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Fresh Cucumber Flavor in Refrigerated Pickles: Comparison of Sensory and Instrumental Analysis

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The ability of nonacidified, refrigerated pickled cucumbers to produce the fresh cucumber flavor impact compounds (*E*,*Z*)-2,6-nonadienal and (*E*)-2-nonenal declined during storage. Production of these compounds decreased as the pH of refrigerated cucumbers was reduced. Despite the fact that the concentrations of (*E*,*Z*)-2,6-nonadienal and (*E*)-2-nonenal generated were over 10^5-fold greater than the threshold levels, it was possible for a sensory panel to consistently detect differences in the intensity of fresh cucumber flavor, provided the pH difference between samples was 1 unit or greater. The presence of spices did not interfere with the ability of panelists to detect differences in fresh flavor intensity. There was a linear correlation between sensory scores and the amount of (*E*,*Z*)-2,6-nonadienal produced by cucumbers equilibrated at different pH levels.

KEYWORDS: ***Cucumis sativus*; gas chromatography; acidification; (*E*,*Z*)-2,6-nonadienal; (*E*)-2-nonenal**

INTRODUCTION

Nonacidified, refrigerated pickle products are characterized by their ability to retain fresh cucumber flavor notes, perishability, and variability (*1*). These products, commonly called overnight or half-sour dill pickles, have not been previously investigated with regard to production of their characteristic fresh cucumber flavor during storage. Fresh cucumber aroma/flavor is the result of enzymatic degradation of linoleic acid and linolenic acid that occur rapidly after the tissue is disrupted. Schieberle et al. (*2*) found that (*E*,*Z*)-2,6-nonadienal had the greatest fresh cucumber odor impact. (*E*)-2-nonenal was found to be the second most important odor compound, with about 2% the odor impact of (*E*,*Z*)-2,6-nonadienal.

The enzyme system responsible for generating fresh cucumber flavor is unstable at low pH. Fleming et al. (*3*) found that formation of carbonyl compounds was prevented when whole cucumbers were blended with water acidified to pH 1.0. Jang et al. (*4*) extracted cucumber lipoxygenase and observed decreasing activity as the pH decreased from 6.5 to 3.0.

There is little published information on sensory analysis of fresh cucumber flavor. The odor threshold for (*E*,*Z*)-2,6-nonadienal has been estimated to be 0.01 ppb (*5*), whereas the threshold for (*E*)-2-nonenal is somewhat higher at about 0.08 ppb (*6*). These extremely low thresholds are several 100,000-fold lower than the amount present when fresh cucumbers are blended (*7*, *8*). The olfactory sense has trouble accommodating a 100-fold difference between the threshold and the concentration which saturates the receptor (*9*). It is possible, therefore, that people would be unable to detect changes in the intensity of fresh cucumber odor in a product until production of the odor impact compounds was nearly eliminated.

The objectives of this project were to (1) measure changes in the ability of refrigerated cucumber pickles to produce fresh cucumber flavor compounds during storage, (2) determine whether a sensory panel can detect variations in the intensity of cucumber flavor during storage of nonacidified, refrigerated pickles, and (3) determine whether typical spices used in this product would affect the perception of fresh cucumber flavor.

MATERIALS AND METHODS

Preparation of Nonacidified, Refrigerated Pickles. Nonacidified, refrigerated pickles were made from size 2B (3.5 to 3.8 cm diameter) pickling cucumbers obtained from a local processor. Cucumbers were washed in a reel washer and then packed into 1360-mL jars and covered with an equal weight of brine to give a 50:50, cucumber/brine, pack-out. Cover solution was prepared with 4% NaCl and 0.2% sodium benzoate to equilibrate in the product at 2% and 0.1%, respectively. When spices were added to the cucumbers, one crushed garlic clove and 10 g of dry spices were added per jar. The spice mixture was a blend of dill seed, coriander, mustard seed, cloves, allspice, bay leaves, cassia, peppercorns, and anise seeds (National Foods, Inc., Bronx, NY).

pH Adjustment of Refrigerated Pickles. The pH of refrigerated pickle samples was reduced below the natural pH of about 5.5 by addition of appropriate amounts of either acetic acid or HCl, determined by titration, to lower the pH to predetermined levels.

pH Adjustment Procedure. A sample of 10−12 cucumbers was blended into a slurry. A sample of the cucumber slurry (100 g) was mixed with 100 mL of cover solution (4% NaCl, 0.2% Na benzoate). This mixture was titrated by addition of aliquots of acid (glacial acetic acid or 3 N HCl) to the slurry until the pH was <3.5. The amount of acid required to reach the target pH in a 1360-mL jar was calculated from the titration curve by interpolation and multiplication by the appropriate volume factor.

