during fermentation (Figure 4). Twenty-seven identified compounds changed less than 2-fold during fermentation.

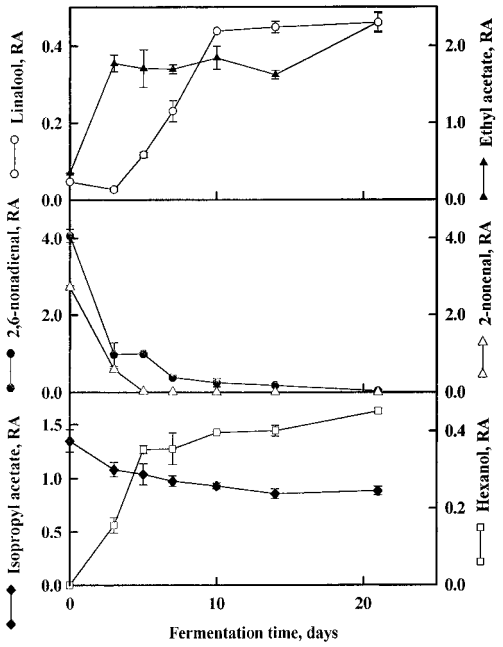

Figure 4. Changes in the relative area (RA) compared to the internal standard of linalool, ethyl acetate, 2,6-nonadienal, 2-nonenal, isopropyl acetate, and hexanol during cucumber fermentation. Data for linalool, 2,6-nonadienal, or 2-nonenal at day 0 were from fresh cucumber slurries. The day 0 data for the other compounds were obtained by purge and trap analysis of 2-fold diluted cover brine. Error bars show the standard deviation in RA at each sampling point.

Differences in relative peak areas between fermented cucumber brine and slurry were generally less than 2-fold (Table 1) as well. However, hexanal and ethylbenzene were higher in the brine than in the slurry.

α-Caryophyllene, 2-undecanone, and (*E*,*Z*)-2,6-nonadienal were present in fermentation brine at a lower concentration than in the fermented cucumber slurry.

Concentrations of seven compounds were determined after calibration with three levels of standards. The amounts present before and after fermentation, along with published odor threshold concentrations, are given in Table 2. Ethyl acetate and linalool increased substantially during fermentation. Several aldehydes were quantitated due to their possible importance to flavor. Hexanol was analyzed to observe the amount relative to hexanal under the anaerobic conditions of fermentation.

Figure 4 shows the types of changes that occurred with volatile compounds during the course of fermentation. Linalool increased rapidly from day 3 to day 10 of fermentation and then remained stable. A small amount of ethyl acetate was present in fresh cucumber slurry. A large increase after 3 days of fermentation was due to the ethyl acetate present in the glacial acetic acid used in the fermentation cover brine. It remained stable during the active fermentation but increased after 14 days. In contrast to linalool and ethyl acetate, the amount of (*E*,*Z*)-2,6-nonadienal and 2-nonenal in cucumber slurries declined rapidly during the first week of the fermentation. After 5 days, 2-nonenal was not detected. Isopropyl acetate was introduced into the fermentation brine as an impurity of glacial acetic acid, and hexanol, which presumably was present in the fresh cucumber, diffused into the fermentation brine as fermentation proceeded.

Table 1. Comparison of Volatiles Identified in Fresh Cucumber Slurry, Fermented Cucumber Slurry, and Brine

