Inhibition of Formation of Oxidative Volatile Components in Fermented Cucumbers by Ascorbic Acid and Turmeric†

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Two naturally occurring antioxidants, ascorbic acid and turmeric, were effective in inhibiting formation of hexanal, (*E*)-2-penenal, (*E*)-2-hexenal, (*E*)-2-heptenal, and (*E*)-2-octenal when slurries of fermented cucumber tissue were exposed to oxygen. Added ascorbic acid prevented formation of most of these oxidative aldehydes at 175 ppm or greater. Turmeric, which is used commercially as a yellow coloring in cucumber pickle products, was found to almost completely prevent aldehyde formation at 40 ppm.

Keywords: *GC−MS; hexanal; Curcuma longa; tartrazine; curcumin*

INTRODUCTION

Many food products undergo quality losses due to oxidative reactions when they are exposed to air. Development of oxidative rancidity in foods is due to formation of lipid hydroperoxides from unsaturated fatty acids, which break down to form a variety of compounds, including aldehydes, ketones, alcohols, acids, and hydrocarbons. Some of these compounds are associated with development of rancid flavors (St. Angelo, 1996).

When fermentation tanks are emptied, cucumbers may be exposed to air for several hours as they are moved from tank yards through final processing operations. Also, the industry is increasing the use of plastic packaging materials, which allow some oxygen diffusion into the products. Off-flavors may result from this exposure to oxygen. Previous investigation found that increases in the concentration of several aldehydes were closely correlated with the development of oxidized odor in fermented cucumber slurries exposed to oxygen (Zhou et al., 2000).

The usual approach to prevent or at least slow development of lipid oxidation induced off-flavors in foods is to use appropriate antioxidants. Of particular interest for fermented cucumbers is the use of ascorbic acid, which is naturally present, and turmeric oleoresin, which is used commercially as a yellow coloring agent in pickle products. Curcumin, the major pigment in turmeric, has been reported to have antioxidant activity in several test systems (Sarma, 1976; Ammon and Wahl, 1991; Gorman et al., 1997). However, there is no evidence that it is effective as an antioxidant in foods at reasonable use levels.

The objective of this work was to evaluate the ability of ascorbic acid and turmeric to prevent formation of oxidative aldehydes when fermented cucumbers were exposed to oxygen.

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MATERIALS AND METHODS

Turmeric coloring (7% curcumin content) was obtained from Kalsec, Inc. (Kalamazoo, MI). Chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI). Cucumbers (size 2B, 35−38 mm diameter) were obtained from a local processing plant.

Fermentation Procedure. Cucumbers were brined in 1360 mL jars. A 50:50 cucumber:cover liquid pack-out ratio was used. Brine solutions were prepared to cover the cucumbers, so the equilibrated concentrations of the added compounds were 18 mM $Ca(OH)_2$, 53 mM acetic acid, and 2% NaCl (Fleming et al., 1988). The jars were inoculated with 10^6 CFU mL^{-1} *Lactobacillus plantarum* MOP-3 culture immediately after the jars were filled with cucumbers and cover solution. Starter culture was prepared by growing the organism overnight at 30 °C in MRS broth (Difco Labs, Detroit, MI) containing 5% salt. Cells were collected by centrifugation and then resuspended in physiological saline solution (0.85% NaCl). Jars were sealed with lids that had a 15 mm diameter rubber septum inserted. Inoculated cucumbers were incubated at 26−30 °C for 3 weeks. After fermentation, antioxidant compounds were added through the septum in the jar lid (see below) and allowed to equilibrate in the absence of oxygen.

Antioxidant Treatments. Cucumbers without added antioxidants were compared to those in identical jars with added L-ascorbic acid or turmeric. An aqueous solution of ascorbic acid (8.0 mL) was injected into the fermented cucumber jars 3 days before slurry preparation. Turmeric extract containing Tween 80 was dispersed in 8.0 mL of water and added 3 days before preparation of the slurry. Several concentrations of L-ascorbic acid and turmeric were added. Fermented cucumbers (400 g) were blended with 400 g of brine from the same jar in a Waring blender for 20 s. After blending, 200 g of slurry was transferred to four 236 mL jars, which were closed with lids containing two rubber septa. The jars were flushed at 40 mL min^{-1} with O_2 or N_2 for 10 min. This was done by placing the tip of an 18 gauge needle near the bottom of the cucumber slurry. Gas escaped from the jars by placing another 18 gauge outlet needle in the headspace of the jars. After being flushed with gas, slurry samples were placed in a water bath at 30 °C for up to 96 h to allow autooxidation to occur.