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Storage Experiments. Sufficient jars of nonacidified cucumbers without added spices were prepared to sample triplicate jars four times over a 40-d storage period. The jars were stored at 5 °C. The equilibrated pH and the ability of the cucumber tissue to produce (*E*,*Z*)-2,6-nonadienal and (*E*)-2-nonenal were determined on each jar. An identical experiment, except for the fact that the cucumbers were acidified to pH 3.5 with acetic acid and that sampling was done over an 8-d storage period, was also carried out.

Sensory and Instrumental Measurement of Fresh Cucumber Flavor. Cucumbers were prepared with and without spices with the pH target levels of 5.5 (no HCl added), 5.0 (1.26 mL of 3 N HCl added per jar), 4.5 (2.88 mL of 3 N HCl), and 4.0 (5.12 mL of 3 N HCl). After 7 d storage at 5 °C to allow equilibration, the cucumbers at each pH were evaluated by a trained panel of 24 people for the relative intensity of fresh cucumber flavor. On the next day, triplicate jars at each target pH were analyzed by SPME-GC for their ability to produce (*E*,*Z*)-2,6-nonadienal and (*E*)-2-nonenal. In addition, the pH of the cover solution in each jar was measured. This experiment was replicated 2 weeks later with another lot of cucumbers.

Measurement of (*E*,*Z*)-2,6-Nonadienal and (*E*)-2-Nonenal Formation. The procedure described by Palma-Harris et al. (*7*) was used, except that calibration of the procedure was done with heat-treated cucumbers which had been equilibrated during storage at 5 °C to contain 2% NaCl and 0.1% sodium benzoate. Decanal was used as the internal standard. As cucumbers were blended, volatile components were collected on a solid-phase microextraction (SPME) fiber. The volatile components were then analyzed by gas chromatography with an FID detector.

Sensory Analysis Procedure. A forced choice/pairwise ranking test was used to evaluate the intensity of fresh cucumber flavor (*10*). A balanced incomplete block design with individual panelists as the blocks was employed. Four samples, prepared to equilibrate to the four target pH values described above, were evaluated. The six possible pairs from the four samples were presented randomly to a group of 24 trained panelists. Randomization of samples within pairs, between pairs, and among panelists was done with the PROC PLAN procedure in SAS (SAS Inc., Cary, NC). A different random three-digit number was assigned to each sample each time it was presented to each panelist. To minimize fatigue, panelists were presented with three pairs of samples in a morning session and three sample pairs in an afternoon session. The panelists did duplicate evaluations on each of the six sample pairs. The complete experiment was replicated with a second lot of cucumbers. The same group of panelists again did duplicate evaluations of the six sample pairs.

Training of panelists was done in two sessions to familiarize the participants with the fresh cucumber flavor attribute. Panelists were asked to smell freshly cut cucumber slices and a mixture of (*E*,*Z*)-2,6-nonadienal and 2-nonenal at concentrations similar to that found in fresh cucumbers. They tasted cucumbers with and without added spices with different levels of flavor compounds. Panelists were asked to use a uniform sampling procedure by chewing cucumber slices the same number of times during their evaluations. None of the panelists were specifically anosmic to fresh cucumber flavor. The ballot had only one question, "Which sample has more fresh cucumber flavor?". There was no option for "no difference" verdicts. Panelists were told to guess if they were uncertain which sample had more fresh flavor. The sample of a pair judged to give the lowest flavor intensity was assigned a score of 1. The sample judged to have the higher flavor intensity was given a score of 2. The rank sum for a sample was calculated by multiplying the number of times it was judged to be less intense by 1 and adding that to the number of times it was judged to be more intense multiplied by 2.