peak no.	CAS Registry No.	compound	identification	relative area[a] brine	pickle slurry	cucumber	*P/C* ratio[b]
1	141-78-6	ethyl acetate	*c*	2.400	2.300	0.290	7.93
2	64-19-7	acetic acid	*c*	18.100	17.700	0.850	20.82
3	6032-29-7	2-pentanol	*c*	0.059	0.041	0.041	1.00
4	109-60-4	propyl acetate	*c*	0.170	0.250	0.200	1.25
5	624-92-0	dimethyl disulfide	*c*	0.056	0.071	0.065	1.09
6	123-51-3	3-methyl-butanol	*c*	0.350	0.330	0.310	1.06
7	137-32-6	2-methyl-butanol	*c*	0.220	0.230	0.230	1.00
8	66-25-1	hexanal	*c*	0.250	0.081	0.190	0.43
9	105-54-4	ethyl butyrate	*c*	0.077	0.093	0.090	1.03
10	100-41-4	ethylbenzene	*c*	0.120	0.057	ND	ND
11	111-27-3	hexanol	*c*	0.450	0.320	0.340	0.94
12	624-41-9	2-methyl-1-butanol acetate	*d*	0.039	0.072	0.050	1.44
13	95-47-6	*o*-xylene	*c*	0.075	0.054	ND	ND
14	543-49-7	2-heptanol	*c*	0.100	0.070	0.090	0.78
15	18829-55-5	(*E*)-2-heptenal	*c*	0.035	0.038	0.040	0.95
16	100-52-7	benzaldehyde	*c*	0.039	0.032	ND	ND
17	3658-80-8	dimethyl trisulfide	*d*	0.063	0.072	0.055	1.31
18	124-13-0	octanal	*c*	0.042	0.033	0.038	0.87
19	142-92-7	hexyl acetate	*c*	0.047	0.034	0.055	0.62
20	104-76-7	2-ethyl-1-hexanol	*c*	0.037	0.038	0.042	0.90
21	470-82-6	eucalyptol	*c*	0.015	0.015	0.013	1.15
22	3779-61-1	3,7-dimethyl-(*E*,*E*,*E*)-1,3,6-octatriene	*d*	0.062	0.032	0.085	0.38
23	111-87-5	octanol	*c*	0.034	0.038	0.050	0.76
24	821-55-6	2-nonanone	*c*	0.028	0.040	0.046	0.87
25	25773-40-4	2-methoxy-3-(1-methylethyl)pyrazine	*c*	0.013	0.012	0.021	0.57
26	78-70-6	linalool	*c*	0.830	0.460	0.047	9.79
27	124-19-6	nonanal	*c*	0.049	0.061	0.070	0.87
28	16409-43-1	tetrahydro-4-methyl-2-(2-methyl-1-propenyl), 2*H*-pyran	*d*	0.110	0.120	0.095	1.26
29	557-48-2	(*E*,*Z*)-2,6-nonadienal	*c*	0.004	0.029	4.020	0.007
30	112-31-2	decanal	*c*	0.100	0.064	0.080	0.80
31	106-24-1	geraniol	*c*	0.018	0.017	0.011	1.54
32	112-12-9	2-undecanone	*c*	ND	0.025	0.080	0.31
33	1189-09-9	methyl 3,7-dimethyl-(*E*,*E*)-2,6-octadienate	*d*	0.092	0.130	0.085	1.53
34	23726-93-4	1-(2,6,6-trimethyl-(*E*,*E*)-1,3-cyclohexadien-1-yl)-2-buten-1-one	*d*	0.023	0.015	0.019	0.79
35	629-59-4	tetradecane	*c*	0.025	0.027	0.021	1.28
36	87-44-5	α-caryophyllene	*d*	0.005	0.017	0.009	1.89
37	544-76-3	hexadecane	*c*	0.016	0.015	0.017	0.88

[a] Relative area = (peak area of compound)/(peak area of internal standard). [b] *P/C* ratio = (relative peak area in pickle slurry)/(relative peak area in cucumber slurry). [c] Identification based upon a match of the mass spectrum and Kovats index with authentic compound. [d] Identification based upon interpretation of the major ions in the mass spectrum.

Table 2. Comparison of Some Volatile Compound Concentrations in Fresh Cucumber Slurry, Fermented Brine, and Fermented Cucumber Slurry

peak no.	compound	brine (ppb)	fermented cucumber (ppb)	fresh cucumber (ppb)	odor threshold in water (ppb)	ref
1	ethyl acetate	365	345	43	3000	Keith and Powers, 1968
8	hexanal	38	12	29	20	Pyysalo, 1977
11	hexanol	25	18	20	500	Fazzalari, 1978
15	2-heptenal	9.4	10	11		
18	octanal	4.4	3.4	3.8	0.5	Ahmed et al., 1987
26	linalool	81	44	4.6	3.8	Ahmed et al., 1987
29	(*E*,*Z*)-2,6-nonadienal	2.93	22.3	3093	0.01	Buttery, 1981

DISCUSSION

The flavor of cucumber pickle products varies widely, depending upon the spices and flavorings added to the final products (Fleming et al., 1995b). A clean flavor of cucumbers after fermentation and storage is considered important for manufacture of high-quality products. The lack of available information on volatile compounds formed during a normal cucumber fermentation led us to develop a procedure to analyze volatile compounds that would be relatively rapid and provide high sensitivity, good resolution of components, and good reproducibility.