Duplicate 1360 mL jars of cucumbers with each antioxidant treatment and nontreated controls were blended, sparged, and distributed into 236 mL jars. Then duplicate jars were sampled at 0, 24, 48, and 96 h after being sparged with oxygen. Each jar was sampled at only one time point and then discarded.

10.1021/jf990669t CCC: $19.00
Published on Web 08/10/2000

A similar procedure was used to determine the effect of yellow no. 5 and curcumin on inhibition of aldehyde formation, except aldehydes were only analyzed 96 h after the jars were flushed with oxygen. Yellow no. 5 (34 mg) was dissolved in 8.0 mL of water, and curcumin (9.5 mg) was dissolved in 8.0 mL of absolute ethanol. Both solutions were injected into fermented cucumber jars 3 days before slurry preparation.

GC−MS with a Purge and Trap Sampler. To measure volatile changes which occurred during exposure of fermented cucumber slurry to oxygen, a 10 g sample of slurry was placed in a fritted glass sparger (Angel Inc., Panorama City, CA) along with fully deuterated toluene (94.3 ng) internal standard in 1 μL of methanol. The slurry was sparged for 30 min with helium (40 mL min^{-1}) on a CDS 6000 purge and trap sampler (CDS Analytical Inc., Oxford, PA). The volatile components from the slurries were absorbed on a Tenax trap (Supelco Inc., Bellefonte, PA). After the purge period, the Tenax trap was sparged for 3 min with dry helium to remove water. Trapped volatile components were transferred to the GC column by heating the Tenax trap for 6 min at 180 °C with a carrier gas flow rate of 4.0 mL/min through the trap. An HP-5 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness, Hewlett-Packard, Palo Alto, CA) was used for separation of the volatiles. The GC−MS system consisted of a HP 5890 II GC and HP 5972 mass selective detector (Hewlett-Packard, Palo Alto, CA). The GC oven temperature was programmed from −20 °C to +220 °C at 15 °C min^{-1} with initial and final hold times of 6 and 1 min, respectively. The carrier gas was electronically controlled at a linear velocity of 42.4 cm s^{-1} (1.5 mL min^{-1}). The ionization voltage for the mass spectrometer was 70 eV. The mass scanning range was 35−350 Da.

Ascorbic Acid Analysis of Fresh Cucumbers. The HPLC method of Vanderslice and Higgs (1993) was modified for measurement of ascorbic acid. A C_{18} column (PLRP-S 100 Å, 5 μm, 250 mm/4.6 mm, Polymer Lab, Amherst, MA), together with a PLRP-S guard cartridge (part no. 1612-1801), was maintained at 4 °C in a refrigerator. The column was eluted at 0.5 mL min^{-1} with 0.2 M NaH_2PO_4 solution (pH adjusted to 2.14). A Rheodyne (Cotati, CA) model 9125 PEEK injector with a 10 μL loop was used for sample injection. Instead of a fluorescence detector (Vanderslice and Higgs, 1993), a pulsed amperometric detector (Dionex, Sunnyvale, CA) with a gold electrode with a continuous potential of +0.8 V was used for detection of ascorbic acid. Detector settings were t_1 = 300 ms and t_2 and t_3 = 0, with a full-scale output range set at 300 nA. Chromatograms were collected and analyzed using ChromPerfect software (Justice Innovations, Inc., Mountain View, CA) installed on a Gateway 486-33 computer (Gateway, Sioux City, SD). Calibration was done on the basis of the peak area of three standard solutions with ascorbic acid concentrations of 17.6, 35.2, and 70.4 ppm.