Statistical Analysis. The Friedman test statistic (*10*) was calculated as an overall test of significance for a group of four samples. Tukey's "honestly significant difference" test (*11*) was used to determine differences between pairs of samples.

Linear regression analysis was done on the production of (*E*,*Z*)-2,6-nonadienal as a function of the pH of refrigerated pickle samples with and without added spices. The ANOVA procedure of SAS was used to test whether the slopes of the lines with and without spices were the same.

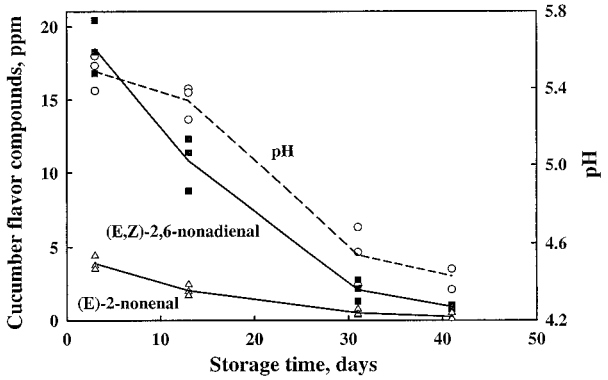

Figure 1. Changes in pH and the ability of cucumber tissue to produce fresh flavor compounds during refrigerated storage of nonacidified cucumbers.

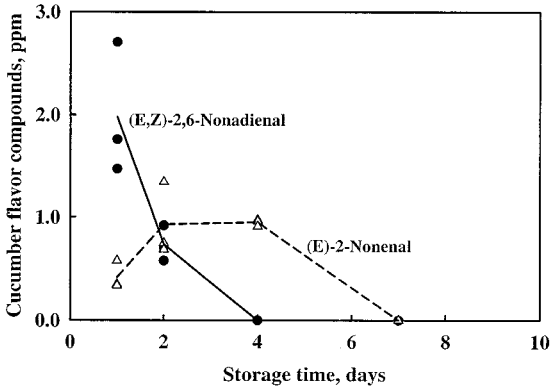

Figure 2. Changes in the ability of cucumber tissue to produce fresh flavor compounds during refrigerated storage of cucumbers initially acidified to pH 3.5.

The relationship between sensory rank sum scores for fresh cucumber flavor and the production of (*E*,*Z*)-2,6-nonadienal was also compared by linear regression analysis.

RESULTS AND DISCUSSION

Nonacidified, refrigerated cucumbers gradually declined in pH when stored for several weeks at 5 °C. This was probably a result of a small amount of acid production by microorganisms during refrigerated storage. Along with the decline in pH, there was also a decline in the ability of the stored cucumbers to produce (*E*,*Z*)-2,6-nonadienal and 2-nonenal, the major odor impact compounds of fresh cucumbers (**Figure 1**). However, when cucumbers were acidified to pH 3.5 at the beginning of the storage period, the ability to produce these compounds was much reduced (**Figure 2**). Production was not detectable after 1 week. Reduced production of flavor impact compounds with lower pH is consistent with the pH dependence of the enzymatic flavor generation system (*4*, *12*). Also, Zhou and McFeeters (*8*) found that the ability of cucumber tissue to produce these compounds was lost after 5 d of an active lactic acid fermentation.

Figure 3 shows the relationship between the production of (*E*,*Z*)-2,6-nonadienal when cucumbers were stored for 1 week in cover solutions with acid added to adjust to different pH levels in the range of 4 to 6. A second experiment with a different lot of cucumbers resulted in a similar degree of variability of this flavor component. Addition of garlic and a commercial spice mixture did not affect the production of the flavor impact compounds. The R^2 value for the linear regression indicated that pH accounted for about 75% of the variability in the

