Solid-Phase Microextraction (SPME) Technique for Measurement of Generation of Fresh Cucumber Flavor Compounds

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Investigations were carried out to determine whether flavor compounds characteristic for fresh cucumbers could be rapidly determined using a solid-phase microextraction (SPME) dynamic headspace sampling method combined with gas chromatography and flame ionization detection. Cucumbers were sampled, during blending, for fresh cucumber flavor compounds (*E,Z*)-2,6-nonadienal and (*E*)-2-nonenal. The GC was such that the two target compounds were separated and baseline-resolved. Relative standard deviations for analysis of both (*E,Z*)-2,6-nonadienal and (*E*)-2-nonenal using this SPME sampling method were ±10%. Utility of the analytical method was demonstrated by determining the effect of heat treatments on the ability of cucumbers to produce these flavor impact compounds.

Keywords: *Cucumis sativus; solid-phase microextraction; blanching*

INTRODUCTION

Flavor impact compounds responsible for fresh cucumber flavor are formed within seconds by enzymatic reactions that begin when the tissue is disrupted (*1, 2*). Such rapid formation of flavor compounds after tissue disruption is common in fruits and vegetables, including apricot (*3*), tomato (*4, 5*), bell pepper (*6*), strawberry (*7*), oriental melon (*8*), and *Allium* species, such as garlic, onion, and leek (*9*).

Isolation and identification of volatile components from blended cucumber tissue has shown that (*E,Z*)-2,6-nonadienal and (*E*)-2-nonenal are the major components present (*10*−*12*). Radioactive labeling studies with cucumber tissue have demonstrated that the nonenals are derived from linoleic acid, whereas the hexenals and nonadienals are formed from linolenic acid (*13, 14*). Schieberle et al. (*11*), using aroma dilution analysis, found that (*E,Z*)-2,6-nonadienal was the most important odor impact compound from fresh cucumbers. The second most important odor impact compound, (*E*)-2-nonenal, has only 1.6% of the aroma impact of (*E,Z*)-2,6-nonadienal.

The flavor compounds, (*E,Z*)-2,6-nondienal and (*E*)-2-nonenal, are in a constant state of flux as they are formed and enter the gas phase (*15*). This creates a complex analytical situation in which the analytes are changing concentrations during the sampling process. Currently, there are no convenient techniques available to determine the ability of cucumber tissue to form these flavor impact compounds. Purge and trap sampling, followed by gas chromatography (GC), would be expected to work for such an analysis. However, analysis times are relatively long, and foaming by cucumber slurries that generate these compounds makes reproducibility difficult (*16*). Therefore, it is difficult to assess the ability of different cucumber cultivars or sizes to generate fresh flavor components or to determine the effect of processing treatments on the loss of flavor generation capability.

Solid-phase microextraction (SPME) was developed for sampling water samples for GC analysis of volatile components (*17*). Considerable research has been done on the application of this technique for volatile flavor analysis (*18, 19*). Krumbein and Ulrich (*20*) found that headspace SPME gave results comparable to those of dynamic headspace trapping onto Tenax for analysis of tomato aroma. Recently, Järvenpää et al. (*21*) used SPME to qualitatively determine the time course of release of volatile compounds from fresh-cut onion.

The objective of this study was to investigate the use of a SPME sampling technique to assess the ability of cucumber tissue to produce the flavor impact compounds (*E,Z*)-2,6-nonadienal and (*E*)-2-nonenal.

MATERIALS AND METHODS

Chemicals. All chemicals were obtained from Aldrich Chemical Co. (Milwaukee, WI). The methanol used as the solvent for standard solutions was purge and trap grade (cat. no. 41-481-6).

Dynamic Headspace Sampling of (*E,Z*)-2,6-Nonadienal and (*E*)-2-Nonenal. Whole, size 2B cucumbers (3.5−3.8 cm dia) obtained from a local processor were equilibrated at 30 °C for 20 min in a water bath. Cucumbers (500 g) were sliced into approximately 15-g pieces and placed in a 2.5-L, single speed, stainless steel blender (Robot Coupe, model # RSI 2Y1, Robot Coupe USA, Inc., Jackson, MS). A 1-mL volume of 500 ppm decanal in methanol was added as an internal standard onto the cucumber tissue before the blender was closed. This gave a 1.0 ppm decanal concentration in the cucumber sample. The internal standard solution was prepared fresh for each experiment and stored in a freezer.