RESULTS AND DISCUSSION

Among over 40 fruits and vegetables, cucumbers have been found to have the lowest natural antioxidant activity (Cao et al., 1996). However, they do contain some ascorbic acid. In these experiments there was about 80−100 ppm ascorbic acid in the fresh cucumbers, which, after dilution with cover liquid, was reduced to about 40−50 ppm after fermentation. Figure 1 shows that, with the natural ascorbic acid level (0 ppm added ascorbic acid), five aldehydes, hexanal, (*E*)-2-pentenal, (*E*)-2-hexenal, (*E*)-2-heptenal, and (*E*)-2-octenal, were formed. Zhou et al. (2000) previously showed these aldehydes are formed nonenzymatically in fermented cucumber slurries. Added ascorbic acid decreased the concentrations of all five aldehydes, even after 96 h of exposure to oxygen (Figure 1). At 175 ppm or greater added ascorbic acid, formation of aldehydes was almost completely inhibited. The time course for formation of the most abundant aldehyde (hexanal) with different concentrations of added ascorbic acid is shown in Figure 2. These results showed that ascorbic acid was an effective inhibitor of oxidative off-flavor development in fermented cucumbers, even though substantial oxidation occurred with only the natural concentration of ascorbate.

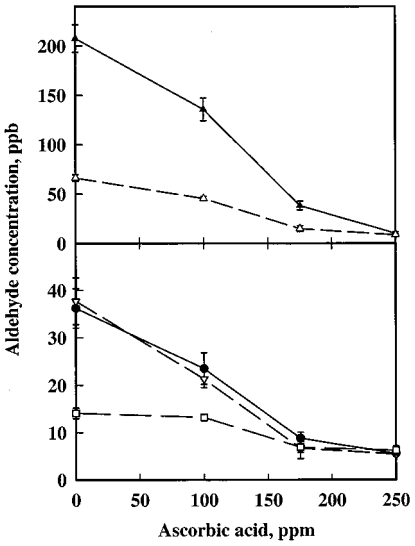

Figure 1. Effect of added ascorbic acid on formation of hexanal (▲), (*E*)-2-heptenal (△), (*E*)-2-octenal (▽), (*E*)-2-pentenal (●), and (*E*)-2-hexenal (□) after exposure of fermented cucumber slurries to oxygen for 96 h. Error bars show the standard deviation for duplicate samples analyzed at each sampling point. If error bars are not visible, the standard deviation was within the range of the data point.

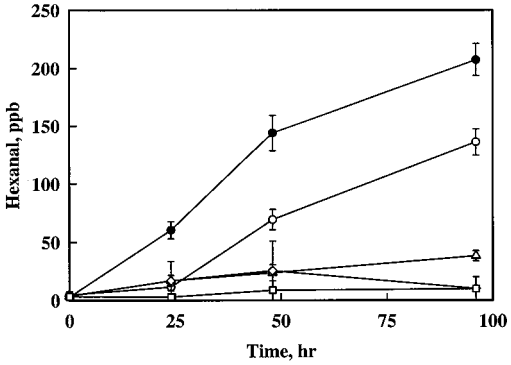

Figure 2. Time course of hexanal formation in fermented cucumber slurries exposed to oxygen with different concentrations of added ascorbic acid. The natural level of ascorbic acid was 48 ppm. Added ascorbic acid concentrations were 0 ppm (●), 100 ppm (○), 175 ppm (△), and 250 ppm (◇). Control slurry samples (□) without added ascorbic acid were flushed with nitrogen. Error bars show the standard deviation for duplicate samples analyzed at each sampling point.

Turmeric is a natural spice extract from *Curcuma longa* that is used as a yellow coloring agent in cucumber pickle products. The yellow pigment, which is present at concentrations of 7−9% in commercial turmeric preparations, is curcumin. Figure 3 shows the effect of turmeric concentration on formation of the same five aldehydes as were analyzed for the ascorbic acid experiment. The turmeric inhibited formation of all of these aldehydes at very low concentrations. Only 5 ppm reduced the formation of the aldehydes between 72% ((*E*)-2-hexenal) and 84% (hexanal). Minimal formation of aldehydes occurred at 40 ppm turmeric.