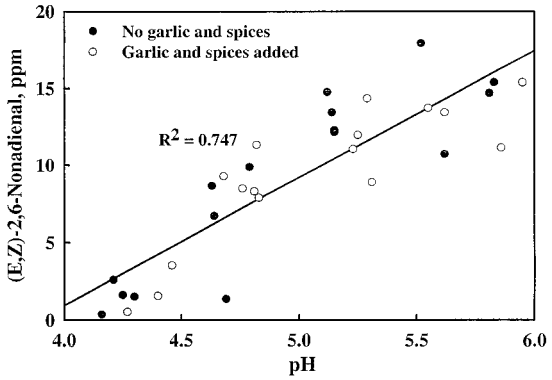

Figure 3. Relationship between storage pH and the ability of refrigerated cucumbers to form (*E*,*Z*)-2,6-nonadienal. Samples with and without added spices were not significantly different in their ability to produce this flavor compound.

Table 1. Detection of Differences in Fresh Cucumber Flavor Intensity between Pairs of Cucumbers Adjusted to Equilibrate at Different pH Levels[a]

	no spice or garlic		added spice and garlic	
pH of sample pairs	lot 1	lot 2	lot 1	lot 2
5.5 and 5.0	-	-	+	-
5.0 and 4.5	-	+	-	-
4.5 and 4.0	-	+	+	+
5.5 and 4.5	+	+	+	+
5.0 and 4.0	+	+	+	+
5.5 and 4.0	+	+	+	+

[a] Tukey's "honestly significant difference" test: +, indicates rank sum scores between sample pairs are different at $P \leq 0.05$ significance level; –, indicates no difference between the pairs at $P \leq 0.05$.

production of (*E*,*Z*)-2,6-nonadienal. Given the relatively low R^2 value for the linear regression, there may have been an undetermined secondary variable that affected the ability of the cucumber tissue to produce this compound.

Sensory evaluation of the intensity of fresh cucumber flavor in two lots of refrigerated cucumbers adjusted to four pH values from 4 to 6 showed that significant differences in flavor intensities were perceived when the pH difference was one unit or greater (**Table 1**). The presence of garlic and spices typically used in these products did not affect the ability of panelists to detect differences in fresh flavor intensity between pairs of samples (**Table 1**). Comparison of rank sum sensory scores with the production of flavor compounds as determined by SPME-GC analysis showed a linear relationship over a production range from about 1 to 16 ppm (*E*,*Z*)-2,6-nonadienal (**Figure 4**). The relationship between sensory rank scores and flavor production measured instrumentally was not significantly affected by the presence of added spices. These concentrations range from about 10^5-fold greater than the odor threshold level of (*E*,*Z*)-2,6-nonadienal to well over 10^6-fold above threshold. In contrast to the suggestion of Meilgaad et al. (*9*) of the limited range over which people can detect differences in odor intensity, it was possible for a panel to differentiate the fresh flavor intensity of cucumbers with quite good reliability at levels greatly in excess of what should be "saturating". This differentiation was possible even when other high-odor-intensity spices were added.

This investigation has demonstrated a strong dependence between product pH and the ability of refrigerated cucumbers to produce flavor components that characterize fresh cucumber flavor. It has also been shown that people can detect differences in the intensity of cucumber over the range of variation that can occur during the normal shelf life of this product. Therefore, to the extent that fresh flavor is a desirable attribute for refrigerated, nonacidified pickles, it would be beneficial to minimize the decline in product pH.

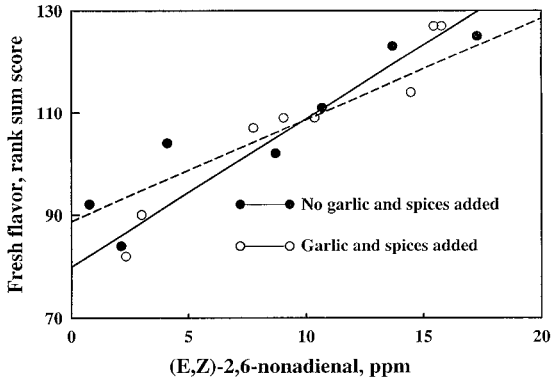

Figure 4. Relationship between sensory perception of fresh cucumber flavor and the production of (*E*,*Z*)-2,6-nonadienal by cucumber tissue.

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