The blender had an inverted 80-mm funnel (15-mm i.d. stem) secured over the 50-mm opening of the cover. A 10-mm length of SPME fiber coated with a 100-μm thickness of poly(dimethylsiloxane) (PDMS; Supelco Co., Bellefonte, PA) was held in the 25-mm funnel stem by a ring stand. The fiber was exposed to the volatile compounds as they escaped through

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the funnel while cucumber tissue was converted to a slurry in the blender. Cucumbers were blended for 4 min at 3,450 rpm to give a uniform slurry.

Calibration. Standard mixtures were prepared by diluting (*E,Z*)-2,6-nonadienal and (*E*)-2-nonenal with methanol to give four concentrations of 5,000, 2,500, 500, and 50 ppm. A 1-mL aliquot of these four levels of standard solutions was pipetted directly onto 500 g of cucumbers (heated to prevent flavor volatile formation) immediately before blending them together with 1.0 mL of 500 ppm decanal internal standard solution. This resulted in final diluted levels in the cucumber samples of 10, 5, 1, and 0.1 ppm. Heat inactivation of the formation of cucumber flavor compounds was accomplished by placing whole cucumbers into an 80 °C water bath for a period of 20 min.

Gas Chromatography. The system consisted of an HP 5890 GC with a flame ionization detector (FID; Hewlett-Packard, Palo Alto, CA). The sample, adsorbed on a SPME fiber, was introduced into the injection port and thermally desorbed for 5 min at 250 °C onto a DB-5 capillary column (30 m × 0.32 mm, J & W Scientific, Folsom, CA). The injection port was equipped with a 0.75-mm i.d. inlet liner to optimize SPME desorption and sample delivery onto the column. There was no detectable carry-over of analytes on the fiber after sample desorption. The GC oven was held at 100 °C for 1 min after exposure of the SPME fiber in the injection port. The temperature was increased at a rate of 1 °C per min to 108 °C and then held at 108 °C for 1 min. The temperature program was completed by heating at a 30 °C per min rate to a final temperature of 200 °C, resulting in a 13-min run time. Chromperfect software (version 4.2.0.18, Justice Laboratory Software, Denville, NJ) was used for data acquisition and analysis.

Identities of (*E,Z*)-2,6-nonadienal and (*E*)-2-nonenal were confirmed by GC/MS. The system consisted of an HP 5890 II GC with an HP 5972 mass selective detector (MSD; Hewlett-Packard.

Evaluation of Sampling Variables. The effect of exposure time of the SPME fiber to volatiles as cucumber samples were blended was evaluated. Blending and exposure of the 10-μm PDMS-coated SPME fiber was carried out for periods of 3, 4, 5, 6, and 7 min. Triplicate samples were analyzed at each sampling time.

Known additions of 10, 5, 1, and 0.1 ppm (*E,Z*)-2,6-nonadienal and (*E*)-2-nonenal were added to fresh cucumber samples. These samples were compared to samples obtained with heated cucumbers to determine if release of the compounds added to fresh tissue was similar to release during blending heated cucumbers.

Exocarp tissue (peel) was removed from size 2B cucumbers with a hand vegetable peeler. Cucumber peel tissue was blended to uniform slurry, heated to 80 °C and held for 20 min to inactivate flavor generation enzymes. Duplicate 500-g samples of peeled cucumbers were sampled with the standard SPME headspace sampling technique with varying amounts of heat-treated cucumber peel added (0, 50, 100, 150, and 200 g). Varying amounts of heat-treated nonpeeled tissue (200, 150, 100, 50, and 0 g) were added in order to make a total sampling weight of 700 g for each analysis.

Reproducibility of SPME analysis of flavor compounds was evaluated by analyzing three lots of cucumbers over a 2-month period. Standard deviation of the analytical method was determined using the generalized linear models procedure (GLM) by SAS (SAS Institute Inc., Cary, NC).