The only previous investigation of volatiles of fermented cucumbers was carried out using packed columns, which allowed separation and detection of highly volatile compounds (Aurand et al., 1965). Only one of the eight compounds they reported, ethyl butyrate, was observed in this study. The other more volatile com-

pounds they reported may not have been detected because Tenax does not efficiently trap them (Reineccius, 1993). About 100 peaks were detected using purge and trap sampling and GC-MS for detection. Thirty-seven compounds were identified along with six esters introduced into the fermentation due to addition of glacial acetic acid in the fermentation brine to prevent the growth of undesirable bacteria and to help buffer the fermentation (Etchells et al., 1973).

The fact that most of the volatile compounds present in a fresh cucumber slurry showed little or no change is a very interesting result of this work. However, the differences which do occur are consistent with the fact that the aroma of fermented cucumbers is very different from that of fresh cucumbers. The characteristic flavor impact compounds of fresh cucumbers, (*E*,*Z*)-2,6-nonadienal and 2-nonenal (Schieberle et al., 1990), are formed enzymatically within seconds when cucumber tissue is macerated (Fleming et al., 1968). Since the ability of cucumber tissue to produce (*E*,*Z*)-2,6-nonadienal and 2-nonenal during blending was lost during fermentation, this change would necessarily be important in the fermentation process. It is generally believed that lipoxygenase is the key enzyme in the formation of fresh cucumber flavor when cucumbers are disrupted. We observed the activity of lipoxygenase in the homogenate of fresh cucumbers, but not in fermented cucumbers (Zhou et al., 1998). The optimum pH of cucumber lipoxygenase is about 5.5, with moderate activity at 3.5 (Wardale and Lambert, 1980). The pH value of brine decreases below 3.8 in the first 5 days of fermentation due to the acid production by lactic acid bacteria (Fleming et al., 1988). Inactivation of lipoxygenase as pH decreases would eliminate the pathway for the biogenesis of (*E*,*Z*)-2,6-nonadienal or 2-nonenal from linolenic or linoleic acids, respectively, (Galliard et al., 1976), which are the two most abundant fatty acids present in cucumbers (Peng and Geisman, 1976).

Methoxy alkyl pyrazines are naturally present in many vegetables and contribute strongly to the aroma of capsicum. A compound from this group, 3-isopropyl-2-methoxypyrazine, was reported to be present in fresh cucumbers by Murray and Whitefield (1975). This work showed that it did not change substantially during fermentation. Since its odor threshold is only 2 ppt (Whitefield and Last, 1991), it may contribute to the overall aroma in fermented cucumbers.

Linalool increased during the fermentation to 44 ppb, which is over 10-fold greater than its threshold level in water (Ahmed et al., 1987). Synthesis of this compound involves a series of reactions (Templeton, 1969). First geranyl pyrophosphate is dephosphorylated to produce geraniol, which was observed in the fermented cucumbers (Table 1). Under acid conditions geraniol can readily undergo nonenzymatic migration of an alcohol group to form linalool (Takeo, 1981). Both the importance of this compound in the flavor quality of fermented cucumbers and the mechanism of its formation during lactic acid fermentation of cucumbers remain to be determined.

CONCLUSIONS

Use of purge and trap sampling followed by GC-MS analysis made it possible to identify many of the volatile compounds present in fermented cucumbers. Most of the identified compounds did not change substantially during fermentation. However, fermented cucumbers lost most of their ability to produce (*E*,*Z*)-2,6-nonadienal when the tissue was disrupted. This is probably one important reason for the difference in odor between fresh and fermented cucumbers. Linalool builds up from low levels to a concentration 10-fold greater than its odor threshold. A few other compounds, absent from fresh cucumber slurries, were also identified in fermented cucumbers. The contribution that these components make to the odor of fermented cucumbers remains to be determined. Future work will attempt to determine which of the volatiles present in fermented cucumbers have the greatest impact on the odor of the product.

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Received for review June 4, 1997. Revised manuscript received March 23, 1998. Accepted March 26, 1998. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or North Carolina Agricultural Research Service, nor does it imply approval to the exclusion of other products that may be suitable.

JF9704726