Yellow no. 5 is used instead of turmeric as a yellow coloring in many pickle products. Its advantage is that

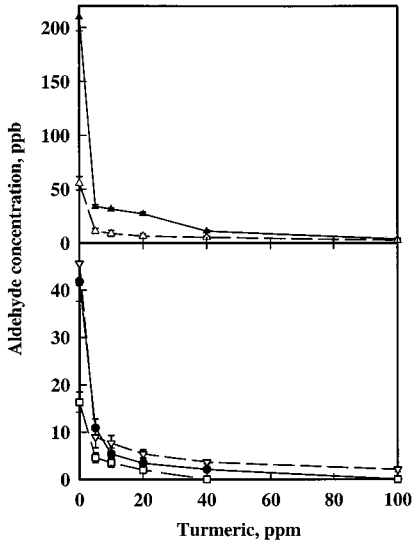

Figure 3. Effect of added turmeric on the formation of hexanal (▲), (*E*)-2-heptenal (△), (*E*)-2-octenal (▽), (*E*)-2-pentenal (●), and (*E*)-2-hexenal (□) after exposure of fermented cucumber slurries to oxygen for 96 h. Error bars show the standard deviation for duplicate samples analyzed at each sampling point.

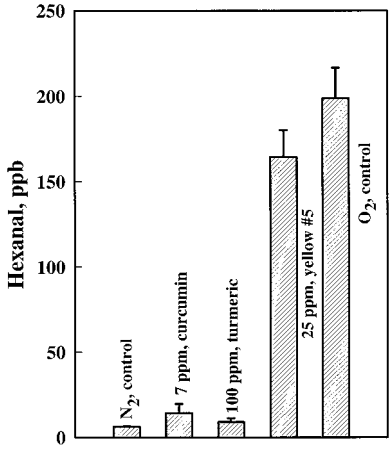

Figure 4. Effect of turmeric, curcumin, and FD&C yellow no. 5 on the formation of hexanal after 96 h of exposure of fermented cucumber slurries to oxygen. Turmeric oil (100 ppm), curcumin (7 ppm), and FD&C yellow no. 5 (25 ppm) were added to jars of fermented cucumbers prior to the slurries being made. These concentrations gave equivalent yellow color intensity. Error bars show the standard deviation for duplicate samples analyzed.

it does not fade in light like turmeric coloring (Price and Buescher, 1996). Figure 4 shows the inhibition of hexanal formation by 25 ppm yellow no. 5, which gave a yellow color intensity equivalent to 100 ppm turmeric extract. Also shown in Figure 4 is formation of hexanal in the presence of curcumin at a concentration equivalent to that present in 100 ppm turmeric. Curcumin inhibited aldehyde formation to about the same extent as the turmeric extract. This indicated that it was the primary turmeric component responsible for prevention of oxidative changes in fermented cucumber slurries. Yellow no. 5 had only a small effect on inhibition of oxidative changes.

In these experiments, cucumber slurries were exposed to rather severe conditions (100% oxygen in the headspace for 96 h at 30 °C), which resulted in nonenzymatic production of sufficient aldehydes to give strong off-odors (Zhou et al., 2000). Both ascorbic acid and turmeric, at concentrations that would be reasonable to add to pickle products, inhibited formation of these aldehydes. On the basis of the current results, it would be reasonable to evaluate use of turmeric and ascorbic acid in products and processing situations where development of off-odors and flavors due to oxygen exposure is a problem. Turmeric is currently used in some commercial pickle products as a coloring agent. The concentrations required for coloring purposes vary among products and processors, but approximately 200 ppm is commonly used (Howard Haley, Kalsec, Inc., personal communication). Since turmeric is effective as an antioxidant at substantially lower concentrations, it could be a useful antioxidant even in situations where coloring is not required.

CONCLUSIONS

Both of the naturally occurring antioxidants investigated, ascorbic acid and turmeric, were effective in preventing formation of several aldehydes that appear to be responsible for off-odors when fermented cucumber tissue is exposed to oxygen. Due to its history of use in pickle products, use of turmeric as an antioxidant in these products might be readily accepted for commercial applications.

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Received for review June 21, 1999. Revised manuscript received June 21, 2000. Accepted June 26, 2000. This investigation was supported in part by a research grant from Pickle Packers International, Inc., St. Charles, IL. 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.

JF990669T