Application of the Volatile Sampling Procedure to Heat Treatments of Cucumbers. Cucumbers were either heated for 20 min to equilibrate at the water bath temperature or blanched for 15 s. Blanch treatments of 15 s have been used previously to reduce the microbial load on fresh cucumbers (*22*).

Whole fruit were equilibrated at room temperature. Cucumbers were placed in a temperature-controlled water bath. Duplicate lots of cucumbers were heated for 20 min at 10 temperatures in the range of 30 to 80 °C. This was sufficient time to equilibrate cucumbers to the bath temperature (*23*).

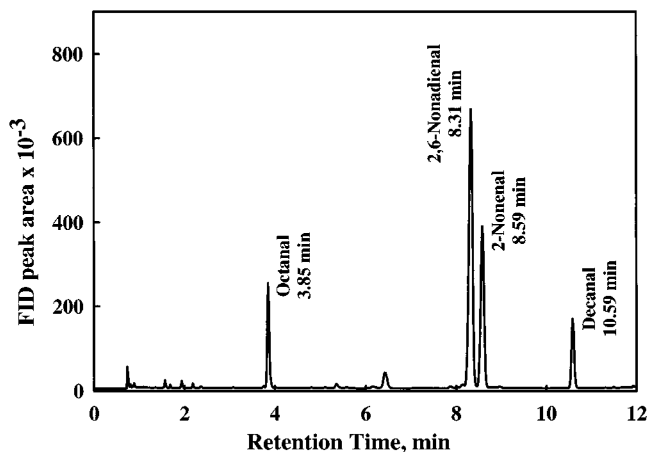

Figure 1. Gas chromatographic separation of cucumber flavor volatiles with added octanal and decanal after dynamic headspace sampling with a SPME fiber.

After heat treatment, cucumbers were placed into a 30 °C water bath for 20 min to equilibrate to a constant sampling temperature. Exactly 500 g of each sample of heated cucumbers was blended and sampled for production of (*E,Z*)-2,6-nonadienal and (*E*)-2-nonenal with an SMPE fiber.

Duplicate lots of whole cucumbers, equilibrated to ambient temperature, were immersed for 15 s in water in a steam-jacketed kettle heated to 60, 70, 80, 90, and 100 °C. Immediately after blanching, the fruit were transferred to 1,360-mL jars and covered with an equal volume of water for at least 15 min to cool the fruit. Production of (*E,Z*)-2,6-nonadienal and (*E*)-2-nonenal from 500-g blanched cucumber samples was measured with the SPME sampling technique.

RESULTS AND DISCUSSION

Figure 1 shows a gas chromatogram of (*E,Z*)-2,6-nonadienal and (*E*)-2-nonenal eluting at 8.31 and 8.59 min, respectively. Octanal and decanal, which were evaluated as internal standards for the SPME sampling method, had retention times of 3.85 and 10.59 min, respectively. Both compounds were suitable internal standards. Decanal was selected because it had a slightly smaller relative standard deviation in peak areas than was observed for octanal.

Formation of fresh flavor volatiles as cucumber tissue is disrupted is a dynamic process with the concentrations of (*E,Z*)-2,6-nonadienal and (*E*)-2-nonenal constantly changing. Therefore, a necessary element for a rapid, convenient method to assess the relative ability of cucumber tissue to form these compounds is to define a sampling procedure that gives reproducible results in a short time period. Selection of a sampling time to determine (*E,Z*)-2,6-nonadienal and (*E*)-2-nonenal production was based upon the requirements that uniform blending of cucumber tissue occurred, sufficient analyte was collected on the fiber to measure the range of production that might occur, and analysis time was kept to a minimum.

It took at least 3 min of blending to consistently produce a uniform cucumber slurry. Figure 2 shows changes in the peak areas of the flavor volatiles, as well as decanal, as sampling times from 3 to 7 min were evaluated. The peak areas of the analytes increased significantly ($P \leq 0.005$) with increasing sampling time, though the changes were not very large. This was expected because of continuing production of volatile components. On the other hand, the peak area of decanal had a slightly negative slope, but the slope was

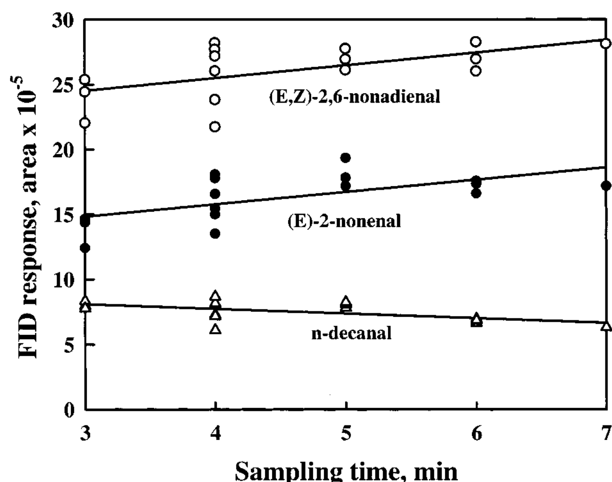

Figure 2. Effect of SPME sampling time on the analysis of cucumber flavor compounds.

not significantly different from zero. The fact that the amount of 2,6-nondienal and (*E*)-2-nonenal adsorbed to the fiber did not increase greatly with sampling time led to the decision to sample for 4 min.

Fleming et al. (*1*) observed that heating cucumbers before blending could reduce or eliminate increases in carbonyl compounds when the tissue was blended. We observed that heating cucumber fruit to equilibrate at 70 °C or higher resulted in no detectable formation of either (*E,Z*)-2,6-nonadienal or (*E*)-2-nonenal (shown later in Figure 5A). Therefore, cucumber tissue heated to 80 °C was evaluated to determine if it could be used as a suitable matrix for addition of known amounts of these compounds for calibration of their production as cucumbers were blended. Figure 3 shows there is a linear relationship between the amount of (*E,Z*)-2,6-nonadienal (Figure 3A) and (*E*)-2-nonenal (Figure 3B) added to heated cucumber pieces and peak area of an SPME sample collected for 4 min. When the compounds were added to nonheated cucumbers, the increase in peak area was also proportional to the added analytes. The slopes were not significantly different from the slopes for the heated tissue. The correlation coefficient for (*E,Z*)-2,6-nonadienal added to nonheated cucumber was not nearly as good as that observed for (*E*)-2-nonenal. This was thought to be due to the fact that amounts were near the upper limit of detection. The conclusion was that (*E,Z*)-2,6-nonadienal and (*E*)-2-nonenal went into the vapor phase and adsorbed to the SPME fiber to the same extent in heated tissue as in nonheated tissue. In subsequent work, calibration of the analysis was done by addition of four concentrations of (*E,Z*)-2,6-nonadienal and (*E*)-2-nonenal, along with decanal internal standard to cucumbers that had been heated to 80 °C.

The exocarp tissue of the cucumber has about 2-fold higher lipoxygenase activity than other parts of the fruit (*24, 25*). Therefore, if the fraction of exocarp tissue in cucumber samples were variable, due to cucumber size or peeling, that factor could affect the amount of flavor impact compounds produced. This technique should be able to measure the effects of such variables as different proportions of tissues on the ability of cucumbers to produce those aldehydes. However, there is likely to be more lipid in the exocarp tissue because of the natural waxy layer on the fruit surface and chloroplasts in the epidermal cells. This could change the distribution

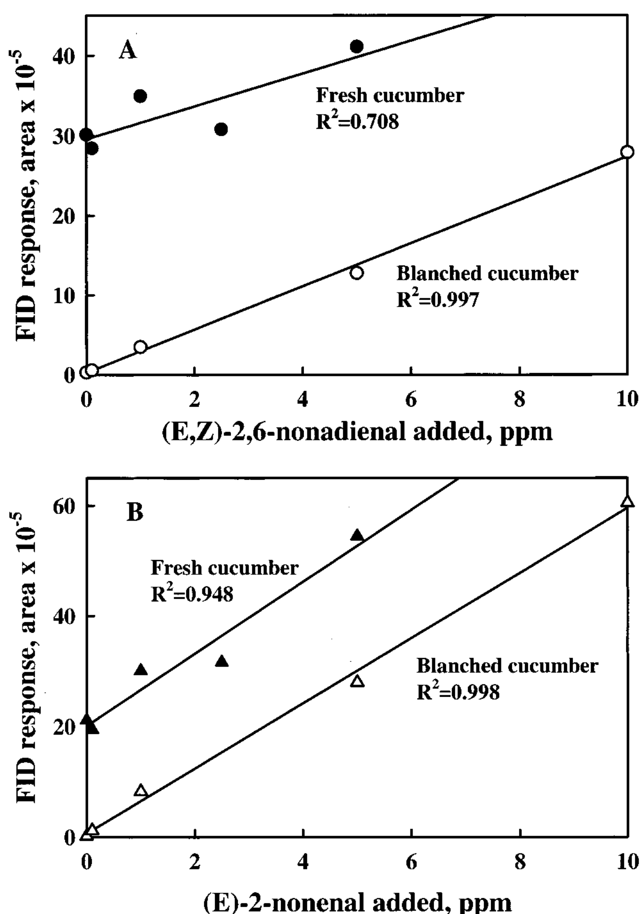

Figure 3. Analysis of (*E,Z*)-2,6-nondienal (A) and (*E*)-2-nonenal (B) added to fresh and heat-treated cucumber tissue.

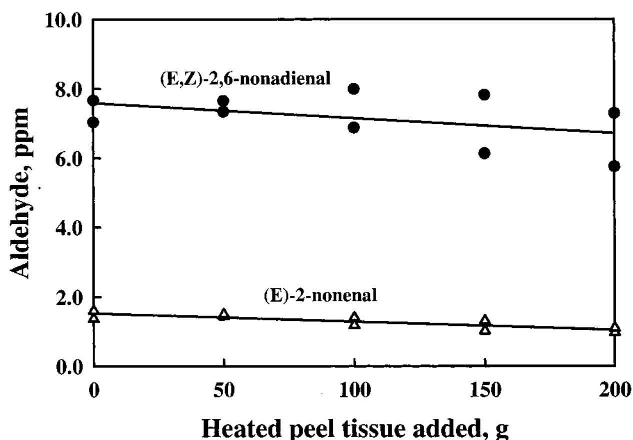

Figure 4. Effect of heated cucumber exocarp tissue on release of fresh cucumber flavor compounds (*E,Z*)-2,6-nonadienal and (*E*)-2-nonenal during SPME sampling.

coefficients of the lipid-soluble flavor compounds produced, which could interfere with measurement of differences in production of these compounds. To evaluate the effect of variable proportions of peel tissue on measurement of the amounts of (*E,Z*)-2,6-nonadienal and (*E*)-2-nonenal produced, increasing amounts of peel from cucumbers heated to 80 °C to inactivate enzymes were added to 500 g of nonheated cucumber tissue.

Figure 4 shows the amount of (*E,Z*)-2,6-nonadienal and (*E*)-2-nonenal found, using decanal as the internal standard, in the presence of up to 200 g of added peel tissue. The size of the chromatographic peaks of both

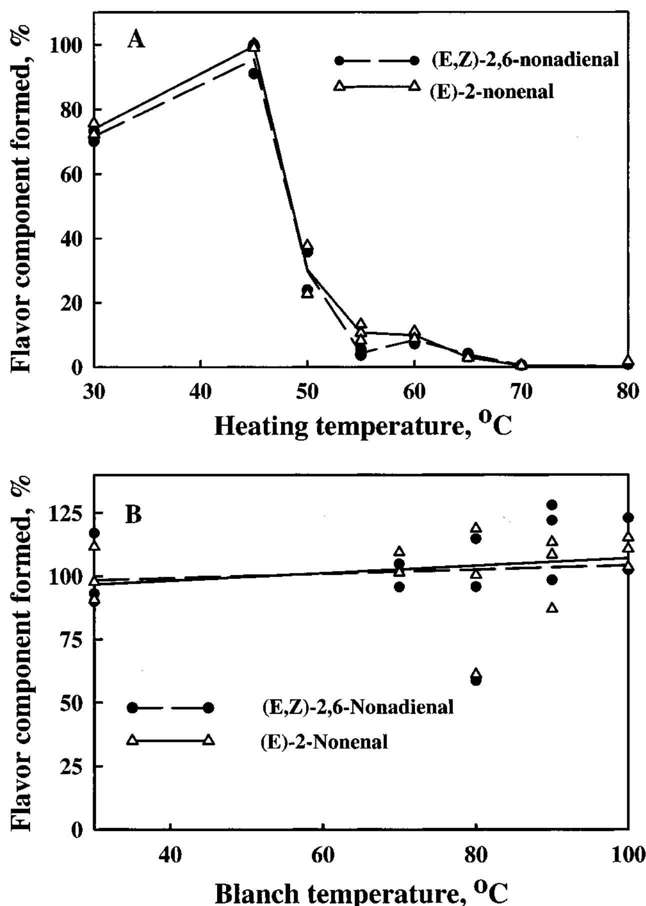

Figure 5. Heat inactivation of the production of (*E,Z*)-2,6-nonadienal and (*E*)-2-nonenal in whole size 2B cucumbers. Cucumbers were either heated for 20 min (A) or blanched for 15 s (B) at the treatment temperatures. 100% generation was 12 ppm for (*E,Z*)-2,6-nonadienal and 4 ppm for (*E*)-2-nonenal.

Table 1. Reproducibility of Formation of (*E,Z*)-2,6-Nonadienal and (*E*)-2-Nonenal in Fresh Cucumber Tissue Slurries[a]

compound	concentration (ppm)	root MSE (ppm)	standard deviation (%)
(*E,Z*)-2,6-nonadienal	10.6	1.1	10.4
(*E*)-2-nonenal	3.0	0.3	10.0

[a] Three different lots of cucumbers were analyzed.

the analytes and internal standards declined as the amount of added exocarp tissue increased. The slopes for the two lines are both slightly negative. However, statistically the slopes of the lines were not significantly different from zero ($P \leq 0.05$). This result suggested that differences in production of these compounds in cucumbers of different sizes or different peel thickness, where the proportion of lipid components would be somewhat different, could be determined using the SPME sampling method with decanal added as an internal standard.

Analysis of multiple lots of cucumbers gave an average formation of about 10 ppm (*E,Z*)-2,6-nonadienal and 3 ppm (*E*)-2-nonenal (Table 1). Zhou and McFeeters (*16*) found about 3 ppm (*E,Z*)-2,6-nonadienal by purge and trap sampling of fresh cucumber slurry. However, the cucumber was blended for only 20 s, so there was less tissue disruption and, as a result, formation of less (*E,Z*)-2,6-nonadienal. The reproducibility of about ±10% for both compounds was thought to be reasonable, considering the dynamic character of both formation of these compounds and the sampling method.

Use of this method to evaluate changes in the ability of cucumber tissue to produce these flavor impact compounds is demonstrated in Figure 5. Cucumbers were either heated for 20 min to equilibrate at the water bath temperature or heated for only 15 s to kill surface microorganisms (*22*). Figure 5A shows there were almost identical changes in the relative amounts of the two flavor impact compounds when cucumbers were heated to temperatures up to 80 °C. This result indicated that, despite the fact that (*E,Z*)-2,6-nonadienal and (*E*)-2-nonenal are produced from different fatty acids (*13, 14*), the same enzyme system apparently catalyzed formation of these compounds. This is consistent with the recent finding that cucumber mesocarp tissue contains a single hydroperoxide lyase that catalyzes lysis of the 9-hydroperoxides of linoleic and linolenic acids more efficiently than the 13-hydroperoxides (*26*). It is also consistent with the earlier suggestions of Gardner (*27*) and Hatanaka (*28*). No detectable amounts of these compounds were observed after heating to 70 or 80 °C. The inactivation of the flavor-generation enzyme system in whole fruit was similar to that observed for heat inactivation of lipoxygenase (*25*).

Brief blanch treatments of cucumbers (15 s) can reduce the number of microorganisms on fresh cucumbers by a factor of 10^3 or greater (*22*). Because lipoxygenase activity in cucumber peel is about twice as high as that in the remainder of the fruit (*24, 25*), it was possible for brief blanch treatments to inactivate lipoxygenase in the peel and reduce the amount of fresh flavor compounds produced when the tissue was disrupted. The results shown in Figure 5B showed that short time exposure, even in boiling water, did not result in significant change in the ability of the tissue to produce flavor impact compounds when it was blended